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Innervation of rabbit heart ventricles: an immunohistochemical approach

P. Alaburda, N. Paužienė, K. Rysevaitė, H. Inokaitis, D.H. Pauža

Institute of Anatomy, Faculty of Medicine, Lithuanian University of Health Sciences

Introduction

In some cases of myocardial infarction, afferent nerve fibres induce sympathetic hyperactivation, which causes the onset of ventricular arrhythmias or even sudden cardiac death. The autonomic nervous system exerts its effects through intracardiac nerves, nerve fibre bundles, cardiac ganglia and even individual neurons. Little is known about the organisation of these structures. The present study immunohistochemically analysed the distribution of the intracardiac nerve plexus of the rabbit heart.

Materials and methods

Rabbit hearts (N = 10) were perfused with PBS and prefixed with a 4% PFA solution. The ventricles were sectioned into three blocks: the upper third or the base, the middle third or the middle and the lower third or the apex. Tissue samples were stained immunohistochemically for protein gene product 9,5 (PGP9,5), tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), nitric oxide synthase (nNOS), calcitonin gene related peptide (CGRP) and substance P (SP). The area, density and distribution of positively stained neuronal structures within the epicardium, myocardium and endocardium were analysed.

Results

The largest nerves were found in the epicardium; a nerve fibre plexus was observed in the myocardium and the endocardium (Fig. 1). In epicardial nerves, TH positive fibres were dominant (64%), while other phenotypes were less pronounced (5-7%) (Fig. 2 and 3). The heterogeneous nerve fibre plexus of the myocardium mainly contained TH positive fibres (1%), while nNOS fibres were more dense than ChAT fibres ($p < 0.05$). A negative correlation was found between the density of myocardial nerve fibres and the distance from the epicardium (Fig. 4). In the endocardium, the nerve fibre density of all fibre phenotypes was 10 times higher than in the myocardium, with SP and CGRP positive fibres exceptionally pronounced (3% and 4%) (Fig. 5).

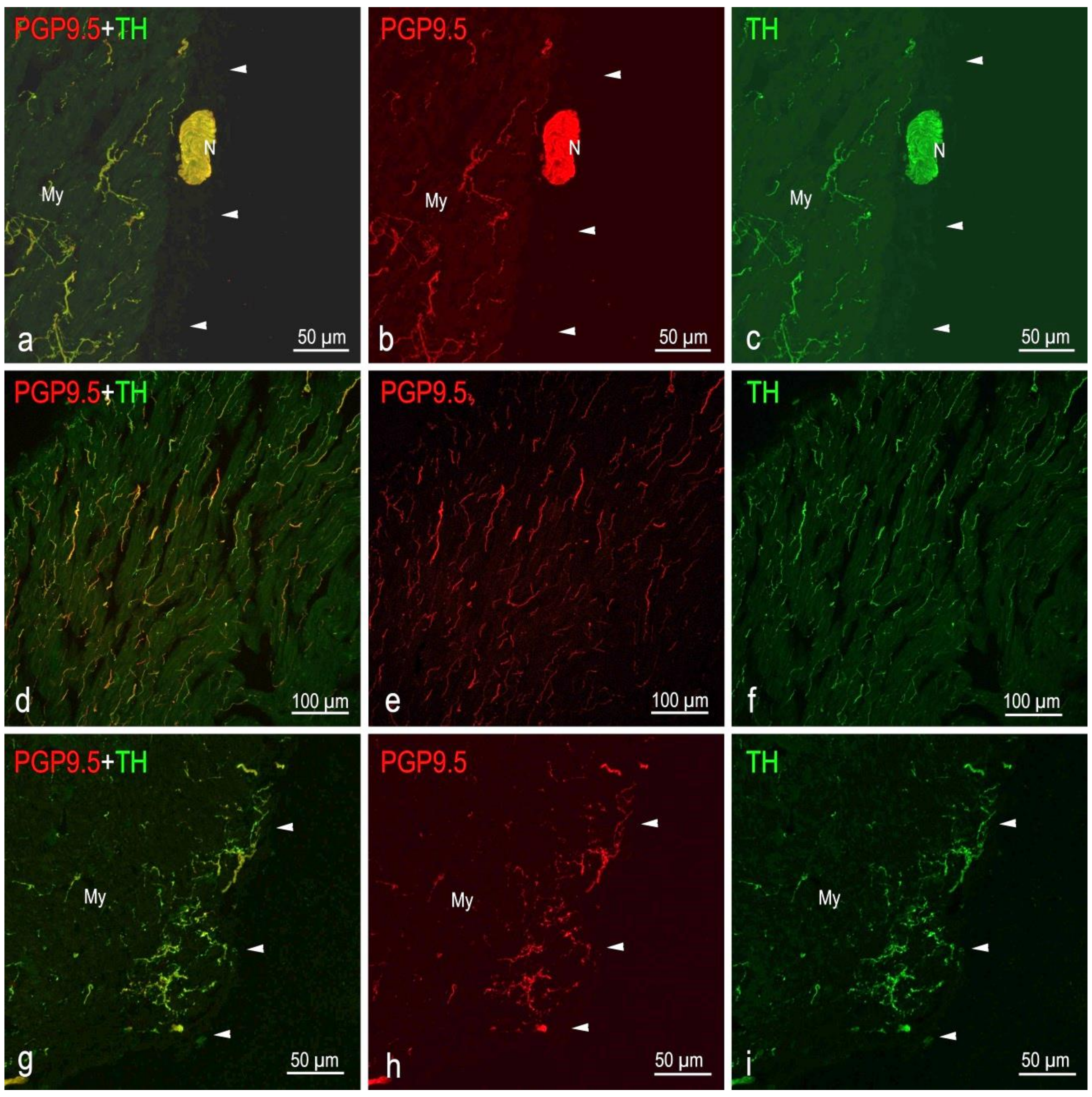

Fig. 1. The distribution of PGP 9.5+ (red) and TH+ (green) nerve fibres in the epicardium (a-c), myocardium (d-f) and endocardium (g-i). A nerve (N) with dominating adrenergic fibres (TH+) is located in the epicardium. In the myocardium, the plexus is composed of relatively scarce nerve fibres; in the myocardium, the plexus is more dense. Arrowheads indicate the border of the epicardium in images a-c and the endocardium in images g-i. My – myocardium.

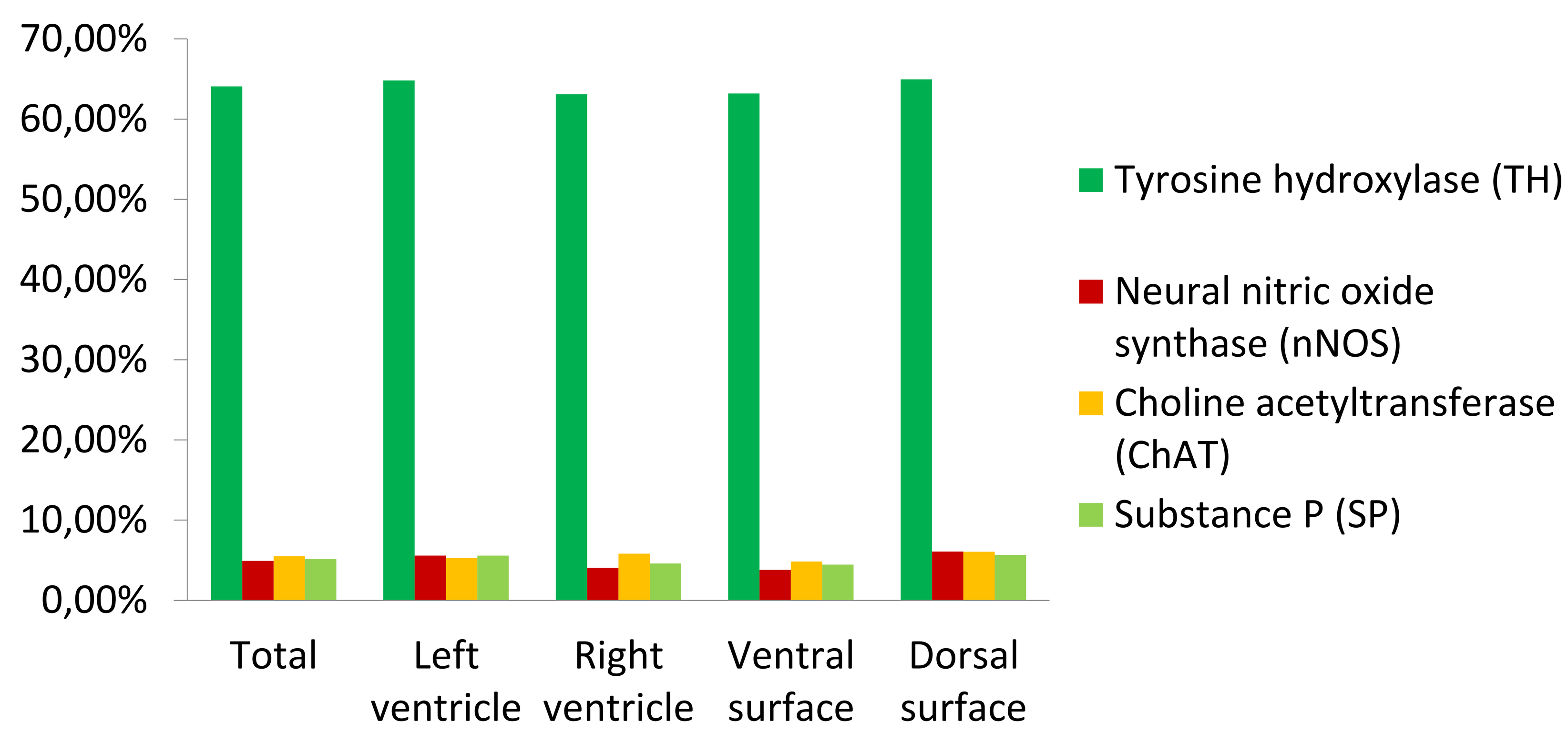

Fig. 3. The composition of epicardial nerves. nNOS+, ChAT+ and SP+ fibres were more dense on the dorsal surface of the heart than on the ventral surface ($p < 0,05$). Statistically significant differences between the left and right ventricles were only found at individual levels.

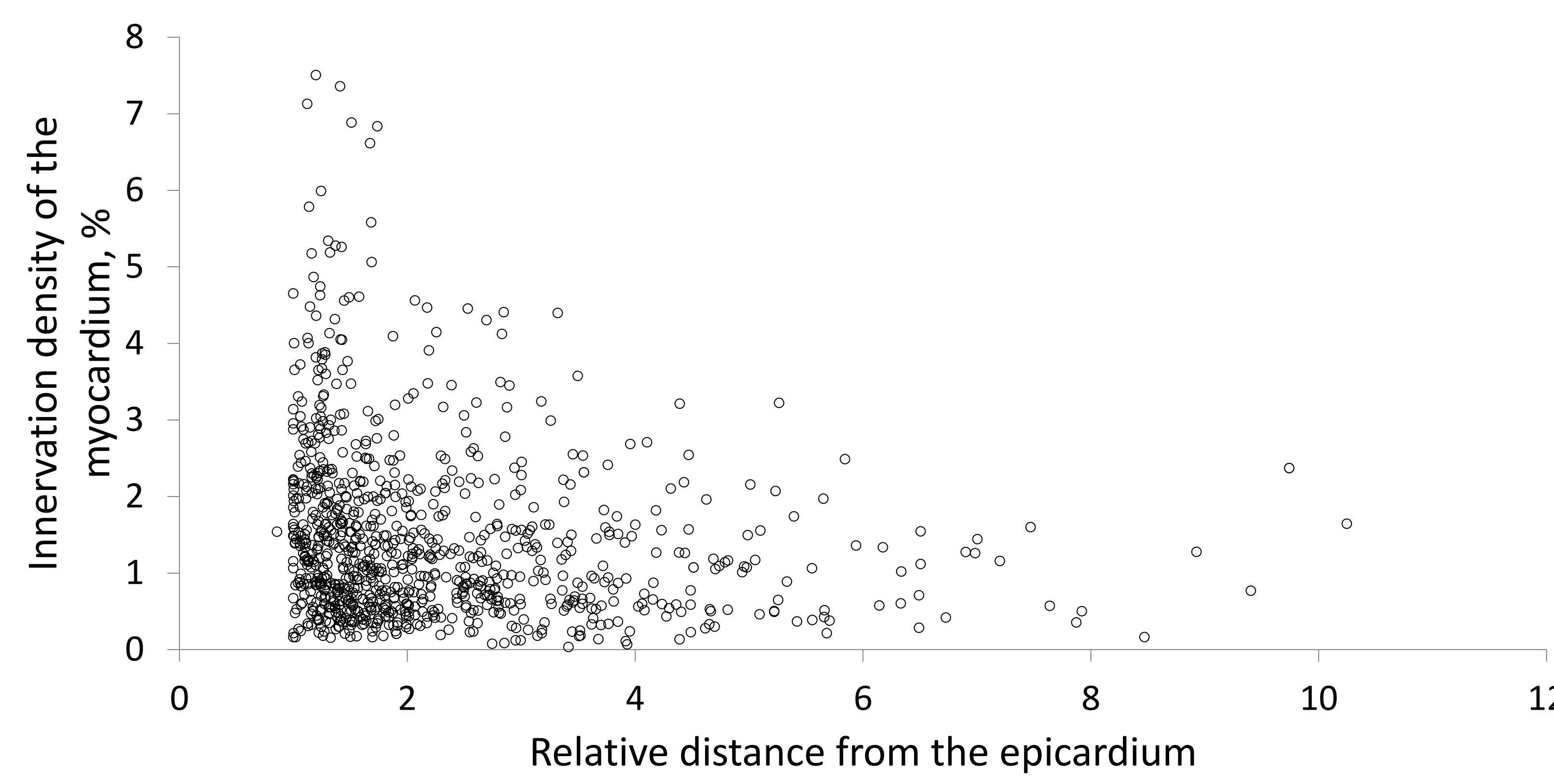

Fig. 4. Scatter diagram of the density of nerve fibres in the myocardium versus the distance from the center of the ventricle. Nerve fibre density is the highest subepicardialy and decreases the closer the myocardium is to the endocardium. A significant ($p < 0,01$) negative correlation was found for PGP9,5+ (Spearman r = -0,240), ChAT+ (Spearman r = -0,319), SP+ (Spearman r = -0,422) and CGRP+ (Spearman r = -0,375) nerve fibres.

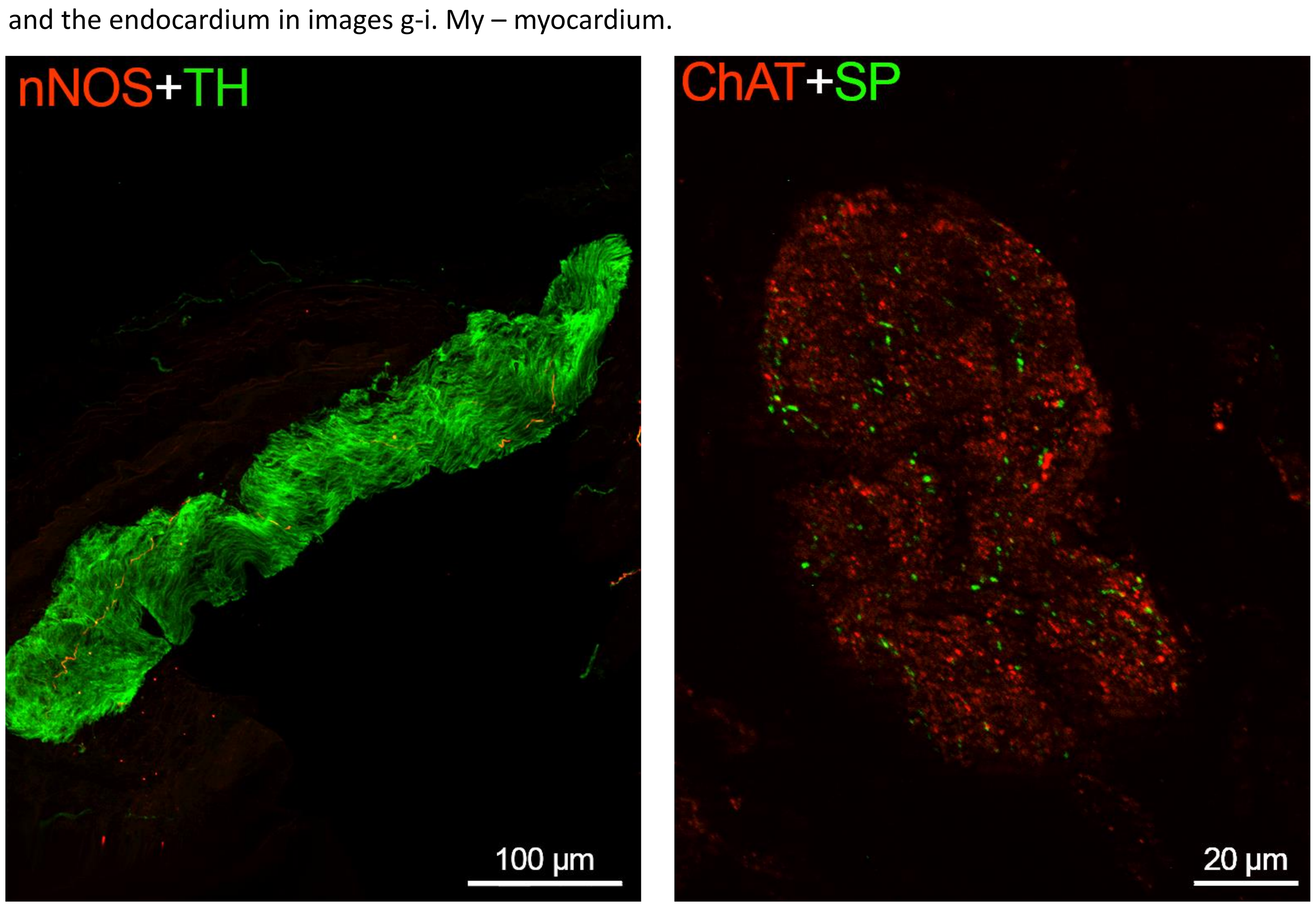

Fig. 2. The distribution of various neural markers within epicardial nerves. The majority of nerve fibres found in epicardial nerves were TH+, while other nerve fibre phenotypes were not as pronounced. The largest cross-sectional nerve area was found on the ventral surface of the left ventricle. The size of nerves decreased from the base of the heart to the apex of the heart.

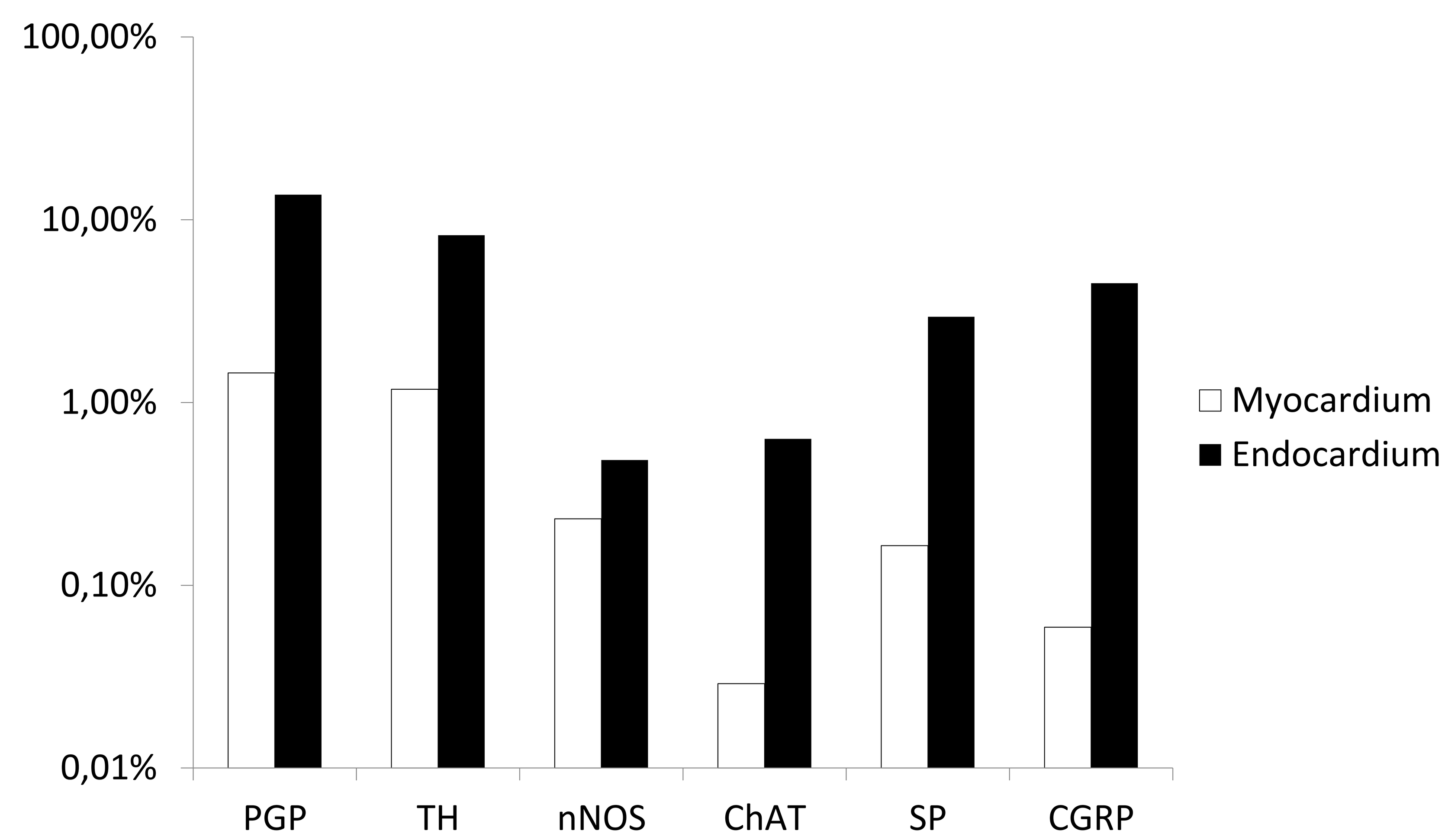

Fig. 5. Log scale comparison of the myocardium and the endocardium. The endocardium contained as much as 10 times more nerve fibres than the myocardium ($p < 0,05$). The density of the myocardium and the endocardium is as follows: PGP9,5 – 1,45% and 13,73%; TH – 1,18% and 8,23%; nNOS – 0,23% and 0,48%; ChAT – 0,03% and 0,63%; SP – 0,17% and 2,94%; CGRP – 0,06% and 4,50%.

Conclusions

1. The largest nerves were found in the epicardium; the nerve plexus observed in the myocardium was scarce; in the endocardium, it was dense.
2. TH positive fibres are dominant in the epicardium, myocardium and the endocardium.
3. The cholinergic innervation in all parts of the ventricles was scarce, with nNOS positive fibres dominating over ChAT positive fibres in the myocardium.
4. The endocardium contains a dense nerve fibre plexus with pronounced CGRP and SP positive fibres. The fibres were relatively abundant in comparison to the myocardium.

Acknowledgements

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