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Atrial and ventricular rat coronary arteries are differently supplied by noradrenergic, cholinergic and nitrergic, but not sensory nerve fibres

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Summary

The present immunohistochemical study set out to determine the extent of perivascular innervation in the rat heart, using markers for noradrenergic sympathetic fibres (tyrosine hydroxylase = TH), cholinergic parasympathetic fibres (vesicular acetylcholine transporter = VAChT), nitrergic fibres (neuronal NO synthase = nNOS), and peptidergic sensory fibres (calcitonin gene-related peptide = CGRP). For each of these antigens, the vascular innervation density was assessed separately in the atria, the basal and the apical parts of the ventricles, and was correlated to the inner vascular diameter. The four major findings are: (1) Each of these neurochemically defined populations shows an individual distribution pattern significantly different from the others with respect to correlation with vascular diameter and occurrence along atrial versus ventricular vessels. (2) Among autonomic efferent axons, nNOS-containing fibres are far less numerous than cholinergic and noradrenergic fibres. (3) Autonomic efferent axons (noradrenergic, cholinergic, nitrergic) are much more abundant around atrial than ventricular vessels, whereas perivascular CGRP-immunoreactive sensory nerve fibres are equally distributed in the various parts of the heart. (4) Noradrenergic and cholinergic axons preferentially innervate small-diameter vessels (negative linear correlation between index of innervation and vascular diameter), whereas the supply with CGRP-immunoreactive sensory nerve fibres does not change with vascular diameter. Collectively, the present study shows individual distribution patterns for each of the neurochemically defined populations of perivascular axons along the atrial and ventricular coronary arteries, indicating a

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highly differentiated nervous regulation of atrial versus ventricular, and large-diameter versus resistance vessels.

Introduction

Arterial tone is under control of multiple determinants including circulating hormones, endothelial-derived factors, metabolic factors originating from the surrounding tissue and perivascular nerve fibres. The relative importance of each of these factors shows a broad extent of variety and is highly specific for individual organs and vascular beds. In the pulmonary circulation, for example, perivascular nerve terminals are rather sparse (Cech, 1969; O'Donnell et al., 1978; Haberberger et al., 1997) and local perfusion is primarily determined by alveolar oxygen tension (West, 1974). In the skin, in contrast, preterminal arterioles are densely innervated and sympathetic vasoconstrictor axons provide the major control on perfusion (Gibbins and Morris, 1990; Jänig and Baron, 2002). Quantitative immunohistochemical investigations on vascular innervation are available for a limited number of individual vascular beds and revealed highly organ-specific patterns of how densely sympathetic, parasympathetic and sensory perivascular axons supply arterial vessels of different size classes (guinea-pig lung: Haberberger et al., 1997; guinea-pig tongue: Henrich et al., 2003; guinea-pig uterus: Morris et al., 1987). Despite its enormous clinical and experimental relevance, however, such data are still missing for the coronary circulation. Hence, the present immunohistochemical study set out to determine the extent of perivascular innervation in the rat heart. Sympathetic noradrenergic axons were visualized by an antiserum against the rate-limiting enzyme of catecholamine synthesis, tyrosine hydroxylase (TH). Parasympathetic cholinergic axons were labelled with an antiserum against the vesicular acetylcholine transporter (VAChT), which imports acetylcholine into synaptic vesicles in cholinergic nerve terminals (Erickson et al., 1994). In several vascular beds, cholinergic parasympathetic axons may, in addition, produce nitric oxide (NO), although this does not apply to all cholinergic axons, depending on individual blood vessel and species (Kummer et al., 1992; Anderson et al., 1997; Haberberger et al., 1997; Kimura et al., 1997). Nerve cell bodies and perivascular axons containing the neuronal isoform of NO synthase (nNOS) have been reported in the heart of several species including the rat (Klimaschewski et al., 1992; Sosunov et al., 1995, 1996; Richardson et al., 2003). Thus, an antiserum against nNOS was also used in this study. Besides sympathetic and parasympathetic axons, a particular class of viscerosensory axons also contributes to regulation of vascular tone. In the rat heart, specifically, these sensory axons contain and release calcitonin gene-related peptide (CGRP) (Mulderry et al., 1985; Wharton et al., 1986; Ferdinandy et al., 1997; Katona et al., 2004) and play an important role in "ischemic preconditioning" (Li et al., 1996; Luo et al., 2004). This term describes the phenomenon that a short, transient period of hypoxia has a beneficial effect on cardiac function in subsequent severe ischemia-reperfusion. This type of nerve fibre was visualized with an antiserum directed against CGRP. For each of these antigens, the innervation density of arteries and arterioles was assessed separately in the atria, the basal and the apical parts of the ventricles. For each of these cardiac regions, the "index of innervation", defined as that part (in percent) of the arterial circumference that was covered with immunoreactive nerve fibres, was plotted against the inner vascular diameter. Thus, region-specific innervation patterns related to vascular diameter are provided for each neurochemically defined nerve fibre population.

Material and methods

The quantitative investigation was performed on hearts of 5 adult male Wistar rats (300–500 g body weight). The animals were killed by inhalation of an overdose of trichloromethane and the hearts were quickly dissected and fixed overnight by immersion in Zamboni's fixative (2% paraformaldehyde, 15% saturated picric acid in 0.1 M phosphate buffer [PB], pH 7.4). Then, atria were dissected from ventricles, and the ventricles were further divided by a cross-section at the midlevel between the base of the heart and the apex. These specimens were extensively washed in 0.1 M PB over 24 h, then immersed for another day in the same buffer containing 18% sucrose, embedded in Tissue Tek OCT compound (Sakura Finetek Europe B.V., Zoeterwoude, NL), and frozen on filter paper, with the