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Regulation of cardiac innervation and function via the p75 neurotrophin receptor

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Abstract

Homeostatic regulation of cardiac function is dependent on the balance of inputs from the sympathetic and parasympathetic nervous systems. We investigated whether the p75 neurotrophin receptor plays a developmental role in cardiac innervation by analyzing sympathetic and parasympathetic fibers in the atria of p75 knockout and wildtype mice at several stages of postnatal development, and examining the effect on control of heart rate. We found that parasympathetic innervation of the atria in p75−/− mice was similar to wildtype at all time points, but that the density of sympathetic innervation was dynamically regulated. Compared to wildtype mice, the p75−/− mice had less innervation at postnatal day 4, an increase at day 28, and decreased innervation in adult mice. These changes reflect defects in initial fiber in-growth and the timing of the normal developmental decrease in sympathetic innervation density in the atria. Thus, p75 regulates both the growth and stability of cardiac sympathetic fibers. The distribution of sympathetic fibers was also altered, so that many regions lacked innervation. Basal heart rate was depressed in adult p75−/− mice, and these mice exhibited a diminished heart rate response to restraint stress. This resulted from the lack of sympathetic innervation rather than increased parasympathetic transmission or a direct effect of p75 in cardiac cells. Norepinephrine was elevated in p75−/− atria, but stimulating norepinephrine release with tyramine produced less tachycardia in p75−/− mice than wild type mice. This suggests that altered density and distribution of sympathetic fibers in p75−/− atria impairs the control of heart rate.

Keywords: Axon outgrowth; Axon maintenance; Sympathetic; Parasympathetic; Development; Autonomic control

1. Introduction

The proper development and modulation of the autonomic nervous system is critical for maintaining internal homeostasis in the midst of a changing environment. The heart is an important target of the sympathetic and parasympathetic nervous systems, and balance between the stimulatory sympathetic inputs and inhibitory parasympathetic inputs are crucial for control of cardiac function. Post-ganglionic sympathetic neurons require the neurotrophin nerve growth factor (NGF) for survival (Francis and Landis, 1999; Levi-Montalcini and Angeletti, 1968) while parasympathetic neurons do not (Airaksinen and Saarma, 2002; Hiltunen et al., 2000). In the heart NGF signaling is required for innervation by sympathetic axons in addition to its effects on cell survival (Glebova and Ginty, 2004). Thus, disruption of neurotrophin signaling would be expected to impact the development of the cardiac sympathetic innervation, while having little effect on the cardiac parasympathetic innervation.

Neurotrophins, including NGF, act through two distinct types of receptors: the Tropomyosin-related tyrosine kinase

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(Trk) receptors, and the lower affinity p75 receptor (reviewed by Glebova and Ginty, 2005; Zampieri and Chao, 2006). NGF and Neurotrophin-3 promote sympathetic neuron survival both *in vitro* (Birren et al., 1993; DiCicco-Bloom et al., 1993) and *in vivo* (Levi-Montalcini and Booker, 1960; Francis et al., 1999) and this survival response is mediated via the TrkA receptor (Belliveau et al., 1997; Fagan et al., 1996; Tessarollo et al., 1997). Both neurotrophins act through TrkA to promote the outgrowth of sympathetic neurons during development, with NGF playing a key role in final target innervation (Kuruvilla et al., 2004; Glebova and Ginty, 2004). The role of the p75 receptor in development of the sympathetic innervation is less clear. In mice lacking p75 many sympathetic targets have normal innervation, while the innervation of other targets is disrupted (Jahed and Kawaja, 2005; Kuruvilla et al., 2004; Lee et al., 1992). Both of these disparate observations have been made concerning the sympathetic innervation of the heart. The lack of p75 during embryogenesis results in significantly fewer sympathetic axons projecting into the heart at E16.5 (Kuruvilla et al., 2004) compared to wild type mice. In contrast, the density of sympathetic innervation in the heart is apparently normal 11–14 days after birth in mice lacking p75 (Jahed and Kawaja, 2005).

We examined the sympathetic and parasympathetic innervation of the right atria in mice lacking p75 at several postnatal time points to determine if there was a developmental delay in the establishment of the cardiac innervation. Furthermore, we asked if this led to functional consequences for the heart by monitoring heart rate. Sympathetic neurons innervating the sino-atrial node (SA node) in the right atria stimulate heart rate through release of norepinephrine (NE) and activation of β adrenergic receptors. Parasympathetic neurons inhibit heart rate at the SA node through release of acetylcholine (ACh) and activation of m2 muscarinic ACh receptors. We found that parasympathetic innervation of p75−/− atria was similar to wildtype at all time points that were examined, but that the density of sympathetic innervation was decreased at postnatal day 4. We observed a transient sympathetic hyperinnervation of p75−/− atria compared to the wildtype at postnatal day 28, and decreased sympathetic innervation in adult p75−/− mice compared to age-matched wildtype mice. The loss of sympathetic fibers in adults corresponded with a lower basal heart rate and an impaired stress response. These functional changes resulted from decreased sympathetic transmission, not increased parasympathetic transmission or altered cardiac responsiveness. These data indicate that p75 is involved in both the development and maintenance of the cardiac sympathetic innervation, and the lack of p75 alters autonomic control of heart rate.

2. Materials and methods

2.1. Materials

Drugs and chemicals were obtained from Sigma (St. Louis, MO) unless otherwise noted. Antibodies were as follows: rabbit anti-tyrosine hydroxylase (TH), sheep anti-TH, and goat anti-vesicular acetylcholine transporter (VAChT) were from Chemicon (Temecula, California); donkey anti-rabbit Cy5, donkey anti-sheep Cy5, and donkey anti-goat Cy3 were from Jackson ImmunoResearch (West Grove, PA). Dobutamine hydrochloride was from Hospira, Inc. (Lake Forest, IL).

2.1.1. Animals

Wild type C57BL/6J and p75 KO mice (B6.129S4-*Ngfr*^{tm1Jae}/J) were obtained from Jackson Laboratories, Bar Harbor, Maine. All animal experiments were carried out in accordance with animal protocols approved by the Brandeis University Institutional Animal Care and Use Committee or by the Portland VA Medical Center Institutional Animal Care and Use Committee. Adult mice were at least 7 months old and ranged from 7 to 15 months of age.

2.1.2. Immunohistochemistry of mouse atria

Sympathetic fibers were identified by staining mouse atrial whole mounts for tyrosine hydroxylase (TH), the rate limiting enzyme in norepinephrine synthesis. Parasympathetic neurons were identified in the same tissue by staining for the vesicular acetylcholine transporter (VAChT).

Mice were sacrificed by CO_2 asphyxiation and hearts were dissected in dishes of phosphate buffered saline (PBS) under low magnification. Intact atria were separated from the ventricles and cleaned of other tissue, and the right atrium was dissected away from the left. Following removal of the left atrium, the interatrial septum was removed and the underside of the right atrium which attaches to the ventricle was cut away, leaving a flattened surface that was used for whole mount immunohistochemistry. Atria were then fixed by submersion in 4% paraformaldehyde at 4 °C. Postnatal day 4 (P4), P28–35, and adult atria were fixed for 4 h. Following fixation, atria were rinsed 3 × 10 min. in 80% ethanol and stored in PBS at 4 °C. Differences in fixation time (and blocking time, see below) were used to minimize background staining. However, there were no significant differences in staining quality between any of the conditions.

Atria from younger mice (P4 and P28–35) were blocked overnight at 4 °C in 5% horse serum (HS)/0.4% Triton X-100 in PBS, while adult atria were blocked in the same solution at room temperature for 30 min. Atria were incubated overnight at 4 °C with primary antibodies diluted in 1% (P4 and P28–35) or 5% horse serum (adults). All double-labeling was done with rabbit anti-TH (1:500) and goat anti-VAChT (1:1000 or 1:4000). In some experiments in which only TH staining was examined a sheep anti-TH antibody (1:500) was used. Staining with this antibody showed results similar to the rabbit TH antibody and the data was combined. Sections were washed 3 × 20 min in PBS, and then incubated for 2 h at room temperature with secondary antisera: donkey anti-sheep Cy5, or donkey anti-goat Cy5 and donkey anti-rabbit Cy3 diluted 1:400 or 1:600. Atria were rinsed 3 × 1 h in PBS at room temperature and placed on