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Involvement of α4 integrins in maintenance of cardiac sympathetic axons

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Abstract

Sympathetic neurons extend and maintain axons that innervate the myocardium, and proper innervation is important for cardiac function. However, the molecular basis for axon outgrowth and maintenance is not well understood. We have shown previously that the integrin α4β1 is expressed on developing axons, and the α4 function is important for the development of innervation in vivo [Wingerd, K.L., Goodman, N.L., Tresser, J.W., Smail, M.M., Leu, S.T., Rohan, S.J., Pring, J.L., Jackson, D.Y., and Clegg, D.O., 2002. Alpha 4 integrins and vascular cell adhesion molecule-1 play a role in sympathetic innervation of the heart. J. Neurosci. 22, 10772–10780]. Here we examine the function of α4β1 integrins in the maintenance of cardiac sympathetic innervation in vitro and in vivo, and investigate integrin expression and function after myocardial infarction and in hypertensive rats. On substrates of vascular cell adhesion molecule-1 (VCAM-1), α4β1 was required for both initial outgrowth and maintenance of neurites in vitro. On fibronectin substrates, initial outgrowth requires only α4 integrins, but maintenance requires both α4 integrins and RGD-dependent integrins. In vivo, in adult Long Evans rats, inhibition of α4 integrins resulted in decreased maintenance of sympathetic fibers innervating the apex of the heart. However, α4 integrins were not detected on most sympathetic axons that sprout after myocardial infarction, and α4 function was not required for sprouting. Spontaneously hypertensive rats (SHR) have increased numbers of cardiac sympathetic fibers compared to the parental Wistar strain, but many of these lack α4 expression, and α4 function is not required for maintenance of these fibers in the heart. These results suggest that developing sympathetic axons and sprouting sympathetic axons use different mechanisms of outgrowth, and that maintenance of cardiac sympathetic innervation involves α4 integrins in some rat strains.

Keywords: Sympathetic; Integrins; Alpha-4; Beta-1; VLA-4; Myocardial infarction; Sprouting; Neurite outgrowth; Reinnervation; Axon; Synapse; Hypertensive

1. Introduction

Sympathetic neurons innervate the heart during the first few postnatal weeks, and axon terminals remain throughout the myocardium in the adult (Lipp and Rudolph, 1972). Maintenance of the distribution of cardiac sympathetic innervation is important, as several pathological states are associated with abnormal sympathetic sprouting. Increased sympathetic function may lead to arrhythmias and contribute to heart attack, hypertension, and, possibly, sudden infant death syndrome (Podrid et al., 1990; Schwartz et al., 1998; Palatini and Julius, 1999).

After a myocardial infarction (MI) or other myocardial injury, sympathetic innervation is lost in the damaged portion of the heart. A regeneration response ensues, and tissue surrounding the scar is greatly hyper-innervated compared to the surrounding tissue (Chen et al., 2001). Newly formed (improper) sympathetic innervation may lead to ventricular tachycardia, fibrillation and Sudden Cardiac Death (Pugsley et al., 1999; Chen et al., 2001). This hypothesis is supported by studies in dog (Zipes, 1990; Chang et al., 2001), rat (Vracko et al., 1990; Du et al., 1999), and by research on human patients (Vracko et al., 1991). For example, MI leads to sympathetic sprouting in

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dogs, and if sprouting is augmented by nerve growth factor (NGF), spontaneous ventricular tachycardia, fibrillation, and SCD follows (Cao et al., 2000). Up-regulation of the low affinity NGF receptor and GAP43 appears to drive the sprouting that occurs after MI (Zhou et al., 2004). In rats, Nori et al. (1995) found that necrotic injury of rat myocardium (induced by freeze–thaw) resulted in robust and persistent sympathetic reinnervation. Du et al. (1999) showed that in infarcted rats (coronary artery occlusion), sympathetic activation is a potent trigger for the onset of ventricular tachyarrhythmias. Sympathetic remodeling after MI has been documented in humans (Stanton et al., 1989; Vracko et al., 1991) and β-adrenergic antagonists are known to reduce the incidence of SCD in humans. In fact, recent clinical trials have indicated that β blockers reduce mortality in congestive heart failure (Doggrell, 2001). Treatments to control sympathetic sprouting after MI may be a novel, more effective way of preventing arrhythmias.

Sympathetic over-activity may also be involved in hypertension. Studies of spontaneously hypertensive rats have shown that there are increased numbers of sympathetic fibers around blood vessels and in the heart (Kondo et al., 1995; Tabei et al., 1995). Furthermore, sympathetic activity may be augmented by increased firing rates, faulty norepinephrine reuptake, or other factors (Rumantir et al., 2000; Schlaich et al., 2003). Increased sympathetic activity can lead to left ventricular hypertrophy, a risk factor in cardiovascular morbidity (Brum et al., 2002).

The adhesive relationship between sympathetic axon terminals and heart tissue and the regulation of sympathetic sprouting are poorly understood. Sympathetic axon terminals often terminate in boutons or varicosities, without recognizable active zones, at some distance from the target cells, and released norepinephrine is thought to diffuse through the tissue and produce a slow, second messenger-coupled response (Landis, 1976; Kitajiri et al., 1993). Synaptic structures have also been identified where sympathetic axons terminate on smooth muscle cells of the vasculature and appear to make close cell–cell contacts (Luff, 1996).

Integrin receptors mediate cell–cell and cell–ECM interactions and may be involved in maintaining stable connections between neurons and their targets (Clegg et al., 2003). Integrins have also been implicated in neurite sprouting and neuronal development (Ekstrom et al., 2003; Hikita et al., 2003). Furthermore, some anti-adhesive molecules that cause axon retraction, such as ephrins, may function by disrupting integrin signaling pathways (Zou et al., 1999; Kullander and Klien, 2002). Integrins are a family of heterodimeric transmembrane receptors consisting of 18 alpha and 8 beta subunits that form 24 known pairs (Siebers et al., 2005). The integrin α4β1, well known for its role in inflammation and hematopoiesis (Lobb and Hemler, 1994; Arroyo et al., 1996), has also been shown to function in neurons (Vogelezang et al., 2001; Wingerd et al., 2002). The α4β1 integrin binds multiple ligands, including vascular cell adhesion molecule-1 (VCAM-1) (Osborn et al., 1989), fibronectin's (Fn) connecting sequence-1 LDV motif (Guan and Hynes, 1990), thrombospondin-1 (Yabkowitz et al., 1993), other α4 integrins (Altevogt et al., 1995), the propolypeptide of von Willebrand factor (Isobe et al., 1997), ICAM-4 (Spring et al., 2001), transglutaminase C (Isobe et al., 1999), and osteopontin (Bayless et al., 1998). Both VCAM-1 and FN are expressed in innervated regions of the heart (Sheppard et al., 1994). Neural cells that express α4β1 include neural crest cells (Kil et al., 1998), retinal cells (Sheppard et al., 1994), dorsal root ganglion neurons (Vogelezang et al., 2001), and superior cervical ganglion (SCG) neurons (Vogelezang et al., 2001; Wingerd et al., 2002).

We have previously shown that integrins play a crucial role in sympathetic innervation of the heart during development (Wingerd et al., 2002). Blockade of α4 integrins leads to a 50% decrease in the number of fibers that reach the heart in Long Evans rats. The α4β1 integrin is tightly regulated during development. The intracellular distribution and isotype of the integrin change as function is down regulated with age (Wingerd et al., 2004). In the adult, α4 immunoreactivity within the superior cervical ganglion cells and on some of the axons in the myocardium persist, suggesting that α4 could play a role in maintenance of fibers.

Here we present evidence that supports the hypothesis that the α4 integrins play a role in the maintenance of sympathetic fibers in the heart. First, an in vitro assay is described that assesses the importance of integrins in maintaining neurites once they have been elaborated on substrates of integrin ligands found in the heart. We show that α4β1 integrins are required for maintaining neuritic projections on VCAM-1 and FN. Next, α4 integrins are shown to be required for maintenance of sympathetic fibers in adult Long Evans rats, but are not required for maintenance in hypertensive rats or for sprouting post MI.

2. Methods

2.1. Quantification of neurite outgrowth

Primary cultures of rat SCG cells (P1-3) were isolated and plated on 96 well culture plates (Corning-Costar, Acton, MA) coated with purified proteins, and incubated as described (Choi et al., 1994; Wingerd et al., 2002). Briefly, the cells were manually dissociated in media, trypsinized (0.05% trypsin), and triturated to eliminate clumped cells. The cells were cultured in serum free L15 media supplemented with ITS (insulin, transferin, selenium; Gibco BRL, Carlsbad, CA), PSF (penicillin, streptomycin, fungizone; Gibco BRL) and 100 ng/ml 7S-NGF (Sigma, St. Louis, MO). The wells were coated overnight with either laminin-2/4 (10 μg/ml LN; merosin, Gibco BRL) as a positive control, 1% BSA as a negative control, plasma fibronectin (20 μg/ml FN, Gibco BRL), or recombinant soluble