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Neuropeptides. Author manuscript; available in PMC 2012 February 1.

Published in final edited form as:
Neuropeptides. 2011 February ; 45(1): 33–42. doi:10.1016/j.npep.2010.10.002.

Cardiac ischemia-reperfusion regulates sympathetic neuropeptide expression through gp130-dependent and independent mechanisms

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Abstract

Cardiac function is regulated by a balance of sympathetic and parasympathetic transmission. Neuropeptide Y (NPY) and galanin (GAL) released from cardiac sympathetic neurons inhibits parasympathetic transmission in the heart. Sympathetic peptides may contribute to autonomic imbalance, which is characterized by increased sympathetic and decreased parasympathetic transmission and contributes to life threatening cardiovascular pathologies. Several gp130 cytokines are increased in the heart after myocardial infarction (MI), and these cytokines stimulate neuropeptide expression in sympathetic neurons. We used mice whose sympathetic neurons lack the gp130 receptor ($gp130^{DBHCre/lox}$ mice) to ask if cytokine activation of gp130 regulated neuropeptide expression in cardiac sympathetic nerves after MI. Myocardial infarction decreased NPY mRNA through a gp130 independent mechanism and increased VIP and PACAP mRNA via gp130, while GAL mRNA was unchanged. Immunohistochemistry revealed a gp130-dependent increase in PACAP38 in cells of the stellate ganglion after MI, and PACAP was detected in pre-ganglionic fibers of all genotypes and surgical groups. VIP was identified in a few sympathetic nerve fibers in all genotypes and surgical groups. GAL and PACAP38 were not detected in sham hearts, but peptide immunoreactivity was high in the infarct three days after MI. Surprisingly, peptides were abundant in cells that co-labeled with macrophage markers F4/80 and MAC2, but were not detected in sympathetic axons. PACAP protects cardiac myocytes from apoptosis, and GAL stimulates axon regeneration in addition to inhibiting parasympathetic transmission. Thus, these peptides may play an important role in cardiac and neuronal remodeling after ischemia-reperfusion.

Keywords

neuropeptide Y; galanin; vasoactive intestinal peptide; pituitary adenylate cyclase activating peptides

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Introduction

The autonomic nervous system regulates cardiac function by balancing the actions of sympathetic and parasympathetic inputs to the heart. Sympathetic neurons from the stellate ganglia stimulate heart rate, cardiac conduction, and the force of contraction through the release of norepinephrine (NE) and activation of β adrenergic receptors. Conversely, parasympathetic neurons of the cardiac ganglia inhibit heart rate through the release acetylcholine (ACh) and activation of muscarinic ACh receptors. Autonomic imbalance, characterized by increased sympathetic and decreased parasympathetic activity, contributes to many life threatening cardiovascular pathologies (Esler, 1992; La Rovere et al., 2001; Lopez-Sendon et al., 2004; Schwartz, 1998). Cardiac parasympathetic transmission is usually depressed after myocardial infarction (MI), and this loss is a powerful predictor of susceptibility to arrhythmia and death (La Rovere et al., 1998; Nolan et al., 1998). Cardiac parasympathetic transmission is inhibited by the release of neuropeptide Y (NPY)(Herring et al., 2008; Pirola and Potter, 1990; Smith-White et al., 2003) and galanin (GAL) (Smith-White et al., 2003; Ulman et al., 1992), from cardiac sympathetic neurons. Increased activation of sympathetic nerves after myocardial infarction (Graham et al., 2002; Karlsberg et al., 1979), and the corresponding increase in peptide release may contribute to the loss of parasympathetic transmission.

Neuropeptide expression is altered in cardiac sympathetic neurons following myocardial infarction, and these changes are similar to neuropeptide changes after axotomy. For example, vasoactive intestinal peptide (VIP)(Roudenok et al., 2001) and Galanin (GAL) (Ewert et al., 2008; Habecker et al., 2005) increase after myocardial infarction in cardiac sympathetic neurons. Axotomy stimulates expression of VIP, substance P (SubP), pituitary adenylate cyclase activating peptides (PACAP), and GAL in the superior cervical ganglion (Bergner et al., 2000; Boeshore et al., 2004; Hyatt-Sachs et al., 1993; Klimaschewski et al., 1994; Rao et al., 1993; Schreiber et al., 1994) by activation of the gp130 cytokine receptor (Habecker et al., 2009; Heinrich et al., 1998). A number of gp130 cytokines are elevated in the heart following myocardial infarction, including Interleukin-6 (IL-6), Leukemia Inhibitory Factor (LIF), and Cardiotrophin-1 (CT-1)(Aoyama et al., 2000; Frangogiannis et al., 2002; Gwechenberger et al., 1999), where they might stimulate neuropeptide expression in cardiac sympathetic nerves.

Cytokines that activate gp130 are important survival factors for cardiac myocytes (Brar et al., 2001; Hirota et al., 1999; Yoshida et al., 1996; Zou et al., 2003), so to determine the effect of cytokines on neuropeptide expression in cardiac neurons, we used $gp130^{DBHCre/lox}$ mice that have a selective deletion of gp130 in neurons expressing dopamine beta hydroxylase (DBH; (Stanke et al., 2006). Injury-induced inflammatory cytokines do not activate STAT3 in sympathetic neurons from these mice, confirming the absence of gp310 signaling (Habecker et al., 2009; Hyatt Sachs et al., 2010). Therefore, we used these mice to ask if cytokine activation of gp130 was critical for regulation of neuropeptide expression in cardiac sympathetic nerves after myocardial infarction.

Materials and Methods

Ethical Approval

All procedures were approved by the OHSU Institutional Animal Care and Use Committee and comply with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996).

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Materials

Antibodies were obtained as follows: rabbit anti-GAL as well as rabbit and sheep anti-TH were from Chemicon/Millipore (Temecula, CA), goat anti-PACAP (C19; raised against C-terminus of human PACAP and presumed to identify PACAP27&38) was from Santa Cruz Biotechnology (Santa Cruz, CA), rabbit anti-PACAP38 was from Phoenix Pharmaceuticals (Burlingame, CA), rat anti-MAC2/Galectin 3 was from Accurate Scientific (Westbury, NY), rat anti-F4/80 was from AbD Serotec (Oxford, UK), guinea pig anti-VIP was a gift from Dr. Story Landis (Tyrrell and Landis, 1994), and rabbit anti-VIP was from ImmunoStar (Hudson, WI). Species-specific secondary antibodies were from Molecular Probes (Carlsbad, CA) or Jackson Immunochemicals (West Grove, PA). RNAlater®, RNAqueous micro RNA isolation kit, TaqMan primer/probe sets, and real-time PCR reagents were all obtained from Applied Biosystems (Foster City, CA).

Animals

Wild type C57BL/6J were obtained from Jackson Laboratories West, Sacramento, CA. $gp130^{DBHCre/lox}$ mice (referred to as "gp130 KO mice") were generated as previously described (Stanke et al., 2006), and lack gp130 signaling in sympathetic neurons (Habecker et al., 2009). All mice were kept on a 12h:12h light-dark cycle with ad libitum access to food and water. Male and female mice 12–18 weeks old were used for all experiments. Animals from the two genotypes were age and gender matched for each experiment.

Myocardial Infarction by ischemia-reperfusion

Ischemia-reperfusion was carried out as previously described (Gritman et al., 2006; Parrish et al., 2009). All surgical procedures were performed under aseptic conditions. Adult mice were placed in an induction chamber and anesthetized with 4% isoflurane. Mice were intubated, mechanically ventilated, and maintained with 1−2% isoflurane mixed with 100% oxygen. Core body temperature was maintained at ~37°C, and a 2-lead ECG was monitored throughout the surgery using a PowerLab data acquisition system (ADInstruments, Inc., Colorado Springs, CO). The left anterior descending coronary artery (LAD) was ligated and ischemia was confirmed by ST elevation, regional cyanosis, and wall motion abnormalities. After 30 minutes the coronary ligature was released, and reperfusion confirmed by visible epicardial hyperemia. Buprenorphine (0.1 mg/kg) was administered as needed to ensure the animals were comfortable following surgery. Sham animals underwent the same procedure except for ligation of the LAD. Animals were retained for 3 or 7 days after surgery since a previous study in rat characterized peptide expression at those time points (Habecker et al., 2005).

Immunohistochemistry

Hearts were fixed for 1h in 4% paraformaldehyde, rinsed in PBS, cryoprotected in 30% sucrose and 10 μm transverse sections were thaw-mounted onto charged slides. Ganglia were fixed for 15 minutes in 4% paraformaldehyde, rinsed, and placed in sucrose. To reduce fixative-induced autofluorescence, sections were rinsed in 10 mg/mL sodium borohydride 3×10 minutes and then rinsed in PBS 3×10 minutes. Sections were then blocked in 2% B.S.A/ 0.3% Triton X-100 in PBS for 1 h, incubated with primary antibodies overnight (diluted 1:300 in blocking solution), rinsed 3 × 10 minutes in PBS, and incubated 1.5 h with AlexaFluor -conjugated species-specific secondary antibodies (1:300). Sections were rinsed 3 × 10 minutes in PBS, incubated in 10mM $CuSO_4$/ 50mM $NH_4C_2H_3O_2$ for 30 minutes to further reduce background staining, coverslipped, and visualized by fluorescence microscopy. A minimum of 6 sections/heart or 3 sections/ganglion were stained with each antibody. At least 4 hearts or 3 ganglia were examined from each experimental group (except for innervation density analysis, see below), with representative pictures shown.

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Innervation density analysis

Innervation density was determined by threshold discrimination using ImageJ as previously described (Lorentz et al., 2010). Three sections at least 150 μm apart were analyzed from each heart and averaged together. The data shown are the average of two independent determinations. Fiber density was quantified in 5–6 hearts from each experimental group, focusing on the infarct and the peri-infarct myocardium as shown in Figure 5. The peri-infarct picture was two fields of view away from the edge of the infarct in order to maintain consistency. Pictures from sham animals were taken in the region that typically corresponded to the infarct.

Real-time PCR

Stellate ganglia, which contain most of the sympathetic neurons that project to the heart, were removed and stored in RNAlater®. RNA was isolated using the RNAquous micro kit, cDNA was generated, and real-time PCR was performed using ABI Taqman master mix and prevalidated Taqman gene expression assays for mouse NPY, VIP, GAL, PACAP and GAPDH. cDNA from a single ganglion was used to quantify all 5 genes in duplicate. Left and right stellates were used interchangeably since previous studies indicated that similar changes occurred in both ganglia (Habecker et al., 2005). Peptide mRNAs were normalized to GAPDH mRNA in the same sample. Standard curves were generated for each gene tested. Peptide mRNAs were quantified in a minimum of 4 mice in each surgical group. Differences between groups were determined by two-way ANOVA with Bonferroni post-test using Prism 5.0 software.

Results

Basal expression of NPY, VIP, and GAL mRNA in the stellate ganglion of unoperated control gp130 KO mice did not differ significantly from wild type (WT) levels (Fig. 1), but PACAP mRNA was significantly lower in stellates from unoperated KO mice compared to WT mice (Fig. 1). Several changes occurred in peptide mRNA expression after myocardial infarction. NPY mRNA decreased significantly in both genotypes three and seven days (Fig. 2) after MI. GAL mRNA did not increase in either genotype after infarction (Fig. 2), in contrast to previous studies carried out in rat (Ewert et al., 2008;Habecker et al., 2005). VIP and PACAP mRNA were elevated significantly after ischemia-reperfusion in WT but not gp130 KO ganglia (Fig. 2). Ganglionic PACAP mRNA was significantly higher in WT shams compared to gp130 KO shams, but all other peptides were similar in the shams of both genotypes.

To determine if increased peptide mRNA corresponded with peptide expression in cardiac sympathetic neurons, we carried out immunohistochemistry for PACAP in stellate ganglia from both genotypes. PACAP immunoreactivity with the C-19 antibody was abundant in pre-ganglionic fibers from both genotypes, but was not detected in post-ganglionic cell bodies of either genotype after sham surgery (data not shown) or myocardial infarction (Fig. 3E,F). PACAP38-specific immunoreactivity was abundant in WT pre- and post-ganglionic neurons 3 days after MI, but was low in sham controls and post-MI gp130 KO ganglia (Fig. 3A–D). All subsequent experiments used the PACAP38-specific antibody. GAL immunoreactivity was detected in fibers and neuron cell bodies within sham and MI stellate ganglia from both genotypes (Fig. 4), consistent with GAL mRNA expression.

TH-immunoreactive sympathetic fibers were quantified in the left ventricle free wall of sham and post-MI mice to determine if the lack of gp130 in sympathetic neurons led to altered nerve density compared to WT mice. Sympathetic fiber density was identical in the two genotypes after sham or ischemia-reperfusion surgery (Fig. 5), consistent with normal

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TH mRNA levels and cardiac NE (Parrish et al., 2009). The infarct was completely denervated three days after MI, but peri-infarct nerves were intact and the density of peri-infarct innervation was similar to sham control hearts (Fig. 5).

Heart sections were double-labeled with TH and antibodies against neuropeptides to determine if sympathetic fibers in the ventricle contained increased peptide content after myocardial infarction. A small subset of TH+ sympathetic nerve fibers stained for VIP (Fig. 6). Rare VIP+/TH+ fibers were present in sham and MI hearts from both genotypes. The overall incidence of TH+/VIP+ fibers was so low in all groups that the fiber density was not quantified. PACAP38 was significantly elevated in the left ventricle free wall of both genotypes three days after MI (Fig. 7). PACAP38 immunoreactivity was localized to the denervated infarct, appearing as diffuse staining in myocardium/extracellular matrix and round-shaped cells in the heart similar to those that labeled with the macrophage marker F4/80 in adjacent sections (Fig. 7). Significant GAL staining was also observed in the heart after MI. Like PACAP, GAL did not co-localize with TH in peri-infarct sympathetic nerve fibers (Fig. 8), but instead appeared in round cells within the denervated infarct (Fig. 9). Some but not all GAL-immunoreactive cells co-labeled with the macrophage markers F4/80 and MAC2, suggesting that some infiltrating macrophages were expressing GAL. Similar GAL staining was observed in both WT and gp130 KO hearts. No GAL+ or PACAP38+ cells were detected in the left ventricle after sham surgery.

Discussion

Cardiac sympathetic neurons in most species produce NPY, which has well-characterized effects on cardiac parasympathetic transmission (Herring et al., 2008) in addition to direct effects on the heart (Allen et al., 1983). NPY expression can be stimulated by nerve activity (Rao et al., 1992) and suppressed by inflammatory cytokines (Lewis et al., 1994). Plasma NPY is increased 3 days after acute myocardial infarction in humans (Omland et al., 1994). This is thought to be due to enhanced release, since cardiac sympathetic nerve activity is increased after myocardial infarction (Graham et al., 2002; Graham et al., 2004), and higher frequency nerve stimulation preferentially promotes peptide release (Shakiryanova et al., 2005). Our observation that NPY mRNA decreased significantly after MI contrasts with elevated plasma NPY in humans, but is consistent with the loss of NPY in rat sympathetic neurons whose axons are damaged by axotomy (Zigmond et al., 1996), and in human cardiac sympathetic neurons after overload hypertrophy (Love et al., 1993) or idiopathic dilated cardiomyopathy (Anderson et al., 1992). Cytokines suppress NPY expression in cultured sympathetic neurons, and we expected that NPY mRNA would not be altered in mice whose sympathetic neurons lack gp130. However, NPY mRNA decreased significantly in cardiac sympathetic neurons from KO mice as well as WT mice suggesting that other factors regulate NPY expression in this model.

In contrast to the surprising gp130-independent change in NPY mRNA, the increase in VIP and PACAP mRNA observed following ischemia-reperfusion required activation of the gp130 cytokine receptor. This is consistent with regulation of these peptides after axotomy (Habecker et al., 2009; Rao et al., 1993), and with the regulation of these peptides in cultured sympathetic neurons treated with inflammatory cytokines (Lewis et al., 1994; Rao et al., 1993; Rao and Landis, 1990). Nerve activity can stimulate expression and release of both peptides (Girard et al., 2002), but we found that the post-infarct increase in cardiac sympathetic nerve activity (Graham et al., 2002; Graham et al., 2004) was not sufficient to stimulate VIP or PACAP mRNA in the absence of gp130.

PACAPs enhance sympathetic neurotransmission (Beaudet et al., 2000; Braas and May, 1999), dilate coronary arteries (Bruch et al., 1997; Dalsgaard et al., 2003; Kastner et al.,

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1995) and protect cardiac myocytes from damage *in vitro* and *in vivo* (Gasz et al., 2006b; Gasz et al., 2006a; Mori et al., 2010; Racz et al., 2008), while VIP released from parasympathetic nerves increases blood flow in coronary arteries and stimulates cardiac contractility (reviewed by (Henning and Sawmiller, 2001). Thus, production of these peptides by cardiac sympathetic neurons might have significant effects in the heart. In addition, preganglionic PACAP release might contribute to the development of autonomic imbalance following myocardial infarction, which is a powerful predictor of susceptibility to arrhythmia and death (La Rovere et al., 1998). Human studies indicate that VIP expression is increased in the stellate following acute myocardial infarction (Roudenok et al., 2001), and we expected to see a significant increase in VIP+ fibers in the left ventricle. We were surprised to find that rare TH+/VIP+ sympathetic fibers were detected in the in the left ventricle of each group examined, regardless of genotype or surgical group. In contrast, PACAP38 peptide was increased in neuronal cell bodies of the stellate ganglion in a gp130-dependent manner, but no TH+/PACAP38+ fibers were observed in the heart. We cannot exclude the possibility that PACAP was present in sympathetic axons, but below the level of detection. PACAP38 immunoreactivity in the heart localized to the infarct, which was devoid of nerve fibers. There were two major types of PACAP38 immunoreactivity in the damaged left ventricle. Diffuse staining that appeared to be in extracellular matrix and possibly myocytes, and cytoplasmic staining in round cells that resembled F4/80+ infiltrating macrophages. The localization of PACAP38 to the infarct, and its apparent expression by immune cells, is particularly interesting given the cardio-protective effects that have been identified for PACAPs in a variety of paradigms (Gasz et al., 2006a; Mori et al., 2010; Racz et al., 2008; Roth et al., 2009; Sano et al., 2002).

GAL was also elevated specifically in the infarct following ischemia-reperfusion. The presence of GAL in the ventricle was expected since we previously showed in the rat that GAL mRNA was increased in the stellate following ischemia-reperfusion and that GAL peptide content, quantified by ELISA, was increased significantly in the left ventricle (Ewert et al., 2008; Habecker et al., 2005). Mouse cardiac sympathetic neurons produce GAL under normal conditions in contrast to rat cardiac sympathetic neurons, which do not (Smith-White, et al., 2003). Given the lack of an increase in GAL mRNA in the stellate ganglion after MI, we did not expect to see significant increases in GAL peptide in the heart. However, immunohistochemistry revealed significant amounts of GAL in the infarcted left ventricle, localized to round cells that appeared to be infiltrating macrophages rather than TH+ nerve fibers. Since physiology experiments indicate that GAL is released from cardiac sympathetic neurons (Smith-White, et al., 2003), we expect that the peptide is present in TH + fibers, but below the level of detection with this antibody. Double label experiments with two common macrophage markers revealed that some of the GAL co-localized with F4/80 and MAC2/galectin. The presence of GAL peptide in macrophages (and potentially other cell types) within the mouse heart raises the possibility that some fraction of the GAL peptide quantified by ELISA in our rat studies (Ewert et al., 2008; Habecker et al., 2005) was in non-neuronal cells rather than nerve fibers. The appearance of GAL in immune cells was surprising but not unprecedented, since GAL has been localized to ED1/CD68+ macrophages during skin inflammation (Ji et al., 1995).

The functional consequences of GAL content in the left ventricle is unclear at this time. GAL inhibits parasympathetic transmission (Potter and Smith-White, 2005), so increased GAL release could also contribute to autonomic imbalance. In addition, GAL stimulates sensory nerve regeneration (Holmes et al., 2000; Mahoney et al., 2003; Suarez et al., 2006) and is positioned to promote the re-growth of cardiac sensory nerves following ischemia-reperfusion. GAL is transported to regenerating sympathetic nerve endings following axotomy (Shadiack and Zigmond, 1998), where it is hypothesized to stimulate sympathetic axon regeneration. Indirect support for this model comes from studies of sympathetic

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neurons that lack gp130, since impaired axon regeneration coincides with the absence of GAL expression (Hyatt Sachs et al., 2010). However, there is currently no direct evidence for GAL stimulation of sympathetic regeneration. All three GAL receptors have been detected in the heart by PCR (Kolakowski et al., 1998; Sullivan et al., 1997; Wang et al., 1997), but it is not clear if GAL has direct effects on cardiac function, in contrast to the clear cardiac effects of PACAPs. Further studies will be required to understand what role GAL and PACAP38 are playing in the remodeling ventricle and how they interact with other factors like neurotrophins that stimulate post-infarct changes in myocytes and nerves.

Acknowledgments

Sources of Support: This work was supported by HL068231 (BAH).

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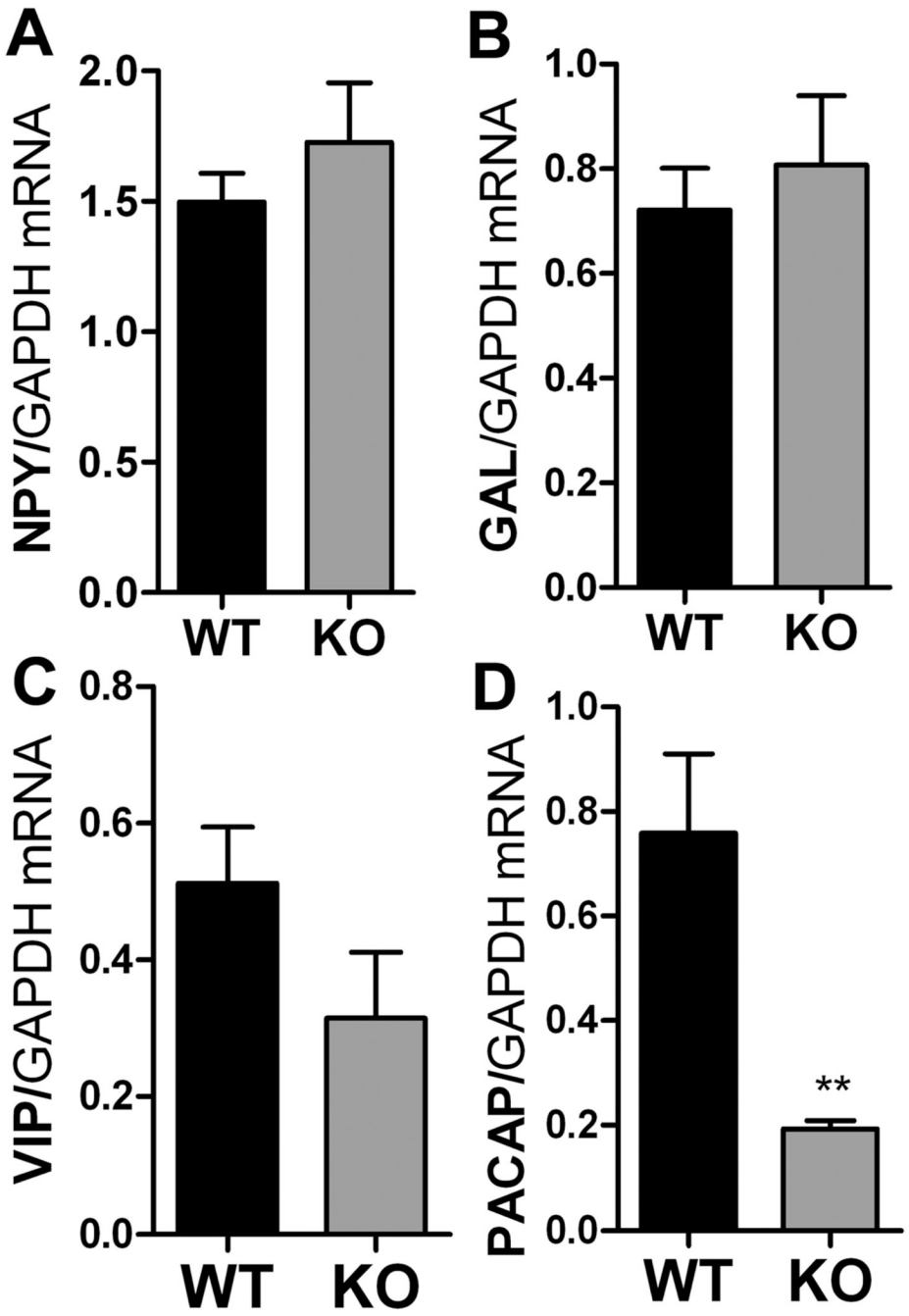

Figure 1. Basal peptide gene expression in cardiac sympathetic neurons

NPY (A), GAL (B), VIP (C), and PACAP (D) mRNA were quantified in WT (black bars) and gp130 KO (gray bars) stellate ganglia. Peptide genes were normalized to GAPDH mRNA in the same sample. Data are mean±sem, n=4, **p<0.01.

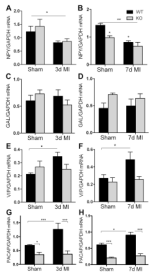

Figure 2. Peptide mRNA 3 and 7 days after myocardial infarction

NPY (A, B), GAL (C, D), VIP (E, F), and PACAP (G, H) mRNA were quantified in WT (black bars) and gp130 KO (gray bars) stellate ganglia 3 and 7 days after sham surgery or myocardial infarction (MI). Data are mean±sem, n=4 except WT 7d MI n=3, *p<0.05, **p<0.01, ***p<0.001 compared to sham of the same genotype. PACAP mRNAs in gp130 KO ganglia were significantly lower than WT mRNA from the same surgical group (two-way ANOVA).

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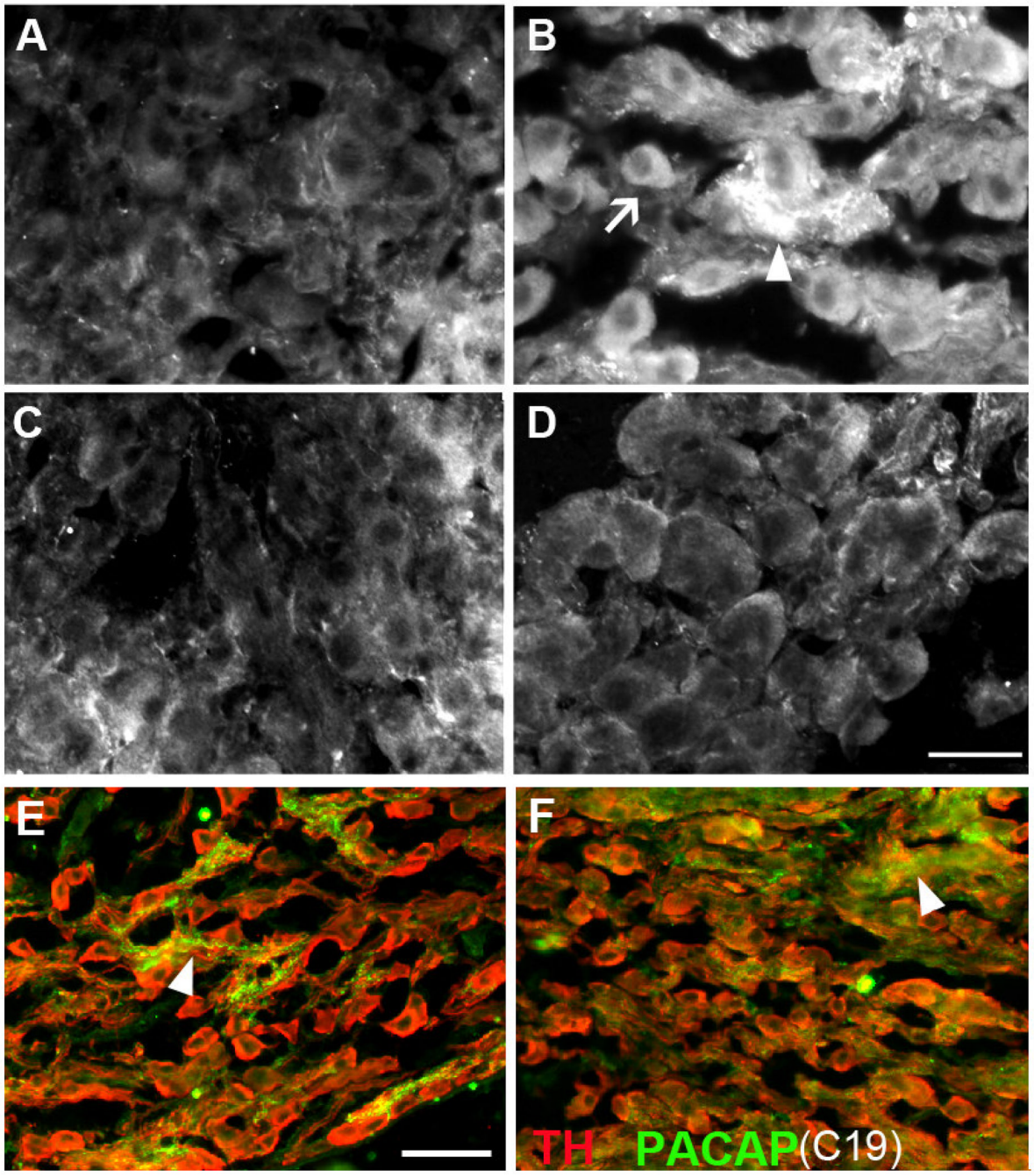

Figure 3. PACAP immunoreactivity in the stellate ganglion

A–D) PACAP38 immunoreactivity in WT (A, B) and gp130 KO (C, D) ganglia. Little PACAP38 is detectable in sham ganglia (A, C) of either genotype. PACAP38 is elevated 3 days after myocardial infarction (MI) in WT but not gp130 KO ganglia. Scale bar A–D is 20 μm. E,F) TH (red) and PACAP (green) immunoreactivity using the C-19 antibody in WT (E) and gp130 KO (F) ganglia three days after MI. Scale bar E–F is 50 μm. In all panels, arrows denote cell bodies and arrowheads identify pre-ganglionic fibers.

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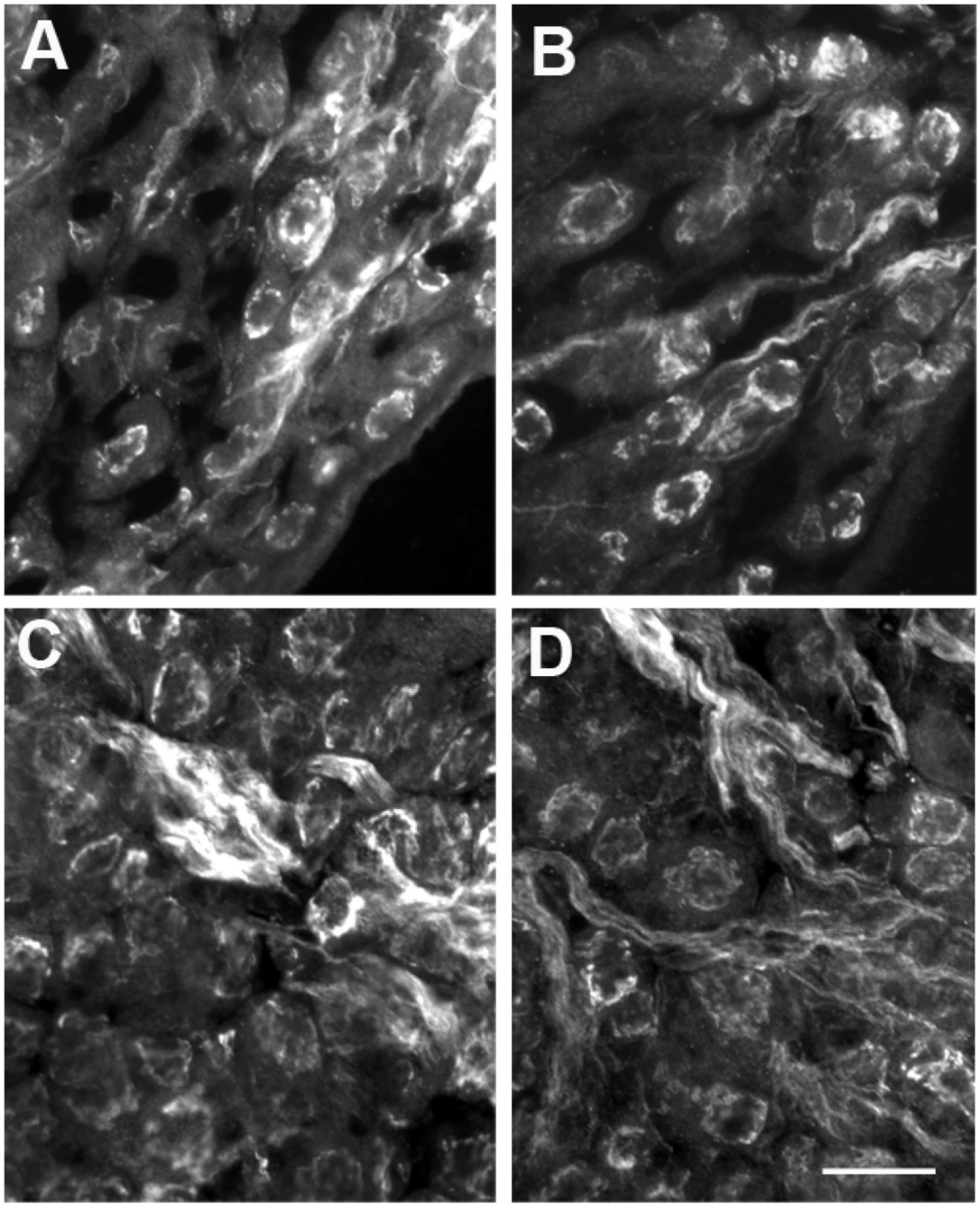

Figure 4. GAL immunoreactivity in the stellate ganglion

Galanin immunoreactivity in WT (A, B) and gp130 KO (C, D) ganglia 3 days after sham surgery (A, C) or myocardial infarction (B, D). GAL is visible in pre-ganglionic fibers in addition to peri-nuclear staining in cell bodies of all ganglia. Scale bar is 20 μm.

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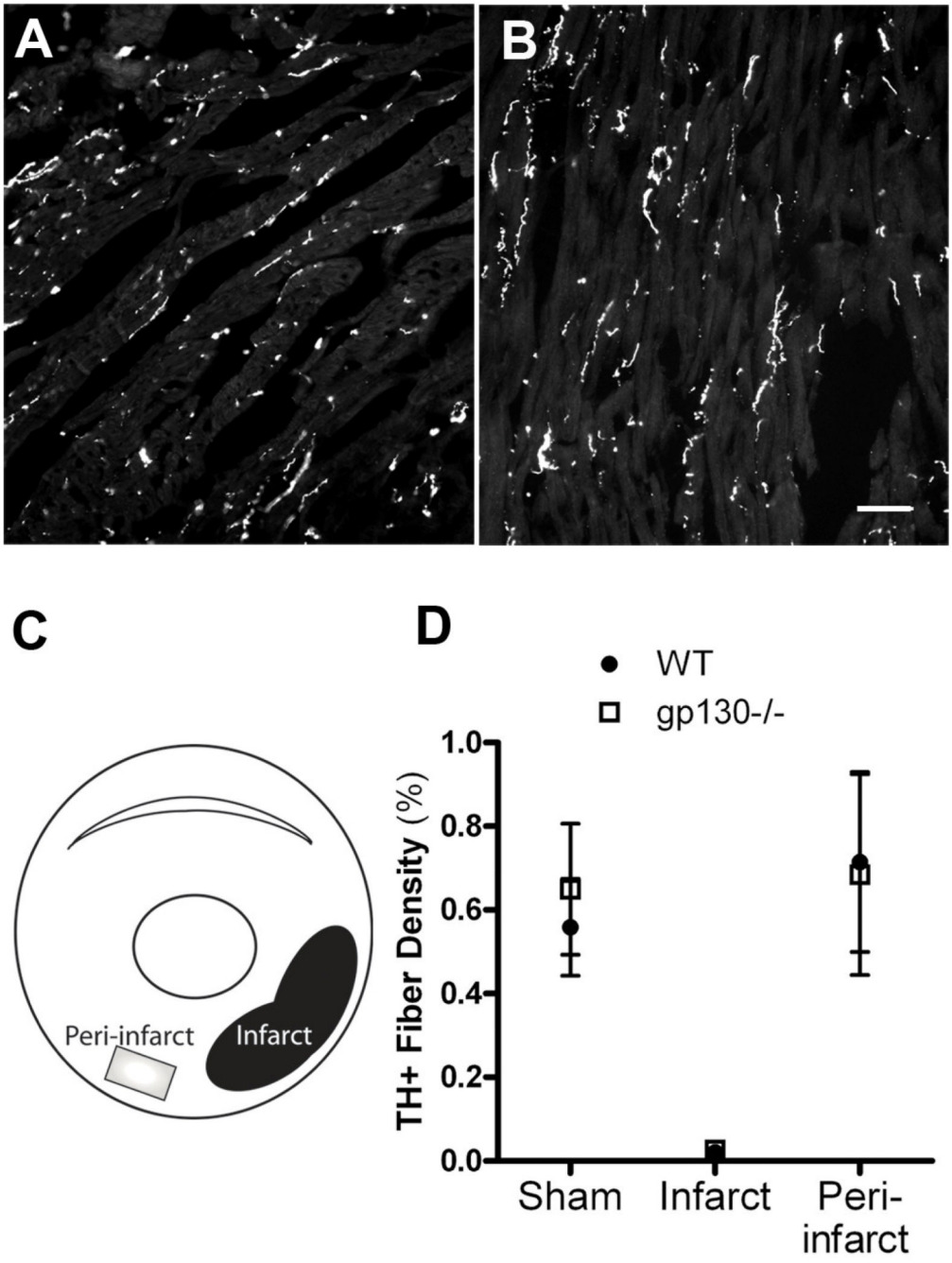

Figure 5. Sympathetic innervation density in WT and gp130 KO left ventricle

A,B) Sympathetic innervation revealed by TH immunoreactivity in sham WT (A) and gp130 KO (B) left ventricle. C) Schematic of the regions for innervation density analysis quantified in panel D. D) Sympathetic innervation density in the infarct and peri-infarct left ventricle was identical in WT and KO hearts 3 days after myocardial infarction. The infarct was essentially denervated in both genotypes. Mean±sem, n=5–6 animals per group.

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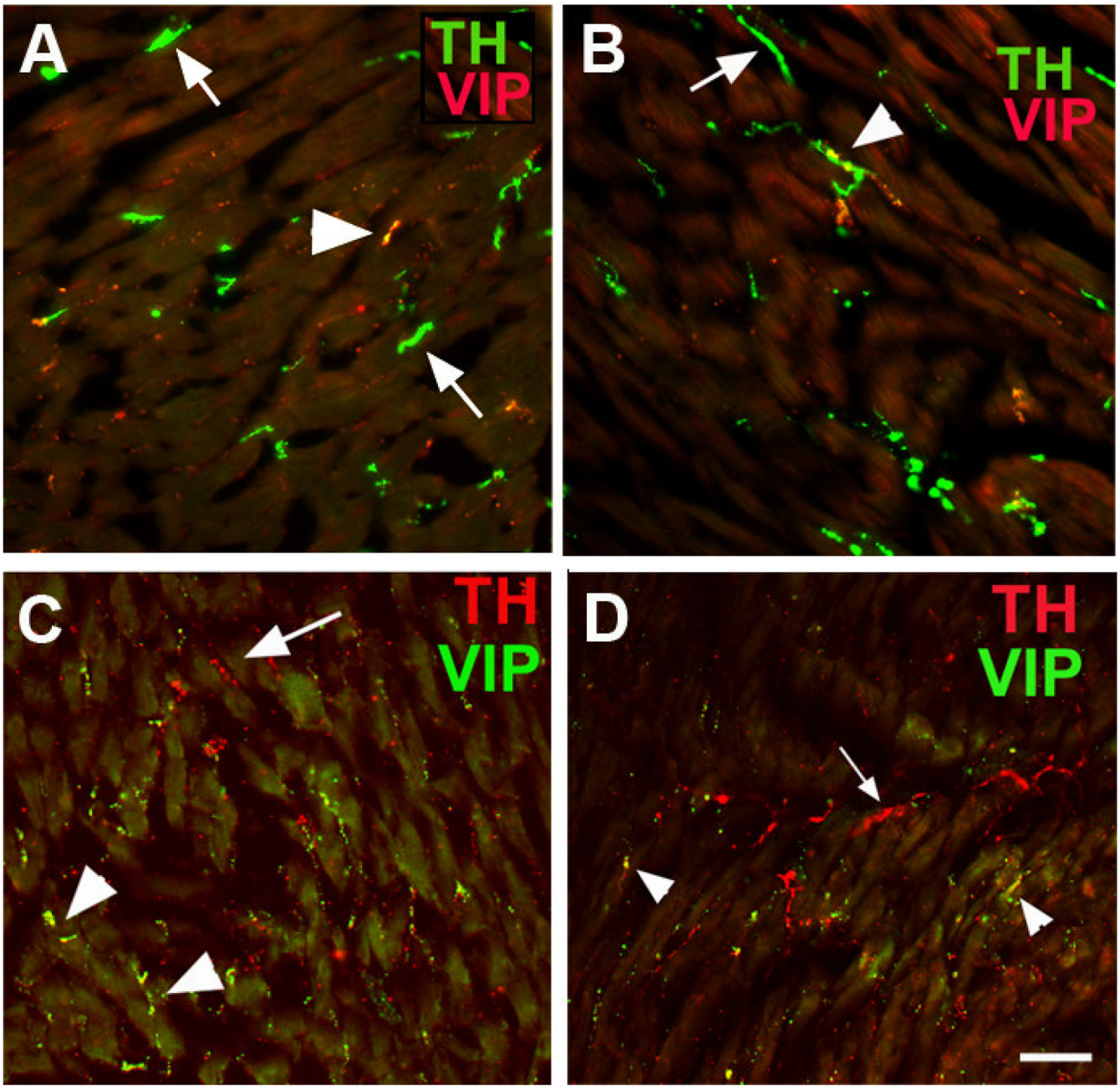

Figure 6. VIP and TH immunoreactivity in the left ventricle

Sections of WT (A, B) or KO (C, D) ventricle were stained for VIP and TH three days after sham surgery (A, C) or myocardial infarction (B,D). Many TH+ fibers are visible, and some of these are marked by arrows. Rare TH/VIP+ fibers are visible and are marked by arrowheads. Scale bars are 50 μm.

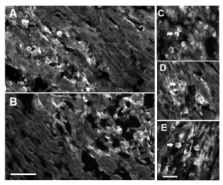

Figure 7. PACAP38 immunoreactivity in the infarcted left ventricle

Sections from WT (A, D) and gp130 KO (B, E) hearts were stained for PACAP38 three days after ischemia-reperfusion. PACAP38 reactivity is localized to the infarct. Scale bar=50 μm. C–E) Higher magnification views of the same sections and macrophage staining. Round cells that have infiltrated into the heart stain for the macrophage marker F4/80 (C). Cells of identical size and shape are immunoreactive for PACAP38 in WT (D) and KO (E) left ventricle after MI.

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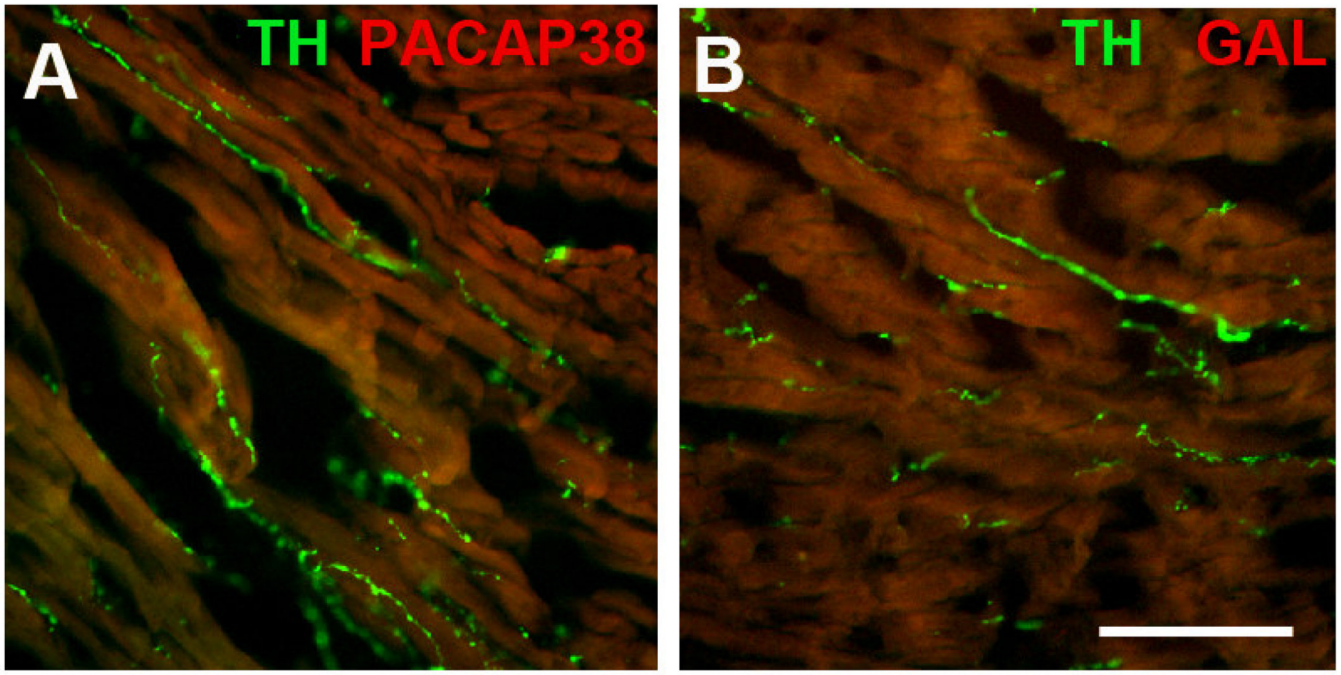

Figure 8. TH/peptide double label in the peri-infarct left ventricle

Sections from WT hearts were stained for TH and PACAP38 (A) or GAL (B) three days after myocardial infarction. Many TH+ fibers are visible but no PACAP38 or GAL is detectable in sympathetic fibers, in contrast to the PACAP and GAL present in the infarcted region of the same heart. Scale bar is 50 μm.

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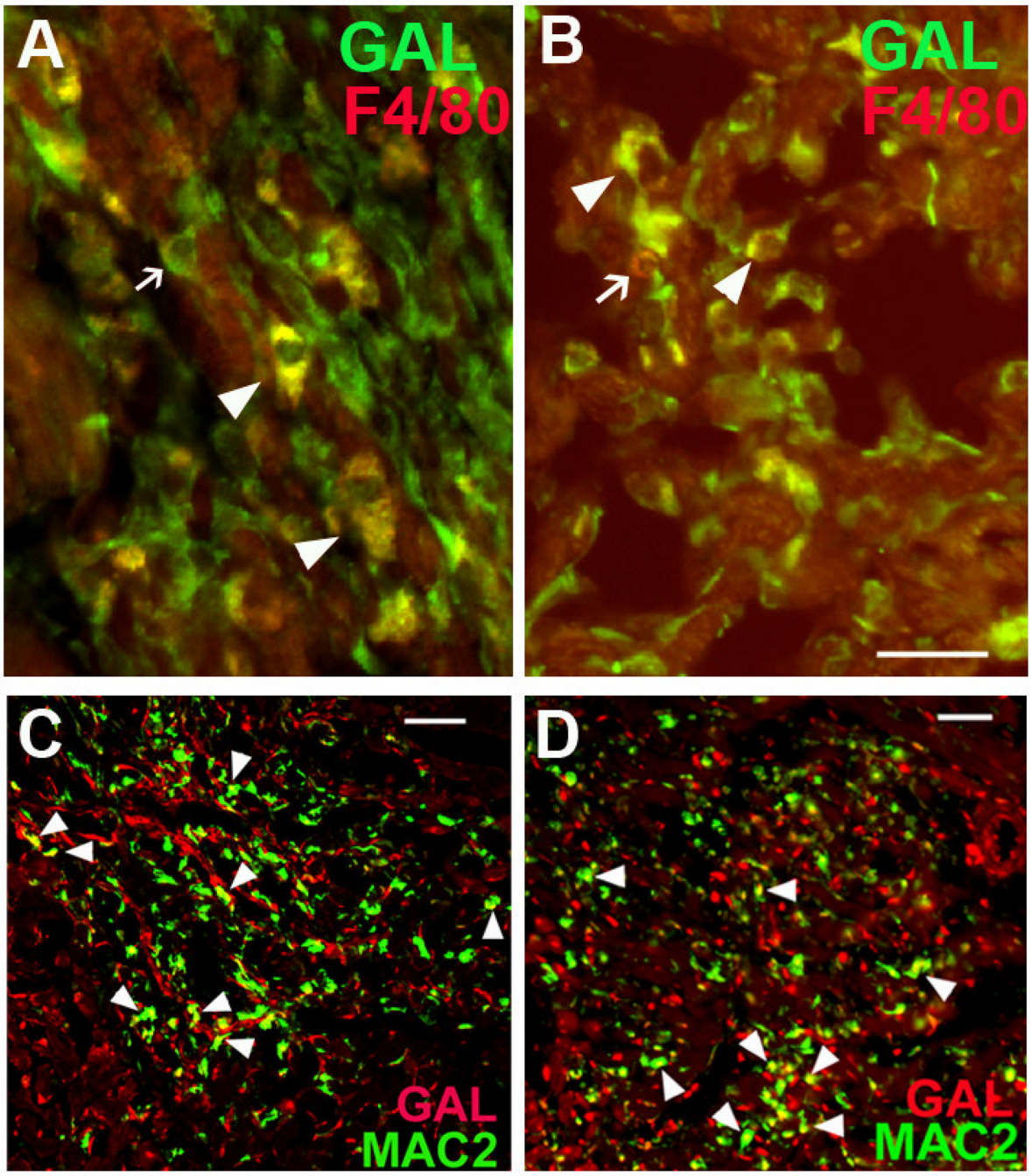

Figure 9. Galanin immunoreactivity in the infarcted left ventricle

Sections from WT (A, C) and gp130 KO (B, D) hearts taken three days after myocardial infarction were double labeled for GAL and the macrophage marker F4/80 (A, B) or MAC2 (C, D). Double-labeled cells are identified with arrowheads and appear yellow. Representative single-labeled cells are identified with arrows. Scale bar for A, B is 25 μm. Scale bars for C, D are 50 μm.