



Post-infarct cardiac sympathetic hyperactivity regulates galanin expression

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ABSTRACT

The neuropeptide galanin is elevated in the cardiac sympathetic innervation after myocardial infarction (MI). Galanin inhibits vagal transmission and may support the regeneration of sympathetic nerves, thereby contributing to the development of arrhythmia and sudden cardiac death after MI. The reason for increased galanin production in sympathetic neurons after myocardial infarction is not known. Cardiac sympathetic neurons are activated chronically after cardiac ischemia–reperfusion, and activation of sympathetic neurons in culture stimulates galanin expression. Therefore, we tested the hypothesis that increased sympathetic nerve activity stimulates galanin expression in cardiac sympathetic neurons after myocardial infarction. To test this hypothesis we used TGR(ASrAOGEN) transgenic rats, which lack brain angiotensinogen and do not exhibit post-infarct sympathetic hyperactivity. Hearts and stellate ganglia were collected 1 week after ischemia–reperfusion. Galanin mRNA was quantified by real-time PCR and peptide content was assayed by enzyme-linked immunosorbent assay. Galanin mRNA increased approximately 3-fold after MI in cardiac sympathetic neurons of both genotypes compared to unoperated and sham controls. Left ventricular galanin content, however, increased after MI only in Sprague–Dawley rats and not in AOGEN rats. These data suggest that post-infarct cardiac sympathetic hyperactivity stimulates galanin peptide production but is not required for increased galanin mRNA expression.

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Galanin is a 29 amino acid neuropeptide that is widely expressed in the brain and peripheral nervous system. Galanin expression is elevated significantly in cardiac sympathetic neurons after myocardial infarction [8], and galanin has important cardiovascular effects [35,32]. However, it is not known what stimulates galanin expression in cardiac sympathetic neurons after ischemia–reperfusion. Galanin expression is stimulated in damaged sympathetic neurons following axotomy [28], and this is due to the presence of inflammatory cytokines like leukemia inhibitory factor (LIF) [23,34] and the loss of nerve growth factor (NGF) [29,30]. Depolarization of cultured sympathetic neurons can also stimulate galanin expression and release [6,12]. The loss of NGF is unlikely to stimulate galanin expression in post-infarct sympathetic neurons since NGF is elevated after ischemia–reperfusion [9]. In contrast, increased sympathetic nerve activity and LIF-related cytokines are likely candidates for stimulating galanin expression in post-infarct cardiac sympathetic neurons.

Depolarization of sympathetic neurons *in vitro* increases galanin production and release [6,12], but it is not known if sympathetic nerve activity stimulates galanin expression *in vivo*. Cardiac sympathetic nerves are activated chronically after myocardial infarction

[7,25], and the increase in cardiac sympathetic nerve activity may stimulate galanin expression. To test this hypothesis we used TGR(ASrAOGEN) transgenic rats (AOGEN rats) [27], which do not have post-MI sympathetic hyperactivity due to the lack of brain angiotensinogen [36].

Adult Sprague–Dawley rats (225–250 g) were obtained from Charles River SASCO and transgenic rats deficient in brain angiotensinogen (AOGEN) were obtained from the Max-Delbrück Center (Berlin-Buch, Germany). All rats were kept on a 12 h:12 h light dark cycle with *ad libitum* access to food and water. Animals from the two genotypes were age-matched and gender matched for each experiment. Three experimental groups were examined: unoperated, sham, and myocardial infarction (MI). A minimum of five animals were assigned to each group for each experiment, and tissue was processed together for each type of analysis. Where the final *n* is less than 5 there were problems with sample recovery. Larger groups were used for peptide analysis by ELISA due to greater variability with that procedure.

Age and gender matched AOGEN and Sprague–Dawley rats (12–18 weeks) were subjected to myocardial infarction by reversible ligation of the left anterior descending coronary artery for 30 min, or sham surgery without artery ligation. Core body temperature was monitored by a rectal probe and maintained at 37°C, and a 3-lead ECG was monitored throughout the surgery using a PowerLab data acquisition system. After surgery, animals were

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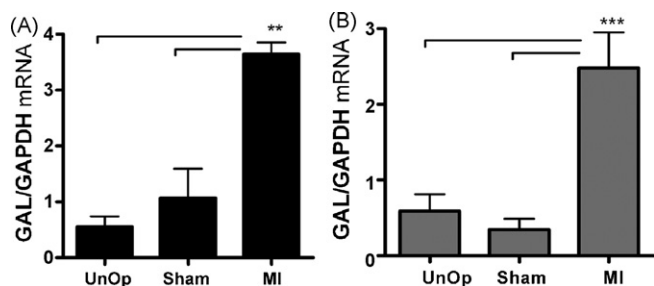


Fig. 1. Galanin mRNA is elevated in cardiac sympathetic neurons after myocardial infarction. Galanin mRNA was normalized to GAPDH mRNA in the stellate ganglia of Sprague–Dawley (A; $n = 3–4$) and AOGEN (B; $n = 4–5$) rats. Ganglia were collected from unoperated control animals (UnOp) or 1 week after sham surgery or myocardial infarction (MI) (** $p < 0.01$, *** $p < 0.001$, mean \pm S.E.M.).

returned to individual cages and given regular food and water for 7 days before euthanasia and tissue harvest. Buprenex (0.1 mg/kg) was administered as needed to ensure the animals were comfortable following surgery. All surgical procedures were performed under aseptic conditions and have been described in detail elsewhere [20]. Infarct size did not differ between the two genotypes [20]. All procedures were approved by the Institutional Animal Care and Use Committee, and comply with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996).

Stellate ganglia were harvested 7 days after ischemia–reperfusion and stored immediately in RNAlater. RNA was isolated from individual stellate ganglia using the Ambion RNAqueous micro kit and 25 ng of each sample reverse transcribed as described previously [20]. Real-time PCR was performed with ABI TaqMan Universal PCR master mix using ABI pre-validated TaqMan gene expression assays for galanin and GAPDH as a normalization control. Standard curves for galanin and GAPDH were generated with known amounts of RNA from control sympathetic ganglia, ranging from 0.39 ng to 100 ng. Values for galanin were normalized to GAPDH from the same sample. Galanin/GAPDH mRNA levels were compared across surgical groups within each genotype by one-way ANOVA with a Newman–Keuls post hoc test using Prism 4.1.

Galanin peptide content was quantified using a competitive enzyme-linked immunoassay kit from Peninsula Laboratories. Hearts were excised and the atria and left ventricles were frozen on dry ice and then pulverized over dry ice using a mortar and pestle. 0.300–0.600 g samples were homogenized in 2 M acetic acid to extract peptides. Samples were assayed in duplicate by ELISA following the manufacturer's instructions. Galanin levels were compared across the surgical groups within each genotype by one-way ANOVA with a Newman–Keuls post hoc test. Basal galanin content was compared between the genotypes by t -test.

The ratio of galanin/GAPDH mRNA was similar in unoperated Sprague–Dawley and AOGEN rats (Fig. 1A and B), and was unchanged by sham surgery. Galanin mRNA increased approximately 3-fold in Sprague–Dawley post-infarct cardiac sympathetic neurons, consistent with previous reports [8]. Similarly, galanin mRNA increased almost 3-fold in post-infarct AOGEN rats, suggesting that sympathetic hyperactivity is not required for the post-infarct induction of galanin mRNA.

Basal galanin peptide content in the atria (SD atria 222 ± 56 ng/gm, $n = 9$; AO atria 363 ± 89 ng/gm, $n = 7$; mean \pm S.E.M.) and left ventricles (LV) of Sprague–Dawley and AOGEN rats (SD LV 89 ± 34 ng/gm, $n = 6$; AO LV 103 ± 24 ng/gm, $n = 5$; mean \pm S.E.M.) were not significantly different between the genotypes. Peptide content did not change significantly in the atria of SD control rats, consistent with previous reports [8], but galanin content decreased in the atria of AOGEN rats after MI (Fig. 2). Peptide levels increased

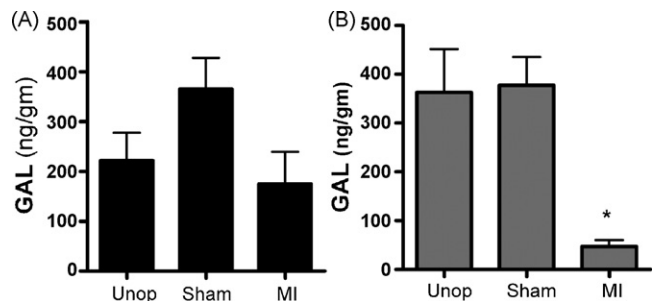


Fig. 2. Galanin peptide content in the atria. Galanin peptide was quantified in atria from unoperated, sham, and MI rats by ELISA. (A) Galanin content did not change significantly in the atria from Sprague–Dawley rats (mean \pm S.E.M., $n = 4–9$). (B) Galanin content decreased significantly in AOGEN atria after MI (* $p < 0.05$, mean \pm S.E.M., $n = 4–7$).

significantly in the LV of Sprague–Dawley control rats (Fig. 3), consistent with previous results [8]. However, galanin content was not altered significantly in the LV of AOGEN transgenic rats after MI, despite the increase in galanin mRNA. Thus, increased nerve activity appears to be essential for the post-infarct induction of galanin peptide, but not for increased expression of galanin mRNA.

Our study reveals important differences in the regulation of galanin expression following myocardial infarction compared to axotomy-induced nerve injury. Galanin mRNA and peptide are elevated following both types of injury. However, our study suggests that increased nerve activity is required for the elevation of galanin peptide content after MI, while galanin levels increase following axotomy without any change in nerve activity. Thus, one difference between the two injury paradigms is the role of preganglionic activation in driving galanin peptide synthesis.

Another important difference in the two types of injury-induced peptide plasticity is the role of NGF. Although the cytokine LIF plays a central role in the regulation of galanin mRNA and peptide following axotomy [23], the increase in cytokine is accompanied by the loss of target-derived NGF. The combination of increased LIF together with decreased NGF is required to recapitulate the induction of galanin mRNA in the absence of nerve injury [30]. LIF is increased significantly in the heart after myocardial infarction [2,13,5], but NGF is also elevated [9,37,1]. Infusion of anti-NGF antibodies acutely following ischemia–reperfusion disrupts sympathetic transmission in the heart, indicating that cardiac sympathetic nerves are capable of taking up NGF after ischemia–reperfusion [1]. Thus, one important difference between axotomy and the nerve injury caused by myocardial infarction is the continued presence of NGF following MI. Increased LIF combined with decreased NGF is required to stimulate galanin mRNA and peptide expression following axotomy [30]. Although inflamma-

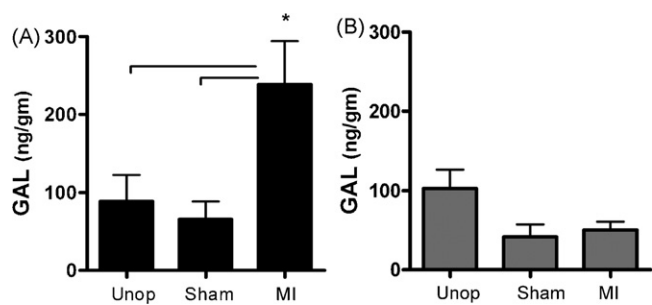


Fig. 3. Galanin peptide content in the left ventricle. Galanin peptide was quantified in left ventricles from unoperated, sham, and MI rats by ELISA. (A) Galanin content increased significantly in Sprague–Dawley LV after MI (* $p < 0.05$, mean \pm S.E.M., $n = 4–6$). (B) Galanin content did not change significantly in AOGEN LV (mean \pm S.E.M., $n = 4–5$).

tory cytokines alone appear capable of stimulating galanin mRNA after MI, it is interesting to speculate that cytokines combined with increased nerve activity are required to stimulate production of galanin peptide.

It is unclear why galanin levels in the atria of AOPEN rats decrease after MI, while galanin content in the LV does not change significantly. Similarly, it is not clear why galanin content is elevated in the Sprague–Dawley LV but unchanged in the atria after myocardial infarction. In both of these situations the ratio of LV:atria galanin is altered following MI. One potential explanation for this regional difference in galanin accumulation is that different populations of sympathetic neurons project to the atria and ventricles and thus are affected differently by ischemia–reperfusion in the left ventricle. Axotomy studies in the SCG indicate that galanin is induced only in the damaged neurons [28]. Therefore, if a subset of cardiac neurons projected to the LV then those neurons would be selectively impacted by ischemia resulting in elevated galanin in the LV but not in the rest of the heart. Detailed mapping of the sympathetic projections to the dog heart indicate a large overlap with nerve branches from each stellate ganglion projecting to both atria and ventricles [22]. Although a similarly detailed map of the rat cardiac innervation has not been completed, Pardini et al. have shown that the atria and left ventricle receive innervation from both the left and right stellate ganglia, and that the cell bodies projecting to the heart are distributed throughout the ganglia [18]. Removal from the rat of one or both stellates resulted in a similar loss of NE content in the atria and ventricles [18,19]. Although these experiments do not rule out the possibility that discrete sub-branches of these nerves innervate the atria vs. the ventricles, there is no clear evidence to support the selective localization of sympathetic neurons to specific chambers of the rat heart. It is possible that galanin is selectively transported to the LV rather than other regions of the heart. However, it is also possible that increased inflammatory cytokines, neurotrophins, oxidative stress, and other post-ischemic changes in the LV impact galanin release [26] or metabolism [11] in a manner that is distinct from its regulation in the rest of the heart.

The increased galanin produced by cardiac sympathetic neurons following myocardial infarction may have important physiological consequences. Galanin promotes the regeneration of sensory and motor neurons following axotomy [16,10,33], and the accumulation of galanin in the left ventricle is consistent with a potential role in sympathetic axon regeneration. Galanin also inhibits the release of acetylcholine from cardiac parasympathetic neurons in a wide variety of species [35,32,4,21,24]. Although galanin peptide content does not accumulate significantly in the atria [8], where sympathetic and parasympathetic axons are in close proximity, galanin release is elevated due to the increased activation of cardiac sympathetic nerves after infarction. This galanin release may contribute to the post-infarct loss of parasympathetic input to the heart that is an important indicator of risk for sudden cardiac death [15,17,14].

Although AOPEN rats provide a useful model to test the role of increased nerve activity on the stimulation of galanin production *in vivo*, they are not a good model for testing the effects of galanin on autonomic control of the heart. Rats are unusual in that their cardiac parasympathetic neurons do not respond to galanin [31], and therefore the decreased atrial galanin seen after MI in AOPEN rats does not alter control of heart rate. We have examined cardiac function and autonomic control of the heart in AOPEN rats, and the differences between those rats and Sprague–Dawley rats before and after MI are due primarily to altered noradrenergic transmission [20,3].

In summary, we used AOPEN transgenic rats which have attenuated post-infarct sympathetic hyperactivity, to determine if increased sympathetic nerve activity was responsible for increased galanin expression in cardiac sympathetic neurons following

ischemia–reperfusion. We found that the MI-induced increase in galanin mRNA observed in Sprague–Dawley rats was present in AOPEN rats, but that the infarction-induced increase in galanin peptide seen in control animals was absent in AOPEN rats. This suggests that increased nerve activity is not required for stimulation of galanin gene expression, but is necessary for increased peptide production. Further studies are required to understand the role of galanin in regulating autonomic transmission and cardiac function after myocardial infarction.

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