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Acute Myocardial Infarction Induces Bilateral Stellate Ganglia Neural Remodeling in Rabbits

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Abstract

Introduction—Myocardial infarction (MI) results in cardiac nerve sprouting in the myocardium. Whether or not similar neural remodeling occurs in the stellate ganglia (SG) is unknown. We aimed to test the hypothesis that MI induces bilateral SG nerve sprouting.

Methods—Acute MI was created by coronary artery ligation in rabbits (n=12). Serum nerve growth factor (NGF) level was measured by enzyme-linked immunosorbent assay (ELISA). The hearts and bilateral SGs were harvested for immunohistochemistry after 1 week in 6 rabbits, and after 1 month in 6 rabbits. Immunostaining for tyrosine hydroxylase (TH), growth-associated protein 43 (GAP43), cholineacetyltransferase (ChAT) and synaptophysin (SYN) was performed to determine the magnitude of nerve sprouting. Tissues from 6 normal rabbits were used as controls. Nerve density was determined by computerized morphometry.

Results—MI results in increased serum NGF levels at 1 week (1519.8 ± 632.2 ng/ml) that persists to 1 month (1361.2 ± 176.3 ng/ml) as compared to controls (89.6 ± 34.9 ng/ml), ($p=.0002$, and $p=.0001$, respectively). Immunostaining demonstrated nerve sprouting and hyperinnervation in both SGs after MI. The nerve densities ($\mu\text{m}^2/\text{ganglion cell}$) in SG 1 week after MI, 1 month after MI and in control groups, respectively, were: GAP43, 278 ± 96 , 225 ± 39 and 149 ± 57 ($p=.01$); SYN, 244 ± 152 , 268 ± 115 and 102 ± 60 ($p=.02$); TH, 233 ± 71 , 180 ± 50 and 135 ± 68 ($p=.047$); ChAT, 244 ± 100 , 208 ± 46 and 130 ± 41 $\mu\text{m}^2/\text{cell}$ ($p=.01$).

Conclusions—MI increases serum NGF levels and induces nerve sprouting and hyperinnervation in bilateral SGs for at least 1 month after MI. The hyperinnervation includes both postganglionic adrenergic axons and preganglionic cholinergic axons in the SG.

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Disclosures
None

Keywords

Myocardial Infarction; Ventricular Arrhythmia; Autonomic Nervous System; Stellate Ganglion; Nerve Sprouting; Sudden Cardiac Death

Myocardial infarction (MI) induces cardiac neural remodeling in both humans and animal models. Post-MI neural remodeling is not limited to the infarcted ventricular myocardium, but also involved the non-infarcted atrial myocardium. In a canine model of MI, Zhou et al demonstrated an immediate rise of nerve growth factor (NGF) in the infarcted myocardium at 3.5 hrs after MI. The NGF level peaked at 1 week and persisted for up to 1 month after MI. That study also found that NGF protein level was increased at the left stellate ganglion (SG) starting at 3 days after MI and remained high up to 1 month afterwards. The increased level of NGF in the left SG is most likely from the retrograde axonal transport of NGF released from myocardium after MI, for there was no change in NGF mRNA level in the left SG. However, because all tissues were used for protein and mRNA analyses, we did not perform histological studies to determine whether or not there is histological evidence of nerve sprouting in the SG. To test the hypothesis that MI can trigger SG nerve sprouting, we use a rabbit model of MI to determine whether MI increases the serum NGF concentration, and causes nerve sprouting in the stellate ganglion.

Materials and Methods

The research protocol was approved by the Institutional Animal Care and Use Committee of Cedars-Sinai Medical Center and followed the American Heart Association guidelines. We studied 19 New Zealand white rabbits (3-5 kg).

Surgical Procedures

Under isoflurane general anesthesia, MI was created by the ligation of the origin of the obtuse marginal branch coronary artery in 13 rabbits via a left lateral thoracotomy in the 4th intercostal space. Surface ECG was monitored to document ST segment elevation. One rabbit died 2 hours after surgery, and the remaining 12 rabbits were used for data analyses. A sham operation was performed, and MI was not created in 6 normal rabbits (Control group). Half of the 12 MI rabbits were euthanized after 1 week (1-w MI Group, n=6), and half after 1 month (1-m MI Group, n=6) of surgery. Both SGs were quickly excised and fixed in 4% formalin for 1 hr followed by storage in 70% alcohol for immunohistochemical studies. Blood samples were obtained from an ear artery at baseline, 1 week, and 1 month after surgery, centrifuged at 4°C and 2×1000 g for 20 minutes. The supernatant was collected and stored at -80°C refrigerator for further processing. Tissue and blood samples were also obtained from the Control Group.

Histological and Immunohistochemical Studies

Infarct size was determined as previously reported. Multiple tissue paraffin blocks were sampled, using sharp skin biopsy punches (Acu-Punch, Acuderm Inc) and a scalpel, from both SG, and routinely processed. Serial 5 µm-thick sections were mounted on slides. The immunohistochemical avidin-biotin complex method was used for immunostaining with antibodies for tyrosine hydroxylase (TH, 1:100, Accurate Chemical BYA90291), choline-acetyltransferase (ChAT, 1:100, Chemicon AB144P-200µl), synaptophysin (SYN, 1:50, Dako, A0010), GAP-43 (1:100, Chemicon, MAB347), and vesicular acetylcholine transporter (VACHT, 1:500, Santa Cruz). The densities of stained nerve fibers were analyzed by light microscopy using computerized morphometry (Image Pro). Stained nerve fiber density was expressed as the total nerve area divided by the total cell bodies per field

examined ($\mu\text{m}^2/\text{cell}$). We quantified and compared the nerve densities in both right SG (RSG) and left SG (LSG). A single blinded investigator randomly selected at least 6 fields for each tissue section and averaged the nerve density counted on the selected fields. The code was then broken for statistical analyses.

Double Immunofluorescence Studies

Double immunofluorescence studies were performed using antibodies TH/ChAT, SYN/TH, and SYN/ChAT (1:200 dilution) to determine if these two nerve markers co-localize in the SG. Dual-labeled sections were visualized with a fluorescent microscope, with digital manipulations restricted to conventional techniques.

Enzyme-Linked Immunosorbent Assay

Serum NGF concentrations were assayed by using sandwich enzyme-linked immunosorbent assay (ELISA) using NGF Emax ImmunoAssay System (Promega) according to the manufacturer's instructions. All assays were performed on F-bottom 96-well plates (Nunc). Tertiary antibodies were conjugated to horseradish peroxidase. Wells were developed with tetramethylbenzidine and measured at 450 nm. NGF content was quantified against a standard curve calibrated with known amounts of protein. All samples (dilution 1:5) were pretreated with acid, assayed in triplicate and expressed as a mean.

Statistical Analysis

Data were presented as mean $\pm$ SD. Student's two-tailed *t* tests were used to compare the means between two groups. Multiple group comparisons were made with ANOVA followed by Newman-Keul post-hoc analysis. Pearson's correlation coefficients were used to test the linear relationship between two groups. A *P* value of ≤ 0.05 was considered statistically significant.

Results

MI Characterization in Rabbits

During the first surgery, ST-segment elevation and frequent premature ventricular contractions were observed immediately after the coronary artery ligation in all rabbits. MI was eventually confirmed by histological examination. The average MI size was $26.3 \pm 3.6\%$ of the total ventricular mass. As compared to controls, MI resulted in a significant increase of serum NGF levels at 1 week (1519.8 ± 632.2 vs. 89.6 ± 34.9 ng/ml at baseline, $p = .0002$), and 1 month (1361.2 ± 176.3 ng/ml vs. 89.6 ± 34.9 ng/ml at baseline, $p = .0001$) after MI.

Timing of SG Neural Remodeling after MI

As shown in Fig. 1, ganglionic cells were almost all TH-positive and nerve fibers structures were mainly either TH-positive sympathetic nerves or ChAT-positive cholinergic nerves. Nerve sprouting in the SG after MI was evident by an increase of GAP-43 immunoreactive nerve fibers. This regenerative remodeling was observed not only in sympathetic nerve but cholinergic nerve fibers as well. The study also showed that the SYN-immunoreactive fiber density was increased. Fig. 2 shows the increased density of nerve fibers that were immunoreactive to GAP43, SYN, TH and ChAT at 1 week after MI. The nerve densities ($\mu\text{m}^2/\text{ganglion cell}$) of SG 1 week after MI, 1 month after MI and in control groups, respectively, were: GAP43, 278 ± 96 , 225 ± 39 and 149 ± 57 ($p = .01$); SYN, 244 ± 152 , 268 ± 115 and 102 ± 60 ($p = .02$); TH, 233 ± 71 , 180 ± 50 and 135 ± 68 ($p = .047$); ChAT, 244 ± 100 , 208 ± 46 and 130 ± 41 $\mu\text{m}^2/\text{cell}$ ($p = .01$).

Bilateral SG Nerve Sprouting

Fig. 3 shows the changes of nerve densities at 1 week and at 1 month after MI. There are significantly increased nerve densities for all 4 stains. Fig. 4 shows LSG nerve density and RSG nerve density separately. Nerve sprouting occurred in both SGs after MI, but to a lesser extent in the LSG as compared to the RSG. The RSG had greater nerve densities in controls, 1 week, and 1 month following MI.

Double Staining

Double staining of TH and ChAT showed no co-localization of TH-positive and ChAT-positive nerve structures (Fig. 1). SYN immunoreactivity in the nerve fibers was mainly co-localized with ChAT, but not with TH (Fig. 5). The co-localization between cholinergic (preganglionic) fibers and SYN suggest that increased cholinergic fibers are most likely functional presynaptic nerve fibers.

Discussion

In the present study, we assessed the characteristics of spontaneous SG nerve sprouting after MI, in terms of type, timing, and distribution. Our main findings include: 1) SG neural remodeling is MI-induced, involves both sympathetic and parasympathetic components, and is NGF-mediated; 2) SG neural remodeling is measurable and peaks 1 week after MI, involving both LSG and RSG to different degrees, leading to a bilateral and asymmetric/differential nerve growth between SG; and 3) SG neural remodeling transforms primarily adrenergic structures into more heterogeneous functional adrenergic and cholinergic ones.

Characteristics of Post-MI Neural Remodeling

Zhou et al have previously reported cardiac nerve sprouting in a canine model of MI. In that model, there is increased NGF protein but not mRNA concentrations in the left SG. However, because all tissues were used for protein analyses, we did not have histological findings to determine the characteristics of neural remodeling in the SG after MI. The present study provides histological evidence of significant SG neural remodeling after MI. An interesting finding is that the post-MI SGs not only have increased postganglionic sympathetic nerve densities but also have significantly increased preganglionic cholinergic nerve densities. The relative densities of preganglionic to postganglionic nerves in the superior cervical ganglion is said to be around 1:4, indicating that the sympathetic ganglion is primarily an adrenergic structure. However, subsequent studies showed that for a given ganglion, there are substantial differences in the ratio index between species, between individuals of the same species, and between stages of development in the same species. While the mechanism of this large variation could be attributed to species and individual differences, it is also possible that neural plasticity could alter the ratio index from time to time even in the same individual. For example, daily NGF injection in neonatal mice for 10 days causes enhanced differentiation of ganglionic neurons, leading to 5-fold higher protein content and a 3.7-fold higher specific activity to TH. In addition to the increased postganglionic fibers, the same authors also showed a doubling of the presynaptic terminals and increased number of axons in the preganglionic nerve. We found that MI resulted in a 20-fold increase of serum NGF levels one week and one month after MI, although we do not know if the same high level is maintained between these two time points. Assuming that there is sustained NGF elevations between one week and one month, the high serum NGF levels can certainly exert trophic effects in inducing sprouting of post-ganglionic sympathetic axons in the SG after MI. The retrograde transported NGF from MI-induced up-regulation of NGF in the myocardium, as suggested by our previous study can also directly contribute to the post-MI collateral sprouting of sympathetic axons in the SG. An interesting finding not anticipated from that study is the 2 fold increase of pre-ganglionic cholinergic

nerve fiber density in the SG after MI. The co-localization of SYN with ChAT indicates the increased cholinergic nerve fibers are functional presynaptic axons, transforming the SG into a structure highly active in the production of both adrenergic and cholinergic markers. Immunohistochemical studies should be performed in other ganglia to assess whether systemic hyperinnervation occurs.

Sympathetic SG Neural Remodeling and Cardiac Arrhythmia

The mechanisms of sudden death after MI are attributable to multiple different factors, including electrophysiological remodeling, structural remodeling and neural remodeling . Direct recording from the LSG in a canine model of sudden death showed that increased nerve activities are immediate triggers of ventricular arrhythmia, including ventricular tachycardia, fibrillation and sudden cardiac death . While increased LSG nerve activity may not be the only mechanism of ventricular arrhythmia, most (86.3%) of ventricular arrhythmia in that study was triggered by increased nerve discharges from LSG. The increased nerve activity could be due to both increased presynaptic nerves that provides more neural inputs to the ganglion cells, and also due to increased postganglionic axons that directly innervate the myocardium. Previous studies have shown that there is a positive correlation between myocardial nerve sprouting and a clinical history of ventricular arrhythmia of transplant recipients , suggesting that increased myocardial innervation is indeed an important factor contributing to ventricular arrhythmogenesis. In canine models, both NGF infusion into the LSG and chronic subthreshold electrical stimulation of the LSG can cause both LSG and myocardial nerve sprouting, and increase the incidence of ventricular arrhythmia and sudden death in ambulatory dogs . Taken together, significant SG remodeling after MI as demonstrated in the present study may contribute significantly to the increased risk of sudden death in patients with MI. However, further studies are required to explore the association between SG nerve sprouting and clinical adverse events.

Limitations

Animal models might be different from the clinical setting. Coronary artery disease in humans is often associated with the development of collateral coronary circulation, and repetitive ischemic insults can potentially have a protective effect. Therefore, our results cannot be interpreted as being representative of the full human clinical scenario. It is possible that NGF and the nerve densities change non-linearly after MI. Therefore, sampling at one week and one month does not necessarily reflect the changes that might occur between one week and one month, and may not be used to project the changes that occur after one month. We did not perform electrical recordings of the stellate ganglion or assess catecholamine serum levels. More studies are necessary to correlate the histopathological findings with autonomic nerve discharges and serum catecholamine levels to gain further insights into the neural mechanisms of cardiac arrhythmia.

Clinical Implications

The autonomic innervation of the heart is important in the genesis of cardiac arrhythmias. Spontaneous initiation of ventricular arrhythmias depends on the presence of both autonomic substrates and triggers. Coupling newly generated sympathetic cardiac and SG nerve fibers with increased cardiac sympathetic tone, in addition to sympathovagal imbalance, and myocardial electrical remodeling, likely contribute to the onset of cardiac arrhythmias. The results of our study partially explain the clinical observations of the efficacy of stellectomy in preventing sudden death after MI. Beta-blockers are known to reduce mortality after MI. Reduction of sympathetic outflow by modification of SG nerve sprouting may provide additional survival benefits to patients who survive acute MI.

Conclusions

MI-induced NGF-mediated neural remodeling involves both SG, with the transformation of the SG into heterogeneous sympathetic and parasympathetic nervous structures with different timing and distribution. These asymmetric and heterogeneous structures might account for the sympathovagal imbalance that predisposes to ventricular arrhythmia. Therapeutic targets to abolish an unbalanced cross-talk between SG and infarcted myocardium in a critical time-frame might prevent sympathetic neural remodeling, and sudden death.

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Summary

We assessed neural remodeling in the stellate ganglia (SGs) after myocardial infarction (MI) in rabbits. MI results in increased serum nerve growth factor levels after 1 week and 1 month, as compared to controls ($p < .05$). Immunohistochemistry demonstrated SGs nerve sprouting 1 week and 1 month after MI, as compared to controls ($p < .05$), involving postganglionic adrenergic and preganglionic cholinergic axons.

Figure 1.
Examples of TH/ChAT double immunofluorescent staining in the stellate ganglia after MI. A and D show TH immunoreactivity (red stain); B and E show ChAT immunoreactivity (green stain); C and F show overlapped images of A and B, and D and E, respectively of TH and ChAT immunoreactivity. A, B, and C show magnification of objective lens x40; D, E, and F show magnification of objective lens x20.

Figure 2.

Examples of nerve sprouting occurring within 1 week following MI (A, C, E, and G) in the stellate ganglia (brown stain, black arrows) compared to controls (B, D, F, and H). A and B show GAP43 immunoreactivity; C and D show SYN immunoreactivity; E and F show TH immunoreactivity; G and H show ChAT immunoreactivity (x400).

Figure 3. Combined LSG and RSG nerve densities in controls, at 1 week, and at 1 month after MI. * indicates statistically significant difference.

Figure 4.
Differential LSG and RSG nerve densities in controls, at 1 week, and at 1 month after MI.

Figure 5.
Examples of SYN/TH and SYN/ChAT double immunofluorescent staining in the stellate ganglia after MI. A shows TH immunoreactivity (green stain); B shows SYN immunoreactivity (red stain); C shows overlapped images of A and B of SYN/TH immunoreactivity (yellow). D shows ChAT immunoreactivity (green stain); E shows SYN immunoreactivity (red stain); F shows overlapped images of D and E of SYN/ChAT immunoreactivity (yellow). The slides show magnification of objective lens x40.