Local β-Adrenergic Stimulation Overcomes Source-Sink Mismatch to Generate Focal Arrhythmia

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Rationale: β-Adrenergic receptor stimulation produces sarcoplasmic reticulum Ca\(^{2+}\) overload and delayed afterdepolarizations in isolated ventricular myocytes. How delayed afterdepolarizations are synchronized to overcome the source-sink mismatch and produce focal arrhythmia in the intact heart remains unknown.

Objective: To determine whether local β-adrenergic receptor stimulation produces spatiotemporal synchronization of delayed afterdepolarizations and to examine the effects of tissue geometry and cell-cell coupling on the induction of focal arrhythmia.

Methods and Results: Simultaneous optical mapping of transmembrane potential and Ca\(^{2+}\) transients was performed in normal rabbit hearts during subepicardial injections (50 μL) of norepinephrine (NE) or control (normal Tyrode’s solution). Local NE produced premature ventricular complexes (PVCs) from the injection site that were dose-dependent (low-dose [30–60 μmol/L], 0.45±0.62 PVCs per injection; high-dose [125–250 μmol/L], 1.33±1.46 PVCs per injection; *P*<0.0001) and were inhibited by propranolol. NE-induced PVCs exhibited abnormal voltage–Ca\(^{2+}\) delay at the initiation site and were inhibited by either sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase inhibition or reduced perfusate [Ca\(^{2+}\)], which indicates a Ca\(^{2+}\)-mediated mechanism. NE-induced PVCs were more common at right ventricular than at left ventricular sites (1.48±1.50 versus 0.55±0.89, *P*<0.01), and this was unchanged after chemical ablation of endocardial Purkinje fibers, which suggests that source-sink interactions may contribute to the greater propensity to right ventricular PVCs. Partial gap junction uncoupling with carbenoxolone (25 μmol/L) increased focal activity (2.18±1.43 versus 1.33±1.46 PVCs per injection, *P*<0.05), which further supports source-sink balance as a critical mediator of Ca\(^{2+}\)-induced PVCs.

Conclusions: These data provide the first experimental demonstration that localized β-adrenergic receptor stimulation produces spatiotemporal synchronization of sarcoplasmic reticulum Ca\(^{2+}\) overload and release in the intact heart and highlight the critical nature of source-sink balance in initiating focal arrhythmias. (Circ Res. 2012;110:1454-1464.)

Key Words: arrhythmia ■ mapping ■ norepinephrine ■ sarcoplasmic reticulum
heart. Experimental evidence suggests that heterogeneous β-AR stimulation can occur in HF, because sympathetic nerve sprouting and remodeling, regional hyperinnervation, and differential release of the neurotransmitter norepinephrine (NE) have all been identified. Activation of β-AR by NE increases SR Ca<sup>2+</sup> load and thus increases the likelihood of spontaneous SR Ca<sup>2+</sup> release. Therefore, localized β-AR stimulation may produce spatial synchronization of SR Ca<sup>2+</sup> overload among neighboring myocytes. Temporal synchronization of the NE-induced SR Ca<sup>2+</sup> release is dictated by the timing of the preceding action potential (AP), Ca<sup>2+</sup> transient (CaT), and recovery from refractoriness of ryanodine receptors (RyR). This combined spatiotemporal synchronization of SR Ca<sup>2+</sup> overload and release is critical for producing a tissue-level DAD in the intact heart. If the DAD magnitude and local depolarizing current are large enough, the source-sink mismatch will be overcome and focal arrhythmia initiated. We further hypothesized that because of the critical nature of source-sink interactions in this scenario, tissue geometry and the degree of cell-cell coupling would contribute to focal arrhythmia propensity in response to localized β-AR stimulation. To test these hypotheses, simultaneous optical mapping of transmembrane potential (Vm) and CaT was performed during local subepicardial application of NE in normal rabbit hearts. The propensity of localized NE to initiate focal arrhythmia was quantified, and the effects of tissue geometry and cell-cell coupling on the inducibility of PVCs were examined.

### Methods

An expanded Methods section is provided in the online-only Data Supplement.

All procedures involving animals were approved by the Animal Care and Use Committee of the University of California, Davis, and adhered to the National Institutes of Health’s “Guide for the Care and Use of Laboratory Animals.” Male New Zealand White rabbits (n=29) were anesthetized with an intravenous injection of pentobarbital sodium (50 mg/kg). Hearts were excised, Langendorff-perfused at 37°C, and loaded with RH237 and Rhod-2AM (Molecular Probes, Eugene, OR) for simultaneous fluorescent imaging of Vm and intracellular Ca<sup>2+</sup>. An ECG was recorded continuously, and pacing was from the basal left ventricle (LV). Blebbistatin (Tocris Bioscience, Ellisville, MO; 10–20 μmol/L) was added to produce partial gap junction (GJ) uncoupling. A further 14 hearts were used to examine the effect of β-AR antagonist propranolol (5–10 μmol/L, n=3), low perfusate [Ca<sup>2+</sup>]<sup>-</sup> (0.33 mmol/L, n=3), the SR Ca<sup>2+</sup>-ATPase (SERCA2a) inhibitor cyclopiazonic acid (CPA; 30 μmol/L, n=2), and ablation of the RV endocardium with Lugol’s solution (n=3) on the occurrence of NE-induced PVCs. Local caffeine injections (10–40 mmol/L, 50 μL, n=4) and global perfusion of isoproterenol (1 μmol/L, n=4) were also studied.

Data analysis was performed with 2 analysis programs (BV Analyze, Brainvision, Tokyo, Japan; and Optiq, Cairn Research, Faversham, UK). Activation time was determined as 50% rise time. For APs, repolarization time at 80% return to baseline was used to calculate action potential duration (APD<sub>80</sub>). For CaTs, duration was measured at 50% (CaT<sub>50</sub>), and the time course of decay was quantified with the time constant (τ) of a single exponential fit of the decline (30%–100%). Vm<sub>act</sub> activation time was subtracted from Ca<sup>2+</sup> activation time to produce maps of Vm<sub>act</sub>-Ca<sup>2+</sup> delay. Phase plots of the Vm<sub>act</sub>/Ca<sup>2+</sup> relationship were generated by plotting the normalized Vm<sub>act</sub> values (x-axis) against the normalized Ca<sup>2+</sup> values (y-axis) for the time course of a single AP, where counterclockwise chirality indicates normal Vm<sub>act</sub>-Ca<sup>2+</sup> coupling and clockwise chirality indicates abnormal Vm<sub>act</sub>-Ca<sup>2+</sup> coupling. Conduction velocity (CV) was calculated as in Bayly et al. Continuous variables are presented as mean±SD. Comparisons between 2 groups of continuous data were made with Student t test, paired where appropriate, and categorical data with a Fisher exact test. Multiple comparisons were made with 1- or 2-way ANOVA with Bonferroni posttest. P<0.05 was considered statistically significant.

### Results

Local NE Application Induces Focal Arrhythmia

Baseline electrophysiological and Ca<sup>2+</sup>-handling parameters before injection and after complete washout of NE are detailed in the online-only Data Supplement and in Online Figure I. Local NE application resulted in PVCs in 15 of 15 hearts, whereas control injections (NT) produced PVCs in 4 of 13 hearts (P<0.001). Examples of focal arrhythmia induced by local NE application are shown in Figure 1A. PVCs were characterized by a broad QRS complex and ventricular-atrial activation sequence and arose from the injection site. Summary data are shown in Figure 1B. A range of NE doses were examined before the data were grouped into high- and
low-dose groups for statistical analysis (Online Figure II). High-dose NE produced significantly more PVCs than NT (NE-high 1.33±1.46 versus NT 0.17±0.43 PVCs per injection, \(P<0.0001\); Figure 1Bi), and a significantly higher proportion of NE applications resulted in ≥1 PVC (NE-high 25/40 [63%] versus NT 8/48 [17%], \(P<0.001\); Figure 1Bi). Low-dose NE gave intermediate responses (NE-low 0.45±0.62 versus NE-high 1.33±1.46 PVCs per injection, \(P<0.01\), Figure 1Bi; NE-low 12/31 [39%] versus NE-high 25/40 [63%], \(P=0.058\), Figure 1Bi).

Because NE stimulates both \(\alpha\)-AR and \(\beta\)-AR, the \(\beta\)-AR antagonist propranolol (5–10 \(\mu\)mol/L, \(n=3\)) was added to the perfusate (Figure 1C). Propranolol reduced the number of PVCs per injection (NE-high 1.00±0.95; with 5 \(\mu\)mol/L propranolol 0.32±0.48; with 10 \(\mu\)mol/L propranolol 0.06±0.25; \(P<0.001\)) and PVC occurrence (NE-high 62%; with 5 \(\mu\)mol/L propranolol 32%; with 10 \(\mu\)mol/L propranolol 6%; \(P<0.05\)) in a dose-dependent manner, which suggests that PVCs were caused by \(\beta\)-AR rather than \(\alpha\)-AR stimulation. The propensity to NT-induced PVCs was unchanged with propranolol (25% versus 21%, \(P=NS\)).

**Local NE-Induced PVCs Are Ca\(^{2+}\)-Mediated**

To investigate whether focal activity was caused by local SR Ca\(^{2+}\) release, maps of \(V_m\)-Ca\(^{2+}\) delay were constructed. Spatial patterns of \(V_m\)-Ca\(^{2+}\) delay during NE-induced PVCs were compared with sinus rhythm, ventricular pacing, and NT-induced PVCs, as shown in Figure 2. Mean epicardial \(V_m\)-Ca\(^{2+}\) delay was similar during sinus rhythm (7.04±0.94 ms), ventricular pacing (7.02±0.95 ms), and NT-induced PVCs (7.39±1.14 ms, \(P=0.65\)). The presence of local NE did not shorten \(V_m\)-Ca\(^{2+}\) delay during sinus rhythm (Online Figure IIIA), nor did \(V_m\)-Ca\(^{2+}\) delay exhibit restitution during shortening of coupling interval (Online Figure IIIB). There were no significant differences in \(V_m\) rise times for sinus rhythm (14.1±4.1 ms), pacing (18.4±4.0 ms), NT-induced PVCs (17.0±2.6 ms), or NE-induced PVCs (17.9±2.2 ms).

During normal excitation-contraction coupling, Ca\(^{2+}\) activation followed \(V_m\) with a relatively uniform delay across the epicardial surface (Figure 2Bi–iii); however, during the majority of NE-induced PVCs, areas of abnormally short \(V_m\)-Ca\(^{2+}\) delay were evident at the site of earliest \(V_m\) activation (Figure 2Biv).

PVCs were caused by local SR Ca\(^{2+}\) release in isolated myocytes,24 in which caffeine (10 \(mmol/L\)) was used to rapidly activate RyR opening and changes in \(V_m\) occurred instantaneously on SR Ca\(^{2+}\) release. Because subsarcolemmal Ca\(^{2+}\) instantaneously drives inward Na\(^{+}\)/Ca\(^{2+}\) exchange current (NCX), \(V_m\)/Ca\(^{2+}\) upstrokes are simultaneous.24 To test whether the same phenomenon occurs in the intact heart, local injections of caffeine were applied (Figures 3A through 3C). Local caffeine-induced PVCs displayed simultaneous \(V_m\)/Ca\(^{2+}\) upstrokes, exactly as observed during NE-induced PVCs. Corresponding negative control experiments were also performed in which the probability of SR Ca\(^{2+}\) overload and release was reduced, either by lowering perfusate [Ca\(^{2+}\)] (0.33 mmol/L) or by administration of the SERCA2a inhibitor CPA (30 \(\mu\)mol/L). As shown in Figure 3D, in the presence of low [Ca\(^{2+}\)], NE-induced PVCs were reduced (0.83±0.70 versus 20±0.46 PVCs per application, \(P<0.0001\); 67% versus 16%, \(P<0.001\)).
Partial SERCA inhibition with CPA (~50% prolongation of $\tau_\text{r}$; data not shown) also reduced NE-induced PVCs (1.21±0.80 versus 0.42±0.66 PVCs per application, $P<0.001$; 87% versus 50%, $P<0.05$; Figure 3E). In contrast, the occurrence of NT-induced PVCs was not changed by either intervention.

Subcellular imaging has shown that spontaneous SR Ca$^{2+}$ release events occur at characteristic times after the prior beat, depending on the recovery of SR Ca$^{2+}$ content, SR Ca$^{2+}$ load, and RyR refractoriness,$^{18,19}$ with a peak at 300 to 500 ms.$^{25}$ To assess whether this same temporal synchronization was evident for NE-induced PVCs, we measured the coupling interval for each PVC with respect to the previous beat (Online Figure IIIC). The latencies of NE-induced PVCs clustered at coupling intervals of 300 to 400 ms, consistent with a temporal synchronizing effect of RyR restitution.

When PVCs were not elicited by NE, subthreshold Ca$^{2+}$ elevation could be observed near the injection site (Online Figure IV). This suggests that local NE application resulted in SR Ca$^{2+}$ release from a group of cells, but in these cases was not large enough to initiate a PVC. Taken together, these data strongly imply that focal activity during localized $\beta$-AR stimulation is mediated by local and relatively synchronous diastolic SR Ca$^{2+}$ release.

**RV Sites Are More Prone to NE-Induced PVCs**

To determine whether differences in tissue geometry or electrophysiological characteristics may play a role in the genesis of focal arrhythmia, PVC inducibility was compared across different sites. There were no significant differences in PVC inducibility between apex and base (NE-high apex 1.29±1.54 versus NE-high base 1.35±1.47 PVCs per injection, $P=\text{NS}$; NE-high apex 64% versus NE-high base 62%, $P=\text{NS}$). In contrast, local NE in the RV produced more PVCs than in the LV (NE-high, RV 1.94±1.66 versus LV 0.82±1.10 PVCs per injection, $P<0.001$; Figure 4A), and a higher proportion of NE injections resulted in at least 1 PVC (81% versus 50%, $P<0.05$). Similar RV/LV trends were observed for low-dose NE. To determine whether the differences in PVC inducibility between LV and RV were caused by underlying electrophysiological heterogeneity, AP and CaT parameters were compared. There were no differences in CV, AP rise time, $\text{APD}_{80}$, dispersion of $\text{APD}_{90}$, or CaT...
dynamics between LV and RV (Table 1). To determine whether functional heterogeneity in the response to β-AR stimulation may exist between the ventricles, 1 μmol/L isoproterenol was added globally. As shown in Table 1, there were no differences between LV and RV in the response to global β-AR stimulation with isoproterenol.

Differences in tissue structure and local vasculature may result in nonuniform NE diffusion within tissue and may contribute to the regional differences in PVC propensity. To address this, the fluorophore R6G was added to NE to visualize the area of myocardium exposed. Figure 4B shows Vm activation time and R6G fluorescence after coinjection of NE and R6G. PVCs arose from the injection site, and R6G fluorescence indicated the spatial extent of NE exposure. The area of epicardial tissue exposed to NE was correlated with PVC inducibility (Figure 4C). Both LV and RV injection sites displayed a similar increase in PVC probability with increasing NE exposure area (regression slopes: LV, 0.0021 ± 0.0008, P < 0.05 and RV, 0.0025 ± 0.0005, P < 0.05; LV versus RV, P = NS). However, for RV sites, PVC probability was higher across the range of exposure areas measured (y-axis intercepts: LV, 0.03 ± 0.19 versus RV, 0.33 ± 0.12, P < 0.05). Transmural histological sections at the injection site were then imaged to determine whether the depth of NE exposure could account for the difference in PVC inducibility. Figure 4D shows LV and RV injection sites with similar epicardial areas of R6G fluorescence. In the LV, the R6G fluorescence extended to the midmyocardium, whereas in the RV, the R6G exposure was fully transmural. These differences in exposure may result in less source-sink mismatch in the RV than in the LV (approximately 2-dimensional versus 3-dimensional) and therefore higher PVC probability.

To investigate whether transmural NE exposure in the RV was activating endocardial Purkinje fibers to initiate focal activity, NE injections were repeated before and after RV endocardial chemical ablation with Lugol’s solution. As shown in Figure 5A, Lugol’s solution produced QRS prolongation and delayed RV activation during sinus rhythm, consistent with disruption of the Purkinje network, which was subsequently confirmed with triphenyltetrazolium chloride staining. The occurrence of NE-induced PVCs in the RV was not affected by chemical ablation of the endocardium (1.0 ± 0.6 versus 1.1 ± 0.6 PVCs per injection, P = NS; 85% versus 85%, P = NS; Figure 5B), which suggests that the Purkinje fibers do not play a large role in initiating focal activity during local β-AR stimulation.

Partial GJ Uncoupling Promotes NE-Induced PVCs

To further investigate the role of source-sink interactions, NE application was repeated in the presence of CBX to induce partial GJ uncoupling. Twenty-five micromoles of CBX per liter resulted in an ≈25% decrease in CV in both the longitudinal (CV_L) and transverse (CV_T) directions (CV_L, 58.7 ± 4.2 versus 38.5 ± 3.1 cm/s, P < 0.001; CV_T, 43.9 ± 3.2 versus 28.2 ± 3.1 cm/s, P < 0.001; Online Figure V). The addition of CBX did not affect CaTD50 (140 ± 9 versus 141 ± 10 ms, P = 0.76) or Vm-Ca2+ delay during sinus rhythm.

![Positive and negative control experiments](http://circres.ahajournals.org/}

**Figure 3. Positive and negative control experiments.** **Left,** Positive control with local caffeine injections. Maps of transmembrane potential (Vm) activation time (A) and Vm-Ca2+ delay (B) during (i) norepinephrine (NE)-induced premature ventricular complex (PVC) and (ii) caffeine-induced PVC (40 mmol/L). C, Vm and Ca2+ upstrokes from the earliest activated sites, showing near-simultaneous upstrokes in both cases. **Right,** Negative control experiments with reduced sarcoplasmic reticulum Ca2+ load. **D**, PVC occurrence after reducing perfusate [Ca2+] to 0.33 mmol/L. **E**, PVC occurrence after 30 μmol/L cyclopiazonic acid (CPA) to partially inhibit sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA2a) activity. NE-CON indicates control norepinephrine experiment. *P < 0.05, ****P < 0.0001.
(7.53±1.41 versus 7.04±0.94 ms, P=0.47) or ventricular pacing (7.12±1.58 versus 7.02±0.95 ms, P=0.89). With CBX, greater numbers of PVCs were induced by both low-dose NE (CBX 1.48±1.33 versus control 0.45±0.62 PVCs per injection, P<0.01) and high-dose NE (CBX 2.18±1.43 versus control 1.33±1.46 PVCs per injection, P<0.05), and a higher proportion of NE injections resulted in ≥1 PVC (NE-low 76% versus 39%, P<0.05; NE-high 88% versus 63%, P=0.06; Figure 6).

Source-Sink Interactions Mediate the Production of NE-Induced PVCs

To determine the mechanism of increased propensity to focal arrhythmia during partial GJ uncoupling, the changes in properties of late phase CaT were measured (Figure 7). PVC occurrence was plotted as a function of distance from the earliest Vm-Ca2+ dynamics were examined (Figure 7). β-AR activation produced more rapid SR Ca2+ uptake and thus faster CaT decline. Shortening of CaT50 around the injection site was observed during NE- but not NT-induced PVCs (Figure 7B). This area of abbreviated CaT50 can be used as a functional measure of the spatial extent of NE effects; however, because of electrotonic coupling, effects on Vm may not be seen across the same area. Thus, the area of abnormal Vm-Ca2+ delay was used as a functional estimate of the spatial extent over which Ca2+ release exerted an effect on Vm (Figure 7C).

To quantify these parameters between hearts, normalized CaT50 and Vm-Ca2+ delay during NT- and NE-induced PVCs were plotted as a function of distance from the earliest Vm activation (Figures 7D and 7E, respectively [longitudinal direction only; transverse profiles shown in Online Figure VII]). During NE-induced PVCs, CaT50 was significantly shorter over the first 4 mm, and this was unchanged with CBX (Figure 7D). Vm-Ca2+ delay was shorter over the first 4 mm under control conditions, and this increased to 7 mm with CBX (Figure 7E). Thus, after uncoupling, a larger effect on Vm was observed for the same NE effect on CaT50. This is consistent with an increased propensity to Ca2+-mediated focal arrhythmia induced by NE during partial GJ uncoupling.

**Discussion**

The present study tested the hypothesis that in the intact heart, localized β-AR stimulation can provide spatiotemporal synchronization of SR Ca2+ release over many cells to overcome source-sink mismatch and produce focal arrhythmia. The results demonstrate that local NE application induces β-AR dependent, Ca2+-mediated focal arrhythmia in normal rabbit.
hearts. Quantification of Vm-Ca$^{2+}$ dynamics established the mechanism of PVC induction. Abnormal Vm-Ca$^{2+}$ delays were exclusively a feature of NE- and caffeine-induced PVCs, which suggests that focal arrhythmias induced by localized β-AR stimulation are mediated by SR Ca$^{2+}$ overload and release. RV sites displayed a higher propensity to focal arrhythmia than LV sites. This difference could not be explained by electrophysiological differences or activation of Purkinje fibers and was thus likely caused by the reduction in source-sink mismatch in the thinner RV wall, which more closely approximates a 2-dimensional geometry. Consistent with this finding, partial GJ uncoupling resulted in a reduction of the current sink and was associated with a higher propensity for focal arrhythmia in response to localized β-AR stimulation.

**Short Vm-Ca$^{2+}$ Activation Delay Is Indicative of Ca$^{2+}$-Mediated Focal Activity**

During normal excitation-contraction coupling, Vm-Ca$^{2+}$ delay was ≈7–8 ms, similar to that reported in rabbits and humans. In contrast, during NE-induced PVCs, significantly shorter Vm-Ca$^{2+}$ delays (≈0–4 ms) were observed at the initiation site. Such short Vm-Ca$^{2+}$ delays were never observed during normal excitation-contraction coupling, which indicates that during NE-induced PVCs, SR Ca$^{2+}$ release likely started before the AP upstroke and opening of L-type Ca$^{2+}$ channels. We tested the possibility that local NE application might accelerate Ca$^{2+}$ release dynamics and abbreviate the Vm-Ca$^{2+}$ delay during normal excitation-contraction coupling. However, localized NE did not alter Vm-Ca$^{2+}$ delay during sinus beats (Online Figure IIIA). Vm-Ca$^{2+}$ delay was also unaltered over a broad range of coupling intervals, which rules out excitation-contraction coupling restitution kinetics as a cause of the abnormally short Vm-Ca$^{2+}$ delay during NE-induced PVCs (Online Figure IIIB). The observations that partial SERCA inhibition with CPA and low [Ca$^{2+}$] perfusate reduced NE- but not NT-induced PVCs (Figures 3D and 3E) and local intracellular

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**Figure 5.** A, Right ventricular (RV) endocardial ablation with Lugol’s solution. (i) ECG, (ii) transmembrane potential (Vm) activation map, and (iii) endocardial triphenyltetrazolium chloride (TTC) staining before (CON) and after (+ Lugol’s) RV endocardial ablation with Lugol’s. Purkinje ablation resulted in QRS prolongation and delayed RV epicardial activation. Tissue ablation was confirmed by the white appearance on TTC. B. Occurrence of PVCs was not affected by endocardial ablation. LV indicates left ventricle.

**Figure 6.** Number of premature ventricular complexes (PVCs) per injection (A) and proportion of injections resulting in ≥1 PVC (B) for low-dose (gray) and high-dose (black) norepinephrine (NE) under control conditions (solid bars) and with carbenoxolone (CBX; hatched bars). *P<0.05 vs control, **P<0.01 vs control.
Ca\textsuperscript{2+} rises during subthreshold NE application (Online Figure IV) all support the conclusion that NE-induced PVCs are Ca\textsuperscript{2+}-mediated. Because short V\textsubscript{m}-Ca\textsuperscript{2+} delays were only observed during NE- and caffeine-induced PVCs, these data suggest that very short V\textsubscript{m}-Ca\textsuperscript{2+} activation delays can be used as an indicator of Ca\textsuperscript{2+}-mediated excitation.

In optical mapping studies of pharmacological QT prolongation\textsuperscript{22} and postshock arrhythmias,\textsuperscript{27} Ca\textsuperscript{2+} upstrokes that precede V\textsubscript{m} upstrokes or slow diastolic Ca\textsuperscript{2+} elevation before V\textsubscript{m} upstrokes have been reported. These models of Ca\textsuperscript{2+}-mediated arrhythmia are characterized by “global” SR Ca\textsuperscript{2+} overload and release, which results in gradual and spatially dyssynchronous Ca\textsuperscript{2+} elevation. In the present experiments, we observed a different phenomenon in that we saw rapid Ca\textsuperscript{2+} release in response to local NE. During NE-induced PVCs, Ca\textsuperscript{2+} and V\textsubscript{m} upstrokes occurred nearly simultaneously at the site of earliest activation. This is highly analogous to rapid caffeine application in isolated cardiomyocytes, in which synchronous SR Ca\textsuperscript{2+} release immediately activates NCX and depolarization occurs simultaneously with the Ca\textsuperscript{2+} rise.\textsuperscript{24} Indeed, local application of caffeine in the intact heart recapitulated these results (Figures 3A through 3C). Thus, although the simultaneous V\textsubscript{m}/Ca\textsuperscript{2+} upstrokes reported here differ from those previously reported for Ca\textsuperscript{2+}-mediated arrhythmia,\textsuperscript{22,27} this is likely attributable to the “local” synchronous SR Ca\textsuperscript{2+} release produced in the present experiments as opposed to “global” dyssynchronous Ca\textsuperscript{2+} release observed in other models.

Induction of a PVC requires the temporal and spatial synchrony of SR Ca\textsuperscript{2+} release. We hypothesized that this temporal synchronization is dictated by the previous AP/Ca\textsuperscript{T} because of the time required for SR Ca\textsuperscript{2+} refilling and RyR restitution.\textsuperscript{18,19} Indeed, in Ca\textsuperscript{2+} overload conditions, there is an increased amplitude, frequency, and synchrony of Ca\textsuperscript{2+} waves.\textsuperscript{25} These factors increase the likelihood that neighboring Ca\textsuperscript{2+} overloaded myocytes will exhibit SR Ca\textsuperscript{2+} release in relative synchrony. We observed a clustering of NE-induced PVCs that occurred 300 to 400 ms after the previous beat (Online Figure IIIC). In contrast, although the number of NT-induced PVCs was small, they appeared to show random coupling intervals. These data are consistent with a temporal synchronization of SR Ca\textsuperscript{2+} release in response to local NE through SR Ca\textsuperscript{2+} refilling and RyR restitution.

**Regional Heterogeneity in Vulnerability to Focal Arrhythmia**

Local β-AR stimulation was more likely to produce PVCs at RV than at LV sites. The finding of increased propensity to PVCs in the RV is congruent with clinical observations. Idiopathic ventricular tachycardia/ventricular fibrillation is...
often found to be triggered by PVCs that arise from a focal RV origin, the ablation of which has been shown to reduce subsequent ventricular tachycardia/ventricular fibrillation.\(^{28}\) Although PVC inducibility was related to the area of epicardial NE exposure, as assessed by coinjection of NE with fluorescent R6G (Figure 4C), differences in the area of NE exposure did not account for higher arrhythmia propensity in the RV. The mechanisms underlying the increased arrhythmogenic potential of the RV are not well understood. If differences in sympathetic drive exist, then this may play an important role. Differences in \(I_{K1}\) may influence the dominant frequency of ventricular fibrillation in guinea pig hearts.\(^{29}\) Although we did not detect significant interventricular electrophysiological or \(Ca^{2+}\) handling differences at baseline or during global \(\beta\)-AR stimulation (Table), it is possible that differences in specific channels, transporters (eg, \(I_{K1}\), NCX, RyR), or \(\beta\)-ARs may contribute to this RV-LV difference. Various degrees of interventricular heterogeneity in ion channel expression have been reported in different species,\(^{29,30}\) but optical mapping studies in intact rabbit hearts are congruent with our observations that no significant differences in overall electrophysiology exist between RV and LV at baseline.\(^{31}\)

Another possible explanation for the RV-LV difference in PVC propensity may be that transmural NE exposure in the RV results in stimulation of the Purkinje network. Because Purkinje fibers may be more prone to focal activity,\(^ {27}\) activation with NE may result in greater PVC incidence in the RV. To test for this, we performed chemical ablation of the RV endocardial surface with Lugol’s solution and observed no significant difference in PVC propensity (Figure 5), which suggests that the higher incidence of PVCs in the RV is likely not attributable to Purkinje activation.

**Tissue Geometry and the Source-Sink Balance**

Another logical contributor to the observed RV-LV differences is inherent geometric source-sink balance. As illustrated in Figure 4D, for the same depth of NE exposure, the RV will experience 2-dimensional radial current sink around the circumference of the exposed area, whereas in the thicker-walled LV, the current sink is 3-dimensional. The importance of dimensionality of the surrounding current sink has been demonstrated in numeric simulations, which have estimated the number of cells with synchronized \(Ca^{2+}\) release required to produce a PVC.\(^ {12}\) Xie et al\(^ {12}\) calculated that in normal, well-coupled 3-dimensional tissue, \(\approx 820,000\) cells with synchronized \(Ca^{2+}\) release were required to produce a PVC, 100-fold more than were required in the equivalent 2-dimensional model.

In simulations, the total current source can be controlled precisely by changing the number of cells with synchronized \(Ca^{2+}\) release. In the present experiments, however, we could neither exactly measure nor systematically vary the total current source, but we could make estimates from functional measures of \(V_m-Ca^{2+}\) dynamics. One way to estimate the current source is to use the area over which local NE abbreviates CaT duration (ie, the area of tissue affected by NE). With strong electrotonic coupling, however, \(V_m\) is “clamped” at the borders of this region, and thus, the alternative estimate, the area over which \(Sr Ca^{2+}\) release drives depolarization (ie, where \(V_m-Ca^{2+}\) delay is short), may be different. Using the dimensions of these functional measurements, along with anatomic surface and depth estimates from R6G imaging, and applying them to a hemi-ellipse (surface radii 0.5–5 mm and depth of 1–2 mm), the volume of current source estimated in the present study is 0.5 to 105 mm\(^3\). If we assume a cell volume of 30 pl,\(^ {32}\) and 30% extracellular space, this equates to 12 200 to 2 400 000 cells. Although this is a relatively crude estimate, the upper and lower limits are very similar to the estimates derived from the mathematical predictions (2-dimensional tissue \(\approx 8000\) cells and 3-dimensional tissue \(\approx 820,000\) cells).\(^ {12}\)

**The Effect of Partial GJ Uncoupling**

Partial GJ uncoupling with CBX resulted in a slowing of CV with preserved anisotropy ratio and AP duration, as has been reported previously in rabbit ventricular myocardium.\(^ {33}\) Partial GJ uncoupling produced a 2-fold increase in NE-induced PVCs (Figure 6). Partial uncoupling facilitates PVC propagation by “insulating” the depolarized region (source) from the surrounding resting myocardium (sink),\(^ {10}\) analogous to initiation of pacemaker activity in the sinoatrial node.\(^ {34,35}\) In the case of local \(\beta\)-AR stimulation, a reduction in electrotonic current flow through GJ reduces the hyperpolarizing current from surrounding cells, which allows the current carried by NCX in response to \(Sr Ca^{2+}\) release to have a larger effect on \(V_m\). This is demonstrated by the significantly larger area of abnormally short \(Sm Ca^{2+}\) delay observed after uncoupling (Figure 7E) despite a similar area of NE effect (Figure 7D). Given that the area of NE effect is not changed, the larger area of abnormal \(Sm Ca^{2+}\) delay with CBX likely reflects poorer voltage clamp at the periphery, caused by reduced coupling of the depolarizing source to the surrounding sink.

**Local \(\beta\)-AR Stimulation as a Pathophysiological Experimental Paradigm**

In the present study, we examined how localized \(\beta\)-AR stimulation produces spatiotemporal synchronization of \(Sr Ca^{2+}\) overload and release across many cells to initiate focal arrhythmia. The data demonstrate that local NE induces \(Ca^{2+}\)-mediated PVCs in intact hearts, which provides a critical link from cellular studies of focal arrhythmia initiation via DADs to the tissue level. Intrasympathetic NE concentrations at vascular nerve terminals have been measured at up to 100 \(\mu\)mol/L, and an inverse relationship between intrasympathetic NE concentration and junctional space was observed.\(^ {36}\) Because cardiac neuroeffector junctions are narrower, the NE concentrations used in the present study (30–250 \(\mu\)mol/L) may be similar to NE concentrations during physiological nerve activation, especially in HF; when sympathetic drive is high. However, sympathetic stimulation does not typically result in PVCs in the normal rabbit or human heart, and no exogenous NE application can fully recapitulate neuronal NE release.

Regionally heterogeneous sympathetic nerve sprouting, nerve remodeling, hyperinnervation,\(^ {13,–15}\) and heterogeneous NE release\(^ {17}\) all occur in HF. Focal arrhythmias are known to occur in animals and humans with HF caused by dilated cardiomyopathy.\(^ {6,7}\) Moreover, GJ uncoupling is a consistent
feature of the electrophysiological phenotype in failing hearts.\textsuperscript{37,38} Therefore, subepicardial NE injection is a useful model of the heterogeneous $\beta$-AR stimulation that occurs in HF, and localized $\beta$-AR stimulation in the presence of GJ uncoupling represents a pathophysiologically relevant paradigm of focal arrhythmia induction. This is the first experimental study to determine a mechanistic link between altered local sympathetic stimulation and the generation of focal arrhythmias in the intact heart. Future studies should examine whether other features of HF remodeling also potentiate focal arrhythmias induced by local $\beta$-AR stimulation.

Study Limitations

A small number of PVCs were induced by NT, likely because of activation of stretch-activated channels.\textsuperscript{39} As demonstrated in Figures 2 and 7, these were clearly distinguishable from NE-induced PVCs on the basis of $V_m$-Ca\textsuperscript{2+} delay. R6G was used to indicate the area of NE exposure, and because the 2 have similar molecular weight (319 versus 479 kDa), we expect that the diffusion kinetics in tissue may be similar. However, R6G\textsuperscript{40} is more lipophilic\textsuperscript{41} and is not taken up by nerve terminals; thus, it does not demonstrate the time course of NE clearance from the tissue. There was variability in the area of tissue exposed to NE despite constant injection volume. The different patterns of R6G staining, both on the epicardium and transmurally, suggest this may be caused by differences in local tissue architecture and microvasculature. Additional effects of $\beta$-AR stimulation (eg, a leftward shift in Ca\textsuperscript{2+} current voltage-dependence, altered RyR properties) may further promote DADs and the development of focal arrhythmias, and these issues merit further study. In the present study, we did not determine the exact delay from NE injection to PVC appearance, in part because in dual $V_m$-Ca\textsuperscript{2+} imaging, we could only record for several seconds. However, the first PVC typically occurred a few seconds after NE injection (as in Figures 1Ai and 1Aii), which is consistent with the expectation that several beats would be required for $\beta$-AR activation to elevate SR Ca\textsuperscript{2+} load and release. As discussed above, focal epicardial application of NE only recapitulates some aspects of neuronal NE release but does allow control and evaluation of exposed area. In future studies, it would be valuable to assess the detailed time course of NE-induced PVCs and to compare responses to local injections to chemical or electric stimulation of endogenous NE release.

Conclusions

Localized $\beta$-AR stimulation produced spatiotemporal synchronization of SR Ca\textsuperscript{2+} overload and release across many cells to produce focal activity in normal rabbit hearts. Source-sink interactions were found to be critically important in the generation of Ca\textsuperscript{2+}-mediated focal arrhythmias. These data provide (1) the first experimental demonstration of localized $\beta$-AR stimulation as a pathophysiologically relevant mechanism of focal arrhythmia initiation; (2) the first experimental evidence of a mechanistic link between sympathetic dysfunction and the generation of focal arrhythmias in the intact heart; (3) the first experimental estimation of the number of cells with synchronized SR Ca\textsuperscript{2+} release required to initiate focal arrhythmia; and (4) a demonstration of the critical nature of the source-sink balance in the initiation of focal arrhythmias in the intact heart.

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Disclosures

None.

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Novelty and Significance

What Is Known?

- In isolated cardiac myocytes, β-adrenergic stimulation can cause spontaneous Ca\textsuperscript{2+} release from the sarcoplasmic reticulum, which may depolarize the membrane and lead to triggered action potentials.
- In the intact heart, strong electrotonic coupling exists between cells, which means that spontaneous Ca\textsuperscript{2+} release in a single cell cannot produce sufficient change in membrane potential to trigger propagating action potentials and arrhythmia (“source-sink mismatch”).
- For spontaneous Ca\textsuperscript{2+} release to trigger arrhythmias in the intact heart, it must be synchronized across many cells, but little is known about how many are required, or the mechanism of synchrony.

What New Information Does This Article Contribute?

- Localized β-adrenergic stimulation by norepinephrine injection leads to Ca\textsuperscript{2+} -mediated focal arrhythmia in the intact rabbit heart.
- Local β-adrenergic stimulation synchronizes spontaneous Ca\textsuperscript{2+} release from the sarcoplasmic reticulum across thousands of cells, overcoming the source-sink mismatch.
- Source-sink interactions are critically important in the generation of Ca\textsuperscript{2+}-mediated focal arrhythmia.

This study addressed the important disconnect between our understanding of arrhythmia mechanisms in isolated cells and the intact heart. β-Adrenergic receptor stimulation produces Ca\textsuperscript{2+}-mediated arrhythmias in cells, but electrotonic coupling in the intact heart means that spontaneous Ca\textsuperscript{2+} release in 1 cell is insufficient to significantly alter membrane potential (V_{m}). We examined how synchronization of spontaneous Ca\textsuperscript{2+} release might occur in order for the source-sink mismatch to be overcome and focal arrhythmia generation. Using dual optical mapping of membrane potential and Ca\textsuperscript{2+}, we demonstrate that local norepinephrine application induces β-adrenergic receptor–dependent, Ca\textsuperscript{2+}-mediated focal arrhythmia in rabbit hearts. The source-sink balance was crucial to focal arrhythmia induction, which was potentiated when the source-to-sink ratio was increased, either by changes in tissue geometry or by reducing electrotonic conduction through gap junctions (both of which modify the sink). The data also allowed the first experimental estimation of the number of cells required to have synchronous Ca\textsuperscript{2+} release in order to initiate focal arrhythmia. These data provide the first demonstration of localized β-adrenergic receptor stimulation as a mechanism of focal arrhythmia initiation and evidence of a mechanistic link between sympathetic dysfunction and focal arrhythmias in the intact heart.
Local β-Adrenergic Stimulation Overcomes Source-Sink Mismatch to Generate Focal Arrhythmia
Rachel C. Myles, Lianguo Wang, Chaoyi Kang, Donald M. Bers and Crystal M. Ripplinger

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SUPPLEMENTAL MATERIAL

Local beta-adrenergic stimulation overcomes source-sink mismatch to generate focal arrhythmia

Myles – Source-sink interactions and focal arrhythmia

Rachel C. Myles, MBBS., Ph.D., Lianguo Wang, M.D., Chaoyi Kang, B.S.,
Donald M. Bers, Ph.D., and Crystal M. Ripplinger, Ph.D.

Detailed Methods

Langendorff perfused rabbit hearts

All procedures involving animals were approved by the Animal Care and Use Committee of the University of California, Davis and adhered to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. Male New Zealand White rabbits (n=29) weighing 3-4kg were anesthetized with a single intravenous injection of pentobarbital sodium (50mg/kg) containing 1000IU of heparin. Following a midsternal incision, hearts were rapidly excised and Langendorff-perfused at 37°C with oxygenated (95% O₂, 5% CO₂) modified Tyrode’s solution of the following composition (in mmol/L): NaCl 128.2, CaCl₂ 1.3, KCl 4.7, MgCl₂ 1.05, NaH₂PO₄ 1.19, NaHCO₃ 20 and glucose 11.1 (pH 7.4 ± 0.05). Flow rate (25-35mL/min) was adjusted to maintain a perfusion pressure of 60-70mmHg. One leaflet of the mitral valve was carefully damaged with sharp forceps inserted through the pulmonary vein to prevent solution congestion after the suppression of ventricular contraction. This also prevented acidification of the perfusate and the development of ischemia in the left ventricle (LV). Two Ag/AgCl disc electrodes were positioned in the bath to record an electrocardiogram (ECG) analogous to a lead I configuration. Bipolar pacing electrodes were positioned on the base of the LV epicardium
for pacing, which was performed at a basic cycle length (BCL) of 300ms using a 2ms pulse at twice the diastolic threshold.

**Dual optical mapping of $V_m$ and $Ca^{2+}$**

Hearts were loaded with the fluorescent intracellular $Ca^{2+}$ indicator Rhod-2 AM (Molecular Probes, Eugene, OR; 0.5ml of 1mg/ml in dimethyl sulfoxide [DMSO] containing 10% pluronic acid) and were subsequently stained with the voltage-sensitive dye RH237 (Molecular Probes; 50μl of 1mg/ml in DMSO). Blebbistatin (Tocris Bioscience, Ellisville, MO; 10-20μM) was added to the perfusate to eliminate motion artifact during optical recordings. The anterior epicardial surface including the right ventricle (RV) and LV was excited using LED light sources centered at 530nm and bandpass filtered from 511-551nm (LEX-2, SciMedia, Costa Mesa, CA) and focused directly on the surface of the preparation. The emitted fluorescence was collected through a 25mm objective (Navitar, Japan) and split with a dichroic mirror at 630nm. The longer wavelength moiety, containing the $V_m$ signal, was longpass filtered at 700nm and the shorter wavelength moiety, containing the $Ca^{2+}$ signal, was bandpass filtered with a 32nm filter centered at 590nm. The emitted fluorescence signals were then recorded using two CMOS cameras (MiCam Ultima-L, SciMedia, Costa Mesa, CA) with a sampling rate of 1kHz and 100x100 pixels with a 35x35mm field of view.

**Experimental protocol**

Baseline electrophysiological parameters were determined during LV epicardial pacing at a BCL of 300ms. The atria were removed to produce a slow junctional rhythm to facilitate the escape of focal activity between beats. Subepicardial injections were delivered via 30G needles with a 90° bend 1.5mm from the tip to control the depth of injection. Needles were connected to a length of PE-10 tubing attached to a 0.5mL syringe to prevent motion at the needle tip during injections. Injections of normal Tyrodes (NT, 50μL: control) and 50μL NE (either low dose [30-60μM] or high dose [125-250μM]) were delivered during the junctional rhythm at different anatomical locations in each heart (LV base / LV apex / RV base / RV
apex). Following each NE injection, the tissue bath was washed out and refilled with fresh NT to ensure that NE did not accumulate in the perfusate. After preliminary experiments with higher volumes (up to 150μL, data not included in analyses), injections for all protocols were limited to 4 x 50μL injections per site in order to avoid tissue damage due to large volumes of fluid. As shown in Supplemental Figure I, there were no functional differences in electrophysiology or changes in AP or CaT morphology following 4 x 50μL injections and complete washout of NE. We performed NT, low dose NE and high dose NE injections at each site and rotated between multiple injection sites to allow a delay between injections at the same site. Beyond the preliminary experiments we performed to develop the protocol, we did not perform multiple injections of the same dose at the same site. Local NE injections were performed in 15 hearts. In a subset of these hearts (n=8), co-injection of NE and the fluorophore rhodamine-6G (R6G, 50μM, 528/547nm ex/em, Sigma, St. Louis, MO) was performed to visualize the epicardial surface area and transmural depth of tissue exposed to NE. During R6G administration, \( V_m \) signals were recorded simultaneously with R6G fluorescence, which utilized the Ca\(^{2+}\) channel (574-606nm); thus, intracellular Ca\(^{2+}\) was not recorded during this protocol. In another subset (n=8), 25μM carbenoxolone (CBX) was added to the perfusate to produce partial gap junction (GJ) uncoupling, and the local injection protocol was repeated. When assessing the impact of CBX, we used the same injections (NT and either low or high dose NE) at the same sites before and after addition of CBX. A further 14 hearts were used for detailed mechanistic studies. These included examining the effect of the \( \beta \)-AR antagonist propranolol (5-10μM, n=3), low perfusate [Ca\(^{2+}\)] (0.33mM, n=3), the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase inhibitor cyclopiazonic acid (CPA, 30μM, n=2) and ablation of the RV endocardium with Lugol’s solution (n=3) on the occurrence of NE-induced PVCs. Lugol’s solution was flushed through the RV cavity for 30 seconds to chemically ablate the RV Purkinje fibers followed by washout with NT. Success of this procedure was confirmed initially by a lack of RV breakthrough during sinus rhythm, evidence of QRS prolongation, and normal epicardial optical action potentials. At the end of the experiments, triphenyl tetrazolium chloride (TTC, 1%, Sigma, St. Louis, MO) staining
was performed to identify ablated tissue. Local caffeine injections (10-40mM, 50 μL, n=4) and global perfusion of isoproterenol (ISO, 1μM, n=4) were also studied.

**Data analysis and statistics**

Data analysis was performed using two different commercially available analysis programs (BV_Analyze, Brainvision, Tokyo, Japan; and Optiq, Cairn, UK). $V_m$ and $Ca^{2+}$ datasets were spatially aligned and processed with a Gaussian spatial filter (radius 3 pixels). For both APs and CaTs, activation time was determined as the time at 50% between peak and baseline amplitude and rise time as the time from 10-90% of the upstroke. For APs, repolarization time at 80% was determined at 80% of the return to baseline amplitude and action potential duration at 80% ($APD_{80}$) as repolarization time – activation time. $APD_{80}$ dispersion was calculated using the 5-95% range of $APD_{80}$. For CaTs, duration was measured at 50% ($CaTD_{50}$) and the time course of decay was quantified using the time constant ($\tau$) of a single exponential fit of the decay portion of the trace (from 30-100% of the downslope). For comparison of $V_m$ and $Ca^{2+}$ activation times, signals were normalized and activation maps were generated for each. $V_m$ activation time was then subtracted from $Ca^{2+}$ activation time for each pixel to produce maps of $V_m$-$Ca^{2+}$ delay. Phase plots of the $V_m$/$Ca^{2+}$ relationship at specific locations were generated by plotting the normalized $V_m$ values (x-axis) against the normalized $Ca^{2+}$ values (y-axis) for the time course of a single AP, where counterclockwise chirality indicates normal $V_m$-$Ca^{2+}$ coupling, and clockwise chirality indicates $Ca^{2+}$ leading $V_m$. Conduction velocity was calculated using the algorithm described by Bayly et al. Continuous variables are presented as mean±SD. Comparisons between two groups of continuous data were made using a Student’s t-test, paired where appropriate, and categorical data using a Fisher’s exact test. Multiple comparisons were made using one- or two-way analysis of variance (ANOVA) with Bonferroni’s post-testing. P<0.05 was considered statistically significant.
Results

Electrophysiological and Ca\textsuperscript{2+}-handling parameters at baseline and following NE injection/washout

Simultaneous optical APs and CaTs recorded during ventricular pacing are shown in Supplemental Figure I. Mean epicardial APD\textsubscript{80} was 183.2±20.9ms and the mean epicardial CaT decay (\(\tau\)) was 65.9±8.6ms. Mean rise time was 14.7±2.7ms for APs and 20.3±1.8ms for CaTs (p<0.001). The mean V\textsubscript{m}-Ca\textsuperscript{2+} delay was 7.02±0.95ms. As shown in Supplemental Figure I, there were no differences in electrophysiology or changes in AP/CaT morphology at injection sites following complete washout of NE, indicating that these protocols did not produce functional tissue damage.

Temporal and site-specific pattern of NE-induced PVCs

All PVCs occurred directly during/after the injection and although the time from injection to PVC was not recorded, no PVCs were observed following washout of NE from the tissue bath, suggesting that the local effect of NE was short-lived. A small number of sites did not respond to any injections (7/52 sites [13%] in 4/15 hearts), although, due to the restrictions we placed on number of injections per site, not all conditions could be tested at each site (e.g., only 3 of these 7 sites had high dose NE administered, and only one had high dose NE in the presence of CBX – the most arrhythmogenic condition). A few sites (4/52 [8%]) displayed PVCs with all injections, whether NT or NE, and many showed PVCs in response to all doses of NE given (18/52 [35%]). A few sites (3/52 [6%]) gave inconsistent responses. The sites where an initial low dose of NE did not produce PVCs 73% (11/15) subsequently produced PVCs in response to higher doses of NE. Where CBX was added 17/20 (81%) of sites produced PVCs with a dose of NE equal to or lower than that which had failed to produce PVCs under control conditions.
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Supplemental Figure I. A) Epicardial optical data before injections. (i) $V_m$ activation time map during ventricular epicardial pacing at 300ms. The white arrows indicate axes along which conduction velocity (CV) was measured and the boxes indicate the needle positions on the epicardial surface. (ii) Superimposed optical signals sampled from the injection sites, with $V_m$ in black and $Ca^{2+}$ in red. (iii) Map of APD$_{80}$. B) Activation time map (i), optical signals (ii) and APD$_{80}$ map (iii) following 4 x 50μl injections (including NT and NE) at each site, following complete washout of NE. There were no changes in $V_m$ or $Ca^{2+}$ signals, CV or APD$_{80}$ as a result of injections.
Supplemental Figure II. Raw data showing dose-response of PVC propensity to individual doses of NE before grouping into low- and high-doses. A) [NE] vs. number of PVCs/injection and B) [NE] vs. percent of injections that produced at least one PVC.
Supplemental Figure III. A) Mean $V_m$-Ca$^{2+}$ delay for sinus rhythm under control conditions (open bar), and in the presence of NE during local injections (black bar). B) Mean $V_m$-Ca$^{2+}$ delay during an S1-S2 restitution protocol. There were no significant changes in $V_m$-Ca$^{2+}$ delay delay during normal EC coupling in the presence of NE or during short coupling intervals. C) Histogram of PVC coupling intervals with respect to the previous beat. NE-induced PVCs are shown in black bars and NT-induced PVCs in white bars.
Supplemental Figure IV. Subthreshold Ca\textsuperscript{2+} elevation following high dose localized NE application. A stable supraventricular rhythm with no PVCs is evident from the ECG. Close to the injection site (left panel, blue box), spontaneous Ca\textsuperscript{2+} elevation (*) can be seen following the application of NE. The highlighted blue area on the image shows the spatial extent of these spontaneous Ca\textsuperscript{2+} elevations. There is no clear evidence of associated
changes in \( V_m \) which exceed the noise level on the \( V_m \) signal. Away from the injection site (right panel, orange box), there is no evidence of spontaneous \( \text{Ca}^{2+} \) release.
Supplemental Figure V. Baseline electrophysiological characteristics before and after partial gap junction uncoupling with carbenoxolone (CBX). A) (i) Longitudinal and transverse conduction velocity (CV) before (solid bars) and after CBX (hatched bars) and (ii) anisotropy ratio. B) Change in (i) mean APD$_{80}$ and (ii) dispersion of APD$_{80}$ before (closed squares) and after CBX (open squares). **p<0.01, ***p<0.001.
Supplemental Figure VI.

A. Transverse CaTD$_{50}$ profiles

A) CaTD$_{50}$ as a function of distance along the transverse axis of conduction during NT-induced PVCs (open symbols, n=11 [6 hearts]), NE-induced PVCs (closed symbols, n=11 [7 hearts]) and NE-induced PVCs in the presence of CBX (red symbols, n=10 [6 hearts]).

B) Corresponding plot for $V_m$-Ca$^{2+}$ delay. * p<0.05.