Nicotine increases ventricular vulnerability to fibrillation in hearts with healed myocardial infarction

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Nicotine increases ventricular vulnerability to fibrillation in hearts with healed myocardial infarction. Am J Physiol Heart Circ Physiol 278: H2124–H2133, 2000.—The vulnerability of the infarcted hearts to ventricular fibrillation (VF) was tested in in situ canine hearts during nicotine infusion. The activation pattern was mapped with 477 bipolar electrodes in open-chest anesthetized dogs (n = 8) 5–6 wk after permanent occlusion of the left anterior descending coronary artery. Nicotine (129 ± 76 ng/ml) lengthened (P < 0.01) the pacing cycle length at which VF was induced from 171 ± 8.9 to 210 ± 14.7 ms. Nicotine selectively amplified the magnitude of conduction time and monophasic action potential (MAP) amplitude and duration (MAPA and MAPD, respectively) alternans in the epicardial border zone (EBZ) but not in the normal zone. With critical reduction of the MAPA and MAPD in the EBZ, conduction block occurred across the long axis of the EBZ cells. Block led immediately to reentry formation in the EBZ with a mean period of 105 ± 10 ms, which, after one to two rotations, degenerated to VF. Nicotine widened the range of diastolic intervals over which the dynamic MAPD restitution curve had a slope >1. We conclude that nicotine facilitates conduction block, reentry, and VF in hearts with healed myocardial infarction by increasing the magnitude of depolarization and repolarization alternans consistent with the restitution hypothesis of vulnerability to VF.

MATERIALS AND METHODS

Surgical Preparation

The research protocol was approved by the Institutional Animal Care and Use Committee of Cedars-Sinai Medical Center and followed the guidelines of the American Heart Association. Eight mongrel dogs of either sex weighing between 23 and 28 kg were anesthetized with intravenous pentobarbital sodium (35 mg/kg), intubated withuffed endotracheal tubing, and ventilated with room air with a Harvard respirator by use of a sterile technique. The chest was opened via the fifth left intercostal space, and the first diagonal branch of the LAD was permanently occluded by single-stage ligation. The chest was then closed in layers, and the dogs were allowed to recover. Five to six weeks postocclusion, the dogs were reanesthetized and studied in the open-chest state (24). Blood pH and gases (Po2 and PCO2) were corrected when necessary (24). In two dogs we ablated the atrioventricular node (radiofrequency energy) to prevent interruption by sinus impulse during regular ventricular pacing. Three dogs with no LAD occlusion served as control.

Stimulation Protocol

Activation maps were constructed during incremental pacing rate by decreasing the cycle length (CL) of stimulation. Ventricular pacing was performed using bipolar silver electrodes coated with Teflon except at the tip (1-mm diameter and 2-mm interpolar distance) from the noninfarcted epicardial base of the left ventricular anterior wall. This electrode location was proximal to the EBZ, allowing the epicardial
paced beats to propagate first from the normal noninfarcted site and then to the EBZ. Pacing started at 300 ms CL and then was progressively shortened, in 10-ms increments, to 250 ms; then in 5-ms increments to 200 ms; and then in 1- to 2-ms increments until VF was induced or one-to-one capture was lost. Each pacing train included 19 beats. There was a nonpacing interval of 30 s between pacing trains. Pacing was stopped when one-to-one capture was lost or a CL was reached that induced VF (VF threshold; VFT) (whichever came first). Pacing from the noninfarcted base of the left ventricle allowed us to determine systematically the characteristics of the wave front propagation (i.e., on a beat-by-beat basis from base to apex) that encompassed the entire EBZ. Because our purpose was to test the effect of nicotine on the restitution hypothesis of vulnerability to conduction block and reentry over the EBZ fibers, no other pacing sites were tested.

Computerized Mapping

Global mapping. Initially, a sock electrode array containing 125 bipolar electrodes was used \((n = 3)\) to construct total epicardial isochronal activation map over both ventricles (6) (Fig. 1A). The sock electrode array was constructed by fixing stainless steel electrode wires (0.4 mm in diameter) to the inner surface of a nylon “sock.” The uninsulated ends of the electrode wires served as the recording electrodes. The interelectrode distance of bipolar electrodes varied between 8 and 10 mm. The sock was pulled over the ventricular surface for global epicardial mapping (6) (Fig. 1A). Epicardial isochronal activation maps of both ventricles were then constructed. The activation time of each deflection was selected by the computerized mapping system according to a voltage change \(\frac{dV}{dt}\) criterion (5, 14, 15). Manual editing was then performed on each channel to eliminate the selection of noise or artifacts.

High-resolution EBZ mapping. After initial global mapping, a high-resolution activation map of the EBZ was then constructed using a 3.2 × 3.8-cm plaque electrode array containing 477 bipolar electrodes, 1.6 mm apart, and two surface electrocardiogram channels (16) (Fig. 1B). The recording plaque array was constructed with the use of similar stainless steel wires. The activation pattern over the EBZ was visualized dynamically as described previously (14–16). Briefly, each electrode site is represented by a dot that becomes illuminated when an activation is recorded. The dot initially illuminates red, then pink, then yellow, then green, and finally blue before fading away. The persistence of each illumination was set at 10 ms, so the total duration of illumination of each dot by one activation was 40 ms; thus each frame (snapshot) represented 40 ms of continuous activation map (isochrone).

Monophasic Action Potential Restitution

At the end of the rapid-pacing protocol, two simultaneous monophasic action potentials (MAPs) were recorded with contact electrodes (17), one from the normal left ventricle (base) near the pacing electrode and another from the center of the EBZ. Dynamic MAP duration and amplitude (MAPD and MAPA, respectively) restitution curves were constructed by two methods. The measured MAPD corresponded to 90% repolarization time. First, the relationship of MAPD and MAPA were plotted against the DIs. Second, MAPD and MAPA were plotted against the pacing CLs (PCLs)(23). The PCL, starting with 300 ms, was shortened in 20-ms steps until 240 ms and thereafter by 10-ms steps until block or VF.
was encountered (whichever came first). At each PCL, a train of 19 beats paced with twice diastolic current threshold current was applied, with 30 s of nonpacing interval between trains. The MAP data were acquired and stored as described previously (32). The effective refractory period (ERP) was determined (6) in the normal zone and the EBZ (6 sites in each zone) before and after nicotine administration.

Nicotine Infusion

Nicotine (Sigma) was infused through the left femoral vein at a rate of 2.5 µg·ml⁻¹·min⁻¹ (n = 5) and 5 µg·ml⁻¹·min⁻¹ (n = 3).

Nicotine Assay

Arterial nicotine and cotinine levels were assayed by the National Medical Services (Willow Grove, PA) with the use of gas chromatographic methods (18).

Histological Studies

Ventricles were sectioned from apex to base into 1- to 1.5-cm-thick transverse slices and were fixed in 10% neutral buffered Formalin for 24 h. Five-micrometer-thick sections of paraffin-embedded sections were stained with hematoxylin and eosin (HE). Infarct size was determined from five to six HE transverse sections with the use of planimetry.

Statistical Analysis

All statistical analyses were performed using GB-STAT (10). Student’s t-tests for single comparison ANOVA for multiple comparisons were used to compare the means. Wilcoxon signed ranks test was used to calculate significance for VFT. The null hypothesis was rejected at a value of P ≤ 0.05. Results are expressed as means ± SD.

RESULTS

VFT

Nicotine at a mean arterial blood level of 284 ± 73 ng/ml had no significant effect on the VFT in dogs without LAD occlusion (167 ± 20 ms at baseline and 171 ± 26 ms after nicotine; n = 3, P = not significant). In contrast, in all eight dogs with LAD occlusion, nicotine either facilitated (5 of 8) or promoted (3 of 8) the induction of VF during rapid pacing. Facilitation in the five of the eight dogs occurred as nicotine significantly (P < 0.01) increased the PCL at which VF could be induced (VFT) from 171 ± 8.9 ms to 210 ± 14.7 ms. The mean nicotine concentration was 129 ± 76 ng/ml (range 57–220 ng/ml, infusion rate 2.5 µg·kg⁻¹·min⁻¹). In the remaining three dogs, VF could not be induced at

Fig. 2. Total epicardial isochronal activation maps (planar projection, see Fig. 1A) of 4 paced beats at a pacing cycle length (PCL) of 210 ms. A: from a dog with no LAD occlusion (similar maps, not shown, were obtained after nicotine). Activation of both ventricles is complete within 63 ms during each of the 4 beats. B: from a dog 5 wk after LAD occlusion. Total epicardial activation time was increased to 152 ms with greatest delay occurring in region that corresponds to EBZ (see Fig 1A). Alternans of conduction time (CT) are evident. A and B: nos. 1–9 on both maps are locations of bipolar electrodes shown next to each map.
baseline during the fastest one-to-one captured CL (175 ± 5 ms). Attempts for pacing at shorter CL resulted in two-to-one and three-to-one block. Nicotine at infusion at 2.5 µg·kg⁻¹·min⁻¹ also failed to induce VF because of the emergence of two-to-one block during faster pacing rates. However, after 5 µg·kg⁻¹·min⁻¹ of nicotine infusion, VF was induced in these three dogs at a mean CL of 196 ± 12 ms, with a mean arterial blood nicotine level of 294 ± 120 ng/ml.

Global Epicardial Activation During Rapid Pacing

In noninfarcted dogs, nicotine did not change the total epicardial activation time at CLs of 250–200 ms compared with baseline (65 ± 12 vs. 68 ± 14 ms; P > 0.1; Fig. 2A). In dogs with infarcts, however, severe and selective conduction slowing and conduction time (CT) alternans developed at baseline in the EBZ as the PCL was decreased below 270 ms (Fig. 2B; see EBZ site in Fig. 1A). Total epicardial activation time at CLs of 265–220 ms alternated between a mean of 123 ± 16 and 140 ± 15 ms (P < 0.01; Fig. 2B). In hearts with MI, nicotine had no significant effect on total epicardial activation time at CLs longer than 270 ms compared with baseline (P > 0.1). However, at CLs between 265 and 220 ms and before reaching the VFT, nicotine significantly (P < 0.01) increased the magnitude of total epicardial CT alternans from 142 ± 11 to 180 ± 18 ms (Fig. 3). The site of the greatest degree of CT slowing at baseline and after nicotine was invariably located in the EBZ (Fig. 3).

High-Resolution EBZ Mapping

Nicotine increased (P < 0.01) the mean CT from 84 ± 9.8 to 101 ± 10.5 ms over the EBZ. Figure 4 shows CT alternans at baseline over the EBZ during pacing at CL of 210 ms. Whereas the CT over the EBZ alternated, no block occurred. After nicotine, however, conduction block evolving to reentry invariably occurred over the EBZ when the VFT was reached in all five dogs that we have mapped. Figure 5 shows that block was preceded by slowing of CV (2nd to 4th) to ~5 cm/s before complete block in the center of the EBZ (the 5th beat). Block in the center of the EBZ occurred across (2.1 cm) and along (0.6 cm) the long axis of the fiber orientation as to form an arc of functional conduction block (Fig. 5). Block was clearly functional, as wave fronts during the preceding second and third beats (Fig. 5) propagated without block. The blocked wave front then skirted from the right upper edge of the line of block and rotated counterclockwise around the line of functional

Fig. 3. Effects of nicotine (57 ng/ml) on epicardial isochronal activation maps in a dog 5 wk after LAD occlusion (same dog as in Fig. 2B). A: epicardial activation during pacing at a cycle length (CL) of 220 ms, with CT delay and alternans occurring in EBZ. B: during pacing at a CL of 210 ms, showing progressive delay in activation at EBZ from 157 to 187 ms (3rd paced beat), which is then followed by ventricular fibrillation (VF). A and B: nos. 1–9 on both maps indicate locations of selected bipolar electrodes shown next to each map.
block (Fig. 5A, curved line with arrow) via the left lateral noninfarcted normal ventricle. The rotating wave front then reentered through the initial site of block (lower site of the arc) to form the first reentrant activation with a period of 120 ms (4th to 5th beat in Fig. 5). During the second rotation, however, the reentrant wave front became disrupted by interference with outside waves. A second wave front entered the mapped region from the left lateral side of the EBZ and collided with the reentrant wave front (Fig. 5A, double lines). Another front then entered the mapped region from the bottom of the plaque, signaling the onset of VF (Fig. 5).

Electrophysiological and Structural Properties of EBZ in Healed Infarcts

Because block after nicotine selectively occurred in the EBZ, we evaluated the effects of nicotine on the excitability and restitution dynamics of the EBZ.

ERP. Nicotine increased (P < 0.01) the ERP in the EBZ from 183 ± 26 to 212 ± 17 ms but had no significant effect on the ERP in the normal base and the left lateral wall. However, the ERP in the noninfarcted left ventricle base in the MI hearts was longer (P < 0.05) than the ERP at similar sites in the noninfarcted hearts (181 ± 18 vs. 167 ± 12 ms).

Monophasic action potentials. Dynamic MAPD and MAPA restitution curves were constructed during two simultaneous MAP recordings, one from the base (normal zone) and another from the EBZ. Nicotine caused only modest (~5%) MAPA and MAPD alternans in the normal zone. However, 30–80% reductions in amplitude and duration were observed in the EBZ after nicotine (Fig. 6). Alternans of MAP in the EBZ were associated with alternans in CT. Figure 7 illustrates that during the alternating cycle of decreased MAPA and shortened MAPD, the CV between the two MAP electrodes slowed from 42 to 30 cm/s (Fig. 7). Effects of nicotine on the dynamic MAPD restitution curves are shown in Fig. 8. In the EBZ, nicotine widened the range of DIs over which the slope of the restitution curve exceeded 1 (34.3 ± 3 vs. 21.4 ± 4 ms; P < 0.01; Fig. 8A). Widening of the steeply sloped region was caused by recruitment of relatively late-coupled DIs (31 ± 3 vs. 16 ± 3 ms). This effect of nicotine is compatible with the development of alternans at longer PCLs (Fig. 8B). However, in the normal zone (Fig. 8C), nicotine did not
change the range of DIs over which the slope exceeded 1 (13 ± 3 vs. 18 ± 4 ms; P = 0.1) and also had minimal effect on the PCL that induced alternans (Fig. 8D). Figure 9 shows the relationship of MAPA vs. the PCL. Nicotine increased the PCL at which MAPA alternans developed (P < 0.05), in both the EBZ (Fig. 9A) and the normal zone (Fig. 9B). However, the magnitude of the MAPA alternans at all PCLs was significantly (P < 0.001) larger in the EBZ than in the normal zone.

MAP Alternans and VF

At the PCL at which VF was induced (VFT), the durations of the alternating MAP and its amplitude were at their minimum when the VF was induced. The onset of VF was associated with a 73 ± 12% reduction of the MAPA in the EBZ and only a 15 ± 7% reduction in the normal zone (P < 0.001; Fig. 10).

Reversibility of Effects of Nicotine

In three dogs, 4 h of nicotine-free saline infusion (blood levels 31 ± 26 ng/ml) partially reversed the effect of nicotine on the VFT, ERP, and dynamic MAPD restitution curve. At this time, nicotine levels increased up to 1 µg/ml due to its longer half-life (35).

Histological Analysis

All infarcted hearts had focal replacement fibrosis (scar) with mild focal chronic inflammatory infiltrate, characteristic of chronic MI. There was sparing of variable layers of myocardial cells at the epicardial edge of the infarct (EBZ) consistent with previous reports (25, 27). The long axis of the EBZ fibers was oriented perpendicular to the LAD. The mean size of the scar tissue was 17 ± 6% of the left ventricle.

DISCUSSION

There are three major findings in this study that describe net effects of nicotine on the EBZ of healed MI in open-chest anesthetized dogs. 1) Nicotine increases the PCL at which VF is induced in hearts with healed MI. 2) Nicotine promotes CT alternans, conduction...
block, and reentry in the EBZs that precede the onset of VF. 3) Nicotine makes the APD restitution curve steeper in the EBZ and widens the range of DIs over which the slope exceeds 1.

Nicotine and Restitution Hypothesis of VF

Simulation studies in two-dimensional excitable media have shown that the slope of the APD restitution curve controls wave front stability and wave front breakups (6, 21, 23, 34). More recent work confirmed and extended these earlier findings by showing that conduction block (wave break) was facilitated by increasing the range of DIs over which the slope of the APD restitution curve remained >1 (30). APD restitution curves with steep slopes are inherently unstable because they lead to alternans of wavelength (product of APD and CV). Because the wavelength represents the spatial amount of depolarized tissue available for downstream depolarization (excitation) and propagation, the alternating cycle of the wavelength that becomes critically short offers a diminished depolarizing current strength (i.e., “low safety factor”), causing the propagation to fail (34). Because not all the regions of the wave front have a uniform CV and/or APD, a specific site of the wave front may first manifest such a critical shortening of the wave length, resulting in failure of propagation at that specific location of the wave front. In our case the site that first exhibited such after nicotine was located in the EBZ. While block occurs in a specific segment of the front (EBZ in our model), the edges of the wave front outside the EBZ continue to propagate, resulting in reentry formation. In our model of healed MI, nicotine-exposed EBZ showed an enhanced magnitude of CV and APD alternans during rapid pacing that resulted in conduction block and reentry immediately preceding the onset of VF. Although our results are consistent with the restitution hypothesis of vulnerability to fibrillation, a definitive proof that this hypothesis was indeed operative would have required the use of an optical mapping system, as both depolarization and repolarization characteristics preceding the VF could be tracked by the optical action potentials.

At baseline, however, rapid pacing-induced VF was not preceded by conduction block and reentry in the EBZ. This observation suggests that regions outside the mapped area might manifest greater vulnerability to wave break than the EBZ during heart rate acceleration in hearts with healed MI. Potentially vulnerable myocardial sites at baseline might have steeper APD restitution curves than the EBZ. Our total epicardial activation maps with the sock electrode array suggest that such sites might be located in the left anteroseptal
However, because of the limited resolution and the two-dimensional aspect of these maps, we could not determine the precise site of the wave break at baseline during heart rate acceleration.

Previous studies designed to investigate conduction characteristics over the EBZ during the healing phase of the MI (5 days after LAD occlusion) suggested diminished excitability (29) and increased refractoriness (11) of EBZ cells in the induction of conduction block. Decreased cellular coupling caused by disrupted and/or decreased gap junctional connexin43 distribution was also suggested to play an important role in promoting conduction slowing and block in the healing (27) and the healed phase (25) of canine MI. It is possible that decreased cellular coupling in the EBZ might amplify the vulnerability to conduction slowing and block caused by the altered restitutional properties of the EBZ cells. The direct and indirect positive inotropic effects of nicotine (20, 22) on ventricular myocytes increase the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\text{i}), leading to increases in gap junctional resistance, conduction slowing, and eventual block (33). Because EBZ cells develop [Ca\(^{2+}\)] oscillation during rapid pacing (1), a rise in [Ca\(^{2+}\)]\text{i} induced by nicotine may enhance the amplitude of oscillation ( alternation) with enhanced cyclic changes of gap junctional resistance, causing MAPA alternans and oscillatory transfer of action potentials between adjoining cells (19, 33). When the MAPA during oscillation becomes critically low, conduction failure results. Finally, nicotine-induced prolongation of EBZ refractoriness could also contribute to conduction slowing and block in the EBZ (8, 11).

Limitation of Study

It may be asked whether the anesthetized open-chest model is appropriate to evaluate the effect of nicotine, because important autonomic nervous system influences of nicotine (4, 26) may be blunted. However, the demonstration that during a given autonomic state (anesthesia, open-chest), nicotine consistently promotes conduction block, reentry, and VF at longer PCL is of great significance. Although we do not know the precise underlying mechanism(s) (i.e., direct cardiac and/or indirect mediated via the autonomic nervous system), it is evident that the net effect of nicotine was a uniform facilitation of conduction block and reentry in the EBZ that evolved to VF. Another limitation of our study might be the fact that our relatively high spatial resolution maps were two and not three dimensional, raising a doubt on the interpretation of the EBZ maps. However, the EBZ is a relatively thin rim of tissue made of 30–100 cell layers (sometimes much less), with the tissue beneath made of nonviable scar tissue.

Fig. 8. Dynamic monophasic action potential duration (APD) restitution curves to 90% repolarization (APD\(_{90}\)) in a dog 5 wk after LAD occlusion. A: relationship between diastolic interval (DI) and APD\(_{90}\). Nicotine (100 ng/ml) shifted curve upward in EBZ and widened range of DI over which slope exceeded 1 (double-headed arrow) to a greater extent than in NZ (C). B: relationship between PCL and APD\(_{90}\) (bifurcation diagram) in same dog. Note that nicotine causes APD alternans in EBZ (B) to develop at longer PCL than in NZ (D).
Our findings were comparable with previous reports during the healing phase of the MI (27). As the result, the EBZ remains relatively immune to interference by transmurally conducted wave fronts, validating the interpretation of the EBZ maps. Third, it may be asked whether the MAP alternans observed in the in situ hearts may reflect alternate paths of activation rather than the consequence of intrinsic restitutional and/or gap junctional characteristics. However, the demonstration of constant sequence of activation over the EBZ at the VFT strongly supports the intrinsic rather than the alternate sequence of activation mechanism. Furthermore, rapid pacing-induced cellular alternans in isolated canine ventricular cells either during quinidine-induced increased slope of the APD restitution (20) or by partial uncoupling of cells with heptanol (7) suggest that active and passive changes promote the phenomenon of MAP alternans seen in the present study. Finally, it may be questioned as to why no conduction block occurred in the EBZ at baseline as shown in prior studies (9). The lack of conduction block over the EBZ at baseline in the present study may be due to age of the infarct. Whereas we evaluated the healed phase of the infarct (5–6 wk), prior studies showing baseline conduction block over the EBZ were conducted during the healing phase (5–6 days after LAD occlusion) of the infarct. More studies are needed to clarify this important issue.

Clinical Implications

Although nicotine mimics the major cardiovascular effects of tobacco smoke (4, 26), we do not know whether the effects of smoking would be similar to the effects of intravenous nicotine infusion. Epidemiological studies in a high-risk cohort of patients with healed MI have shown that smoking was associated with a significant increase in the incidence of VF and sudden cardiac death and that cessation of smoking reduced the incidence of VF in these high-risk patients (28). The results of the present study show that nicotine, in some cases (three dogs), promoted VF when blood nicotine levels ranged between 50 and 100 ng/ml. This finding suggests a mechanistic basis for the epidemiological findings of increased incidence of VF in smokers with chronic MI, as similar nicotine levels (30–85 ng/ml) also develop in the arterial blood of the smokers (12). Species differences, however, may complicate comparison. More work is needed to ascertain whether the responsiveness and sensitivity of surviving human myocardial tissue to nicotine are similar to those seen in the present canine model of chronic MI.

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Fig. 9. Relationship between MAP amplitude (MAPA) and PCL in a dog 5 wk after LAD occlusion (same dog as in Fig. 8). Nicotine causes MAPA alternans to develop at longer PCL and with greater amplitude in the EBZ (A) than in NZ (B). APA, action potential amplitude.

Fig. 10. Two simultaneous MAP recordings during pacing at 195 ms that induced VF after nicotine (100 ng/ml) in a dog 6 wk after LAD occlusion. Top recording is from NZ, and bottom recording is from epicardial BZ. Top signals are stimulus artifacts (arrow), and lowest recording is lead II ECG. Note emergence of selective and progressively increasing MAPA and MAP duration alternans in the BZ (3 arrows) but not in NZ. VF was triggered during alternating cycle at which amplitude and duration of MAP were at minimum (3rd arrow in BZ).
REFERENCES


