Age-related sensitivity to nicotine for inducible atrial tachycardia and atrial fibrillation

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Nicotine at concentrations found in the blood of regular smokers (10) exerts differential influence on the atria with different substrates for AT/AF. The aim of the present study was to test this hypothesis by determining atrial sensitivity to nicotine for inducible AT/AF in young and structurally remodeled atria caused by aging.

METHODS

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.

Isolated Langendorff-Perfused Rat Heart Setup

We studied male Fischer 344 rats, a strain that has been extensively used as a model of aging without the presence of other confounding factors such as coronary, vascular, or valvular abnormalities that often accompany aging (17, 18). Twelve male rats consisting of six young (2–3 mo old) and six old (22–24 mo old) were first anesthetized with an intraperitoneal injection mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). After a thoracotomy was performed, the superior vena cava was ligated, and cold Tyrode solution was then infused through the inferior vena cava. The pulmonary artery and the aorta were cut and the pulmonary veins ligated. The isolated heart was then perfused through the aorta with warm (37°C) oxygenated Tyrode solution at a constant rate of 5 ml/min. The composition of the Tyrode solution was (in mM) 125 NaCl, 4.5 KCl, 0.5 MgCl2, 0.54 NaH 2PO4, 24 NaHCO3, and 5.5 glucose plus 50 mg/l albumin. The isolated heart was immersed in a tissue bath and was superfused with warm and oxygenated Tyrode solution at a rate of 20 ml/min. Five bipolar silver electrodes coated with Teflon except for their tips (0.35 mm diameter) were used for pacing and recording from the atria and the ventricle. Two pairs of bipolar electrodes were placed on the right atrium (RA) and two pairs on the left atrium (LA) for atrial pacing and recording. The fifth pair of the bipolar electrode was placed on the left ventricle. Interpolar...
distance was 1 mm, and the interelectrode distance between the RA and LA was \( \approx 1 \) cm.

**Atrial Vulnerability**

Atrial vulnerability to inducible AT/AF was tested by a rapid, 3-s burst atrial pacing at cycle lengths (CL) of 100–10 ms with a pulse duration of 2 ms and twice-diastolic current threshold. Pacing started at a CL of 100 ms and was decreased by 10 ms until 10 ms was reached or AT/AF induced. Pacing was applied to the RA, and attempts to induce AT/AF were repeated five times at each CL in each rat in both groups. AT was defined as a periodic activation lasting for \( >30 \) s with constant bipolar electrogram morphology and an isoelectric interval. AF was defined as activation at irregular intervals with constantly changing bipolar electrogram morphology and no isoelectric interval.

**Effective Refractory Period and Interatrial Conduction Time**

The effective refractory period (ERP) was measured by the S\(_2\) extrastimulus method using eight regularly paced S\(_1\)-S\(_1\) at a pacing CL of 200 ms in the RA. Both S\(_1\) and S\(_2\) consisted of stimuli of 2-ms duration with the twice-diastolic threshold current. ERP was defined as the longest S\(_1\)-S\(_2\) interval at which S\(_2\) failed to induce a propagated atrial response. The interatrial conduction time (IACT) was measured as the time between the RA and the LA during pacing at CLs of 300, 200, 100, and 50 ms. We also calculated the wavelength (WL) as the product of the inverse over a 1-cm distance and the ERP. The conduction velocity was estimated as the time needed for propagation over a 1-cm distance of interatrial tissue.

**Nicotine Perfusion**

After baseline studies, nicotine (Sigma) at concentrations of 10, 30, 50, 80, and 100 ng/ml in Tyrode solution was perfused through the aorta to determine its effects on the IACT, atrial ERP, atrial vulnerability to inducible AT/AF, and atrial activation wavefront patterns during induced AT and AF.

**High-Resolution Optical Mapping**

The hearts were stained with a voltage-sensitive dye, 0.3–0.5 \( \mu \)M 4-(2-(6-(dibutylamino)-2-naphthalenyl)ethyl)-1-(3-sulfopropyl) hydroxide (di-4-ANEPPS, Molecular Probes; Eugene, OR), which was injected through the aorta. The hearts were illuminated with a solid-state, frequency-doubled laser (Verdi, Coherent; Santa Clara, CA) at a wavelength of 523 mm. The emitted fluorescence was acquired with a charge-coupled device camera (CA-D1-0256T, Dalsa, Waterloo; Ontario, Canada) through a 600-nm long-pass filter. The camera acquired the data from 128 \( \times \) 128 sites simultaneously over a 20 \( \times \) 20-mm\(^2\) area with a time resolution of 2.3 ms/frame. To allow visualization of activation patterns in greater detail, only the portion of the mapped region was selected for presentation in the figures. The image frames were color coded and animated according to the amplitudes of the optical membrane potential. The digital images were transferred to a personal computer with a frame grabber (Roadrunner, Bitflow). No electromechanical uncoupler was used in this study (9).

**Statistical Analysis**

All statistical analyses were performed using GB-Stat. The data are expressed as means ± SD. Statistical tests were performed using Student’s \( t \)-test, \( \chi^2 \)-test, and one-way ANOVA for repeated measures. A value of \( P < 0.05 \) was considered significant.

**RESULTS**

**Body and Heart Weights**

The body and heart weights were significantly smaller in the young compared with the old rats (266 ± 32 vs. 452 ± 19 g and 1.3 ± 0.2 vs. 1.8 ± 0.2 g, respectively) \( (P < 0.01) \).

**Effects of Nicotine on AT/AF Vulnerability**

**AT in young rats.** Figure 1 shows the effects of increasing nicotine concentration on cardiac rhythm immediately after burst atrial pacing. At baseline, no AT could be induced in any of the six young rats studied (Fig. 1A) (Table 1). During nicotine perfusion
at 10–30 ng/ml, burst atrial pacing induced AT in five of six rats (85%, P < 0.01) (Fig. 1B) (Table 1). However, when the nicotine concentration was raised >30 ng/ml (i.e., 50–100 ng/ml), nicotine suppressed the induction of AT in all six old rats (Fig. 2D) (Table 1). Furthermore, nicotine perfusion at concentrations of 10–100 ng/ml prevented burst atrial pacing-induced AF in all six old rats (Fig. 2D) (Table 1).

**AT/AF Cycle Length**

The CL of the induced AT in the presence of nicotine was insensitive to nicotine concentrations in both young and old rats. In the young rats, the mean CL of

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<th>Table 1. Incidences of atrial fibrillation and atrial tachycardia</th>
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+ indicates an episode of induction of atrial fibrillation or atrial tachycardia; − indicates no induction of AF or AT.

at 10–30 ng/ml, burst atrial pacing induced AT in five of six rats (85%, P < 0.01) (Fig. 1B) (Table 1). However, when the nicotine concentration was raised >30 ng/ml (i.e., 50–100 ng/ml), a significant decrease (33%) (P < 0.01) in pacing-induced AT developed (2 of 6 rats) (Table 1). These findings indicate that nicotine exerts a biphasic effect on inducible AT in young rats, causing facilitation at low (10–30 ng/ml) while inhibition at high (50–100 ng/ml) nicotine concentrations.

**AF in young rats.** No AF could be induced in any of the young rats at baseline (Fig. 1A). However, nicotine perfusion at 10–100 ng/ml caused the burst atrial pacing to induce AF in four of six young rats (85%, P < 0.05) (Table 1). Figure 1C illustrates an example of burst atrial pacing-induced AF during 80 ng/ml nicotine perfusion in a young rat. The range of nicotine concentrations during which AF could be induced varied in each rat and encompassed the entire 10–100 ng/ml range tested (Table 1).

**AT/AF in old rats.** The incidences of inducible AT/AF at baseline and during nicotine exposure in the old rats were significantly different from young rats. At baseline, burst atrial pacing induced AT and AF in five of the six old rats studied (Fig. 2, A and B) (Table 1). The influence of nicotine on AT inducibility in the old rats was concentration dependent. At 10–30 ng/ml, nicotine preserved AT inducibility as at baseline (i.e., in 5 of 6 old rats AT could still be induced). However, when the nicotine concentration was raised >30 ng/ml (i.e., 50–100 ng/ml), nicotine suppressed the induction of AT in all six old rats (Fig. 2D) (Table 1). Furthermore, nicotine perfusion at concentrations of 10–100 ng/ml prevented burst atrial pacing-induced AF in all six old rats (Fig. 2D) (Table 1).

**AT/AF Cycle Length**

The CL of the induced AT in the presence of nicotine was insensitive to nicotine concentrations in both young and old rats. In the young rats, the mean CL of...
the AT at nicotine concentrations of 10, 30, 50, 80, and 100 ng/ml were 62.3 ± 22.8, 71.3 ± 14.7, 71.3 ± 14.4, 73.2 ± 17.4, and 79.7 ± 4.5 ms, respectively (P = 0.81). AT induced in the old rats in the presence of 10 and 30 ng/ml nicotine had a mean CL of 81.7 ± 17.4, 73.2 ± 4.5 ms, respectively, which were not significantly different (P = 0.38) from baseline values in old (70.8 ± 24.6 ms) and young rats.

The mean CL of the AF in the young and old rats was also insensitive to nicotine concentrations. There were no significant differences in the AF CL among the RA (48 ± 11 ms), LA (50 ± 12 ms), and the pulmonary vein-LA junction (42 ± 6 ms) in both groups (P = not significant). In the young rats, the mean AF CL of both atria was 37.1 ± 4.9 ms at 10 ng/ml and 43.0 ± 2.6 ms at 100 ng/ml (P = 0.97). At intermediate nicotine concentrations (30–80 ng/ml), the mean CL of the AF did not change (33.2 ± 3.7 and 36.4 ± 3.4 ms, P = 0.97). In the old rats, the mean CL of the AF was not significantly different from the AF CL of young rats (35 ± 12.0 vs. 38.2 ± 4.3 ms).

Effective Refractory Period

In the young rats nicotine had a biphasic effect on atrial ERP. Nicotine at 10 ng/ml significantly (P < 0.01) decreased the ERP, whereas increasing its concentration to 30 ng/ml returned the ERP back to its baseline value. However, a significant progressive increase in atrial ERP developed when nicotine concentrations were raised (50–100 ng/ml) (Table 1). In the old rats, nicotine at 10 ng/ml had no effect on the ERP; however, when nicotine concentrations were raised ≥30 ng/ml, a progressive significant (P < 0.01) increase in the ERP developed (Table 1).

Interatrial Conduction Time

Nicotine significantly increased the IACT in a CL-dependent (300–50 ms) and concentration-dependent (10–100 ng/ml) manner in both the young and old rats (Fig. 3) (Table 3). The increase in the IACT was significantly higher in the old rats than in the young rats at all nicotine concentrations (10–100 ng/ml) (Table 3). A 2:1 interatrial conduction block developed at a pacing CL of 50 ms in the old rats (Fig. 3C), whereas 1:1 conduction was still possible in the young rats (Fig. 3A). However, nicotine infusion at 10 ng/ml caused 2:1 interatrial conduction block at a pacing CL of 50 ms in the young rats and higher degrees of block in the old rats (Fig. 3, B and D). A significant rate- and concentration-dependent interatrial conduction block developed in both groups (Table 3). At 100 ng/ml of nicotine, the atria of the old rats could be captured 1:1 only during pacing at a CL of 300 (Table 3). We estimated the WL at a pacing CL of 300 ms in the two groups before and after nicotine perfusion (10–100 ng/ml). Nicotine had no net influence on the WL in both groups; however, the WL in the old rats was significantly smaller than in the young rats (1.35 ± 0.2 vs. 2.2 ± 0.8 cm, P < 0.05).

High-Resolution Optical Mapping

Figure 4 shows optical maps and optical action potentials during normal sinus rhythm, during induced AT, and during induced AF. During sinus rhythm, the
activation first arose in the upper region of the RA and then propagated downward to the LA (Fig. 4A). During an induced AT, a periodic single large activation wavefront originating from the junction between the LA and the pulmonary vein was present in both the young and the old rats that propagated from the LA to the RA (Fig. 4B). During induced AF, multiple independent wavefronts separated by recovered atrial tissues were present in both the young and the old rats (Fig. 4C). These AF wavelets propagated in all directions (Fig. 4C).

**DISCUSSION**

The results of this study show that atrial sensitivity to nicotine for inducible AT/AF is different in the young rats compared with old rats. Nicotine significantly increased atrial vulnerability to inducible AT/AF in the young rats while suppressing AT/AF induction in the old rats, causing complete prevention of inducible AT/AF and high degrees of interatrial conduction block.

**Nicotine Versus ERP and IACT**

Nicotine alters atrial myocyte ion channel conductance either directly by interacting with ion channels or indirectly by release of neurotransmitters, such as acetylcholine and norepinephrine, from atrial cholinergic and adrenergic nerve endings (7). Studies on isolated atrial myocytes showed that nicotine blocks the transient outward potassium current ($I_{to}$) (20) and the rapid component of the delayed rectifier current ($I_{Kr}$) (21), causing prolongation of atrial action potential duration and the ERP. These effects may be accentuated by the ability of nicotine to increase the L-type calcium inward current (12). These effects of nicotine explain the progressive increase in atrial ERP seen in young and old rats with increasing nicotine concentration. The ERP shortening seen in the young rats at the lowest nicotine concentration (10 ng/ml) might result from the indirect effect of nicotine on the transmitter release (acetylcholine and norepinephrine) from atrial autonomic nerve endings (1, 14). The indirect effects of nicotine, however, become offset with increasing nicotine concentrations that directly block $I_{to}$ (20) and $I_{Kr}$ (21), causing a net prolongation of the ERP in the young rats. We do not know why such a biphasic mechanism of nicotine on atrial ERP is absent in the old rats. Potential mechanisms might include aging-related loss or decreased sensitivity of atrial nerve endings to nicotine for the release of neurotransmitter or diminished influence of transmitters on ion channel function in aged atria. Changes in atrial ion channel density might also develop with aging that might lead to the elimination of the biphasic effect of nicotine on atrial ERP in the old rats. Altered ion channel density and function develops in atria with electrical remodeling (25).

The longer IACT at baseline in the old compared with young rats might be related to the increased interstitial atrial fibrosis (9), an effect known to slow atrial conduction velocity in the aged atria (19). It is therefore possible that the greater increase in the IACT and the development of high degrees interatrial conduction block after nicotine exposure in the old rats might result from the combined interstitial fibrosis (9, 19) and ERP prolonging effect of nicotine. The absence of increased interstitial fibrosis (9) in the young rats explains the lesser degrees of increase in the IACT after nicotine and lesser ERP prolonging effects of nicotine, which develops only at concentrations >30 ng/ml.

**Nicotine and Inducible AT/AF**

Nicotine increased inducible AT/AF in the young rats. However, when the concentration of nicotine was increased, a progressive decrease and ultimately a complete suppression of inducible AT/AF occurred in the old rats. At low concentrations, nicotine shortened the ERP in the young rats but not in the old rats and exerted a considerably greater increase in the ERP and IACT in the old compared with young rats. According to the wavelength hypothesis of AF vulnerability (16),
these effects of nicotine might provide an insight into its differential arrhythmic influence in the young versus the old rats. Nicotine exerts opposing effects on the two variables ERP and conduction velocity (related to the inverse of IACT) that control atrial wavelength. Prolonging the ERP increases while slowing the conduction velocity shortens the wavelength (16). In a given atrial tissue mass, it appears that a critical atrial wavelength must be present in the limited atrial tissue mass for nicotine to be anti- or proarrhythmic (i.e., nicotine-induced critical changes in the ERP and conduction velocity) (16, 23). For example, Li et al. (13) have found that dofetilide, an \( I_{Kr} \) blocker, produces a much larger ERP increase in dogs with AF caused by congestive heart failure than in dogs with rapid atrial pacing, an effect that is associated with greater anti-AF efficacy. Whereas the wavelength hypothesis is compatible with the increased incidence of AF in the old rats at baseline, however, nicotine-related AF in the young rats is not. With the relative constancy in the value of the wavelength with increasing nicotine concentrations at a time when AT/AF inducibility progressively declines suggests that changes in the AT/AF vulnerability may be unrelated to wavelengths in the young rats exposed to nicotine. It is likely that nicotine-related inducible AT/AF may be caused by a focal, nonreentrant mechanism that undergoes fibrillatory conduction in the atria producing multiple independent atrial wavelets. Increased focal discharge activity in the pulmonary veins has recently been found to play an important role in AT/AF in both experimental and clinical studies. Digitalis, for example, was found to promote triggered activity in guinea pig myocardial cells in the pulmonary veins but not in atrial myocardial cells (6). Simi-

Fig. 4. Optical activation maps and transmembrane action potentials during sinus rhythm (A), AT (B), and AF (C) in a young rat. During sinus rhythm (A), a single wavefront is initiated from the upper part of the RA that propagates to the LA (arrows). During AT (B), a single wavefront originates periodically from the LA-pulmonary vein (PV) junction that activates both atria at regular intervals with an isoelectrical interval present in the optical action potentials. During AF (C), however, multiple independent wavefronts separated by recovered tissue are present that propagate in different directions. Optical action potentials during AF do not show isoelectrical window. White arrows point to the directions of the propagating waves. Numbers (1–5) adjacent to each yellow dot (lowest frame in C) correspond to the locations of the optical action potentials (1–5) shown at the bottom of each panel (A–C). Color bar shows fluorescence intensity; red represents fully depolarized state, whereas blue represents rested repolarized state.
larly, in the canine model of AF induced by chronic rapid atrial pacing, focal periodic activity in the pulmonary veins was found to cause AF (24). Clinical studies also provided convincing evidence that ectopic activity in the pulmonary veins can induce AF in patients (4, 8, 11). Our optical mapping data showed that during AT and AF, rapid periodic activity originating from the junction between the LA and the pulmonary vein propagated to the atria causing AT and AF. Whereas in our studies the activation rate at the pulmonary vein-LA junction was slightly faster than the atrial rate, this difference, however, was not significant. It is possible that rapid rates of activation emanating from the pulmonary vein-LA junction undergoes fibrillatory conduction (wavebreaks or wave splitting) in the relatively small atrial tissue mass producing multiple independent wavelets that are characteristically present during AF. Whereas in larger animals with larger atrial tissue mass, it is conceivable that multiple wavelets can continuously be generated to maintain the AF, in rats with smaller tissue mass this may not be possible. It is therefore plausible that nicotine may cause focal activity in the pulmonary vein-LA junction (26) by promoting triggered activity (5). More studies are needed to determine the potential of pulmonary vein myocardial cells to generate triggered activity when exposed to nicotine.

Limitations of the Study

This study was done using Langendorff-perfused normal young and aged hearts with no other detectable confounding atrial disease. Applicability of these findings to human clinical settings may not be possible particularly the potential use of nicotine as an anti-AT/AF agent in the aged population because of the detrimental effects of nicotine in causing high degrees of interatrial conduction block. We did not use an electromechanical uncoupler, which prevented us to map the activation pattern in the pulmonary veins due to their intense motion. However, because agents that mobilize cardiac motion also interfere with cardiac electrical activity, a property that could have distorted in determining the effects of low concentrations of nicotine. Finally, the results of the present study also suggest that nicotine at concentration found in the blood of smokers (i.e., 30–85 ng/ml) (10) might increase atrial vulnerability to inducible AT/AF in normal adult atria with no atrial disease.

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DISCLOSURES

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