

# Dabigatran and Thrombin Modulate Electrophysiological Characteristics of Pulmonary Vein and Left Atrium

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**Background**—Dabigatran reduces stroke in atrial fibrillation. Pulmonary veins (PVs) and left atrium (LA) play a critical role in the pathophysiology of atrial fibrillation. We investigated the effects of thrombin, blood clot solution, and dabigatran on PVs and LA.

**Methods and Results**—Conventional microelectrodes were used to record the action potentials in isolated PV and LA preparations before and after the administration of thrombin or blood clot solution in control and dabigatran-treated rabbits. Thrombin (0.01, 0.1, and 1 unit/mL), respectively, reduced the PV (n=6) spontaneous beating rates from  $1.9\pm 0.2$  to  $1.7\pm 0.2$ ,  $1.6\pm 0.2$ , and  $1.4\pm 0.3$  Hz ( $P=0.046$ ). Blood clot solution (0.5% and 5.0%), respectively, reduced the PV (n=5) spontaneous beating rates from  $2.0\pm 0.4$  to  $1.8\pm 0.4$  and  $1.3\pm 0.3$  Hz ( $P=0.044$ ). Thrombin (0.01, 0.1, and 1 unit/mL) and blood clot solution (0.5% and 5.0%) increased LA diastolic tension and the resting membrane potential with decreased action potential duration and contractility. Thrombin (0.01, 0.1, and 1 unit/mL) and blood clot solution (0.5% and 5%) induced delayed afterdepolarization and burst firing in PVs, but not in LA. N<sup>G</sup>-nitro-L-arginine methyl ester (100  $\mu$ mol/L) or a protease-activated receptor type 1 blocker (BMS 200261, 1  $\mu$ mol/L) attenuated the effects of thrombin and blood clot solution in PVs and LA. Dabigatran-treated PVs had slower spontaneous activity ( $1.1\pm 0.1$  Hz; n=10;  $P=0.0001$  versus control). Their electrophysiological characteristics were not changed by thrombin (1 unit/mL) and blood clot solution (5%).

**Conclusions**—Thrombin modulates PV and LA electric and mechanical characteristics, which were blocked by dabigatran. (*Circ Arrhythm Electrophysiol.* 2012;5:1176-1183.)

**Key Words:** atrial fibrillation ■ direct thrombin inhibitor ■ protease-activated receptor ■ pulmonary vein ■ thrombin

Atrial fibrillation (AF) with spontaneous echo contrast or thrombus formation in left atrium (LA) and appendage was reported in 10% to 35% of patients.<sup>1,2</sup> An increased cardiac thrombin concentration associated with a prothrombotic situation was demonstrated during paroxysm of AF.<sup>3,4</sup> Thrombin can modulate the electrophysiological properties of cardiomyocytes and promote the genesis of ventricular arrhythmia with hemostatic thrombosis.<sup>5</sup> Thrombin affects spontaneous automaticity and elevates intracellular calcium and sodium in cardiomyocytes.<sup>5-11</sup> Thrombin also increases cesium-induced early afterdepolarizations in isolated canine Purkinje fibers and induces proarrhythmic events during early reperfusion in intact adult rat hearts.<sup>12-14</sup> These findings indicate that thrombin has direct electrophysiological effects on cardiomyocytes. Thrombin activates protease-activated receptor type 1 (PAR1) and stimulates NO synthase (NOS) with NO production and vascular relaxation.<sup>15,16</sup> Accordingly, thrombin may attenuate vascular stretching and modulate mechanoelectric feedback, which may contribute to the electrophysiological effects of thrombin and blood clots.<sup>17</sup>

Pulmonary veins (PVs) are important AF initiators, and LA is the main AF substrate for reentry.<sup>18-20</sup> PVs contain a mixture of working myocardium and pacemaker cells that can induce atrial arrhythmias.<sup>21</sup> Previous studies indicated that the NO donor has anti-AF effects and reduces PV arrhythmogenic activity.<sup>22</sup> In addition, mechanoelectric feedback plays an important role in the pathophysiology of PV arrhythmogenesis.<sup>17,23</sup> Therefore, thrombin or blood clots may modulate PV or LA arrhythmogenesis.

## Clinical Perspective on p 1183

Dabigatran, a selective direct thrombin inhibitor, reversibly binds to thrombin and reduces thrombus formation and can also reduce stroke in AF patients.<sup>24</sup> However, the electrophysiological effect of dabigatran has not been elucidated. It is not clear whether dabigatran regulates the cardiac effects of thrombin. The purposes of this study were to investigate the electrophysiological effects of thrombin and blood clots in PVs and the LA and to evaluate the potential modulatory roles of NO, PAR1, and a direct thrombin inhibitor.

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## Methods

### Rabbit PV and LA Tissue Preparations

Experiments in this study conformed to the Institutional Guide for the Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Control and dabigatran-treated (6 mg, BID for consecutive 3 days) male rabbits (that weighed 1–2 kg) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). A mid-line thoracotomy was then performed, and the heart and lungs were removed as described previously.<sup>25</sup> The PVs were separated from the atrium at the level of the LA–PV junction and separated from the lungs at the end of the PV myocardial sleeve in Tyrode solution with a composition (in mmol/L) of 137 NaCl, 4 KCl, 15 NaHCO<sub>3</sub>, 0.5 NaH<sub>2</sub>PO<sub>4</sub>, 0.5 mgCl<sub>2</sub>, 2.7 CaCl<sub>2</sub>, and 11 dextrose; the pH was adjusted to 7.4 by titration with NaOH. One end of the preparations, consisting of the PVs and LA–PV junction, was pinned with needles to the bottom of a tissue bath. The other end (distal PV) was connected to a Grass FT03C force transducer with a silk thread. For LA experiments, the LA was isolated and prepared as described previously.<sup>23,25</sup> The adventitial or epicardial side of the preparations faced upward. The PV and LA tissue strips were superfused at a constant rate (3 mL/min) with Tyrode solution saturated with a 97% O<sub>2</sub> and 3% CO<sub>2</sub> gas mixture. The temperature was maintained at 37°C, and the preparations were allowed to equilibrate for 1 hour before electrophysiology assessment.

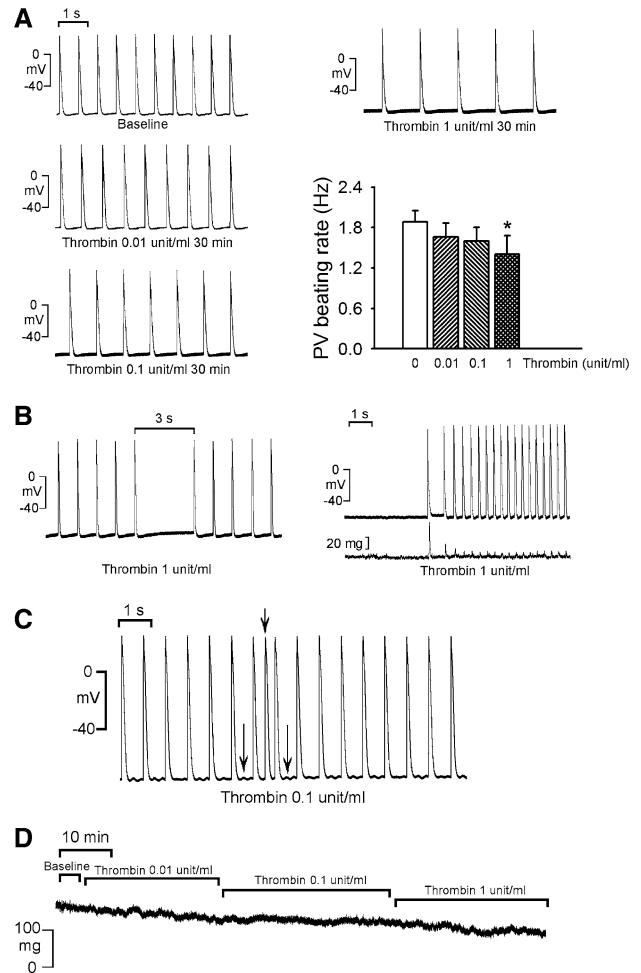
### Electrophysiological and Pharmacological Studies of the PV and LA Preparations

The transmembrane action potentials (APs) of the PVs and LA were recorded using machine-pulled glass capillary microelectrodes filled with 3 mol/L KCl before and after the administration of thrombin (0.01, 0.1, and 1 unit/mL) or 0.5% and 5% blood clot solutions (blood clot from 0.5 or 5 mL rabbit blood and administered in 100 mL Tyrode solution for 30 minutes) in control and dabigatran-treated preparations. Only 1 PV or LA strip from each control or dabigatran-treated rabbit sequentially received different concentrations of thrombin (0.01, 0.1, and 1 unit/mL) or blood clot solutions (0.5% and 5%). The preparations were connected to a WPI model FD223 electrometer under tension with 150 mg, as described previously.<sup>23,25</sup> The electric and mechanical events (contractility and diastolic tension) were simultaneously displayed and recorded on a Gould 4072 oscilloscope and Gould TA11 recorder. The signals were recorded with DC coupling and a 10-kHz low-pass filter cutoff frequency using a data acquisition system. Signals were recorded digitally with 16-bit accuracy at a rate of 125 kHz. PVs with spontaneous activity were defined as the constant occurrence of spontaneous APs with no electric stimulus. To study the molecular mechanisms of thrombin, a BMS-200261 (PAR1 blocker, 1 μmol/L, Sigma) or N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) solution (100 μmol/L) was administered to the PV or LA in the presence or absence of thrombin (1 unit/mL). L-NAME (100 μmol/L) was administered to the PV or LA with a blood clot solution to evaluate the mechanism of blood clots. The electric and mechanical events (contractility and diastolic tension) were continuously and simultaneously displayed and recorded during all the above procedures.

The AP amplitude (APA) was obtained by measuring the difference between the resting membrane potential (RMP) or maximum diastolic potential and the peak of AP depolarization. AP durations at repolarization rates of 90%, 50%, and 20% of the APA were measured as action potential duration (APD)<sub>90</sub>, APD<sub>50</sub>, and APD<sub>20</sub>, respectively. The RMP, APA, APD<sub>90</sub>, APD<sub>50</sub>, APD<sub>20</sub>, and contractile forces were measured under 2-Hz pacing of the LA before and after drug administration. Delayed afterdepolarizations were defined as the presence of a spontaneous depolarization of the impulse after full repolarization.<sup>25</sup>

### Western Blot Analysis of Ion Channel Proteins

Control and dabigatran-treated PVs were washed with cold PBS and lysed on ice for 30 minutes in a radioimmunoprecipitation assay buffer



**Figure 1.** Effects of thrombin on pulmonary veins (PVs). **A**, Example and average data of thrombin (0.01, 0.1, and 1 unit/mL) on PV beating rates ( $n=6$ ). **B**, Examples of thrombin-induced pauses (left) and pause-related burst firing in PVs (right). **C**, An example of thrombin-induced delayed afterdepolarization (DAD) and triggered beats (arrow) in PVs. **D**, An example of the diastolic tension of PV significantly decreasing during the administration of thrombin. \* $P<0.05$  vs baseline.

containing 50 mmol/L Tris, pH 7.4, 150 mmol/L NaCl, 1% nonidet P-40 (NP40), 0.5% sodium deoxycholate, 0.1% SDS, and protease inhibitor cocktails (Sigma-Aldrich Corp, St Louis, MO). The protein concentration was determined with a Bio-Rad protein assay reagent (Bio-Rad, Munich, Germany). Equal amounts of proteins were subjected to an SDS-PAGE. Blots were probed with primary antibodies against Ca<sub>v</sub>1.2 ( $I_{Ca-L}$  subunit; Alomone Labs, Jerusalem, Israel), ryanodine receptor (Thermo, Rockford, IL), sarcoendoplasmic reticulum Ca<sup>2+</sup>-ATPase (Santa Cruz Biotechnology, Santa Cruz, CA), calmodulin kinase II (CaMKII; Abcam, Cambridge, United Kingdom), sodium/calcium exchanger (NCX, Swant, Bellinzona, Switzerland), hyperpolarization-activated cyclic nucleotide-gated potassium channel 4 (Millipore, Billerica, MA), phospholamban (Thermo, Rockford, IL), Ser16 phosphorylated phospholamban (Badrilla, Leeds, United Kingdom), and secondary antibodies conjugated with horseradish peroxidase. Bound antibodies were detected with the ECL detection system (Millipore) and analyzed with ALPHAEASEFC software (Alpha Innotech, San Leandro, CA). Targeted bands were normalized to cardiac  $\alpha$ -sarcomeric actin (Sigma-Aldrich Corp) to confirm equal protein loading.

### Statistical Analysis

All continuous variables are expressed as mean $\pm$ SEM. One-way repeated measures ANOVA followed by the Bonferroni analysis was

used to compare the difference before and after drug administration on PVs and LA. The expressions of ion channel proteins and electrophysiological and mechanical characteristics between the control and dabigatran-treated groups were compared by a Wilcoxon rank-sum test or unpaired *t* test depending on the outcome of the normality test. The McNemar test was used to compare the incidences of a long pause, delayed afterdepolarization, and burst firing before and after drug administration on PVs. A *P*<0.05 was considered statistically significant.

**Results**

**Effects of Thrombin in PVs and LA**

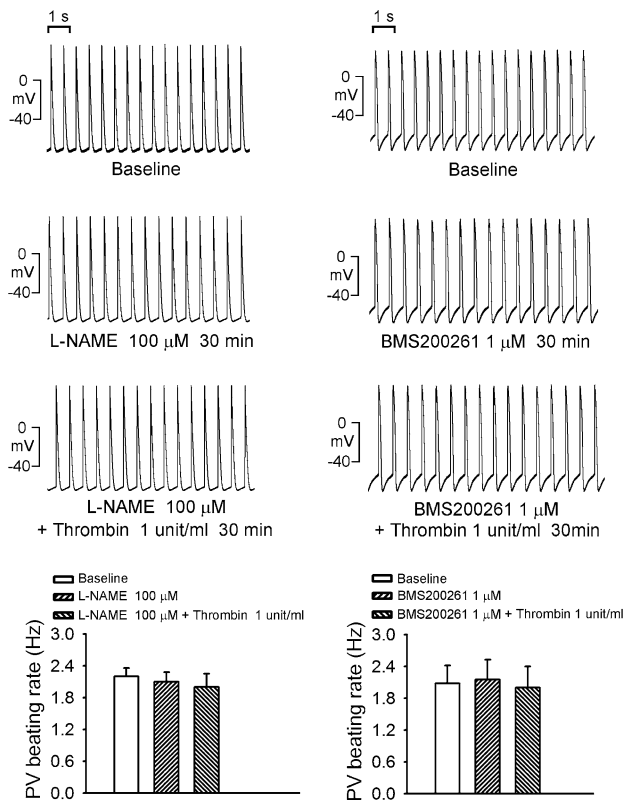
As shown in Figure 1A, thrombin (0.01, 0.1, and 1 unit/mL) concentration-dependently decreased PV beating rates. In addition, thrombin induced long pauses ( $\geq 3$  seconds) in 2 of 6 PVs (33% versus 0% at baseline; *P*=0.157) at 0.01 unit/mL and induced long pauses in 4 of 6 PVs (67% versus baseline; *P*=0.045) at 0.1 and 1 unit/mL, respectively (Figure 1B). Compared with baseline, thrombin (0.01, 0.1, and 1 unit/mL) concentration-dependently induced the occurrences of repetitive PV burst firings in 33% (*P*=0.157), 50% (*P*=0.083), and 83% of PVs (*P*=0.025), which occurred during PV slowing or a long pause (Figure 1B). Thrombin also enhanced the occurrence of delayed afterdepolarizations and triggered beats in 3 PVs (50% versus 0% at baseline; *P*=0.083) at 0.01 unit/mL and in 5 PVs (83%, *P*=0.025) at 0.1 and 1 unit/mL (Figure 1C). Furthermore, thrombin (0.01, 0.1, and 1 unit/mL) concentration-dependently decreased the diastolic tension of

PVs by  $2\pm 7$  mg (*P*=0.452),  $6\pm 7$  mg (*P*=0.265), and  $48\pm 25$  mg (*P*=0.031, Figure 1D), respectively.

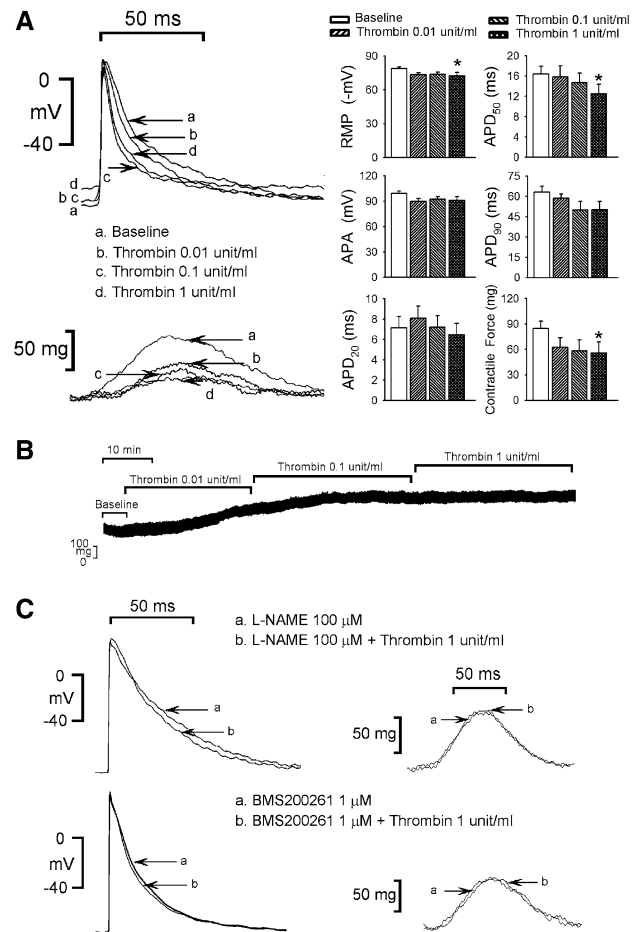
In the presence of L-NAME (100  $\mu$ mol/L), thrombin (1 unit/mL) did not significantly change the PV spontaneous beating rates or diastolic tension. Similarly, in the presence of BMS 200261 (a PAR1 blocker, 1  $\mu$ mol/L), thrombin (1 unit/mL) did not significantly change PV spontaneous beating rates or diastolic tension (Figure 2).

As shown in Figure 3A, thrombin (1 unit/mL) significantly elevated the RMP in LA. However, thrombin did not change APD<sub>20</sub> and APD<sub>90</sub>, but shortened APD<sub>50</sub> at 1 unit/mL and decreased contractile force at 1 unit/mL in LA. Furthermore, thrombin (0.01, 0.1, and 1 unit/mL), respectively, increased the LA diastolic tension by  $477\pm 160$  mg (*P*=0.047),  $543\pm 185$  mg (*P*=0.019), and  $488\pm 190$  mg (*P*=0.034, Figure 3B). In the presence of L-NAME (100  $\mu$ mol/L) or BMS-200261 (1  $\mu$ mol/L), thrombin (1 unit/mL) did not significantly change LA electrophysiological or mechanical characteristics (Figure 3C).

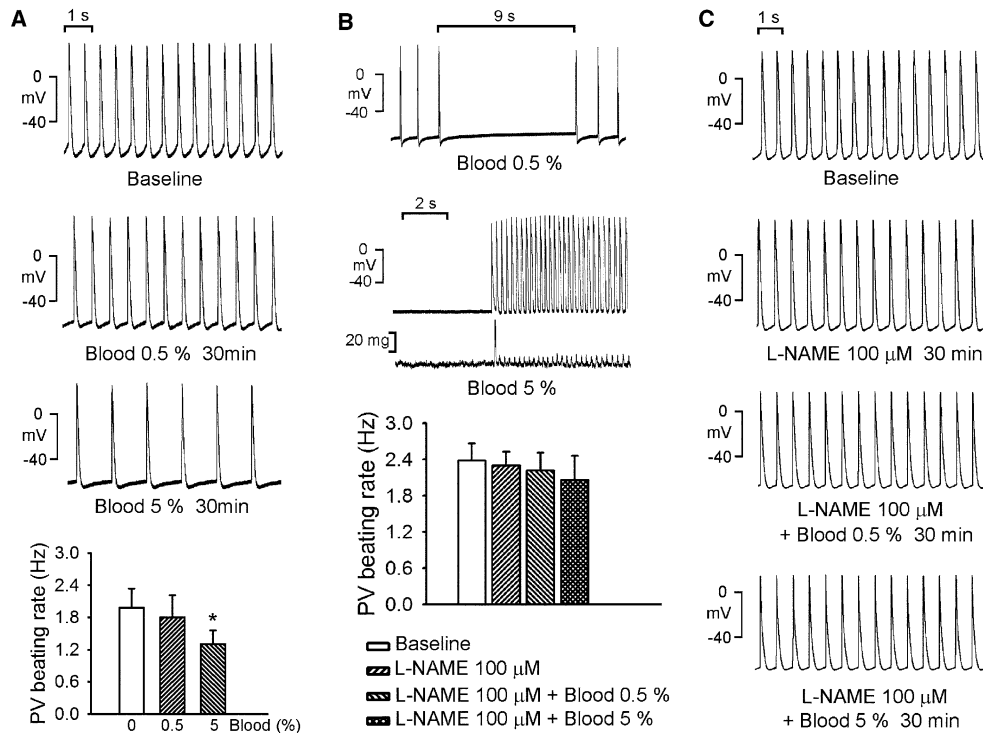
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**Figure 2.** Effects of thrombin on pulmonary veins (PVs) in the presence of N<sup>g</sup>-nitro-L-arginine methyl ester (L-NAME) or BMS-200261. Examples and average data show that thrombin (1 unit/mL) did not change the PV spontaneous beating rates in the presence of L-NAME (100  $\mu$ mol/L, **A**, n=6) or BMS-200261 (1  $\mu$ mol/L, **B**, n=6).



**Figure 3.** Effects of thrombin on the left atrium (LA) in the presence or absence of N<sup>g</sup>-nitro-L-arginine methyl ester (L-NAME) or BMS-200261. **A**, Examples and average data of thrombin (0.01, 0.1, and 1 unit/mL) on the action potential (AP) and contractile forces in LA (n=6). **B**, An example of thrombin increasing the LA diastolic tension. **C**, Examples of thrombin (1 unit/mL) on the AP or contractile force in LA in the presence of L-NAME (100  $\mu$ mol/L) or BMS-200261 (1  $\mu$ mol/L). \**P*<0.05 vs baseline.

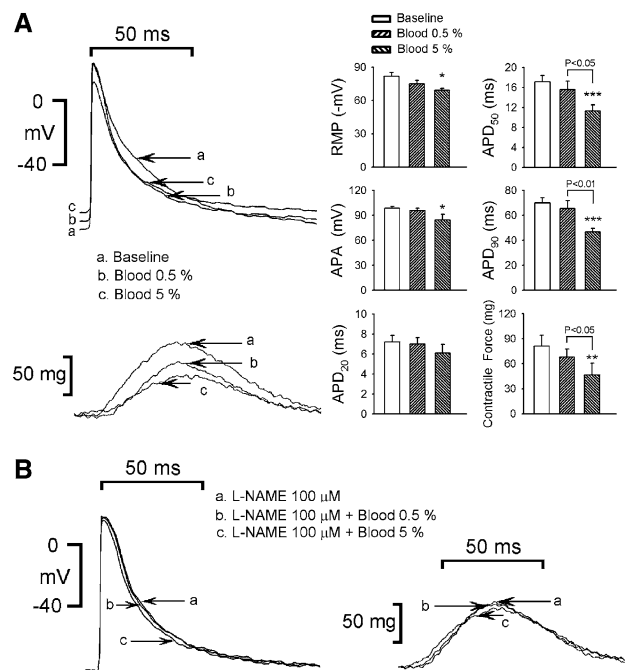


**Figure 4.** Effects of blood clot solution on pulmonary veins (PVs) before and after the presence of  $N^G$ -nitro-L-arginine methyl ester (L-NAME). **A**, Example and average data of blood clot solution (0.5% and 5%) on PV beating rates ( $n=5$ ). **B**, Examples of blood clot solution-induced pauses (**top**) and burst firings (**bottom**) in PVs. **C**, Examples and average data show that the blood clot solution did not change the PV spontaneous beating rates in the presences of L-NAME (100  $\mu\text{mol/L}$ ,  $n=6$ ). \* $P<0.05$  vs baseline.

### Effects of Blood Clot Solution in PVs and LA

As shown in Figure 4A, blood clot solutions (0.5% and 5%) concentration-dependently decreased the PV spontaneous beating rate. Similar to those in thrombin, the blood clot solution induced a long pause in 3 of 5 PVs (60% versus 0% at baseline;  $P=0.083$ ) at a concentration of 0.5% and in 5 of 5 PVs (100% versus baseline;  $P=0.025$ ) at a concentration of 5%. Furthermore, the blood clot solution (0.5% and 5%) induced repetitive PV burst firings in 60% (versus 0% at baseline;  $P=0.083$ ) and 80% of PVs (versus baseline;  $P=0.045$ ), respectively (Figure 4B). Compared with the baseline, the blood clot solution (0.5% and 5%) also induced the occurrence of delayed afterdepolarizations and triggered beats in 60% ( $P=0.083$ ) and 80% of PVs ( $P=0.045$ ). Similar to the effects with thrombin, the blood clot solution (0.5% and 5%) decreased the diastolic tension of PVs ( $n=5$ ) by  $38\pm 13$  mg ( $P=0.147$ ) and  $81\pm 46$  mg ( $P=0.04$ ). In the presence of L-NAME (100  $\mu\text{mol/L}$ ), blood clot solution (0.5% and 5%) did not significantly change PV spontaneous beating rates or diastolic tension (Figure 4C).

As shown in Figure 5A, the blood clot solutions significantly elevated RMP, diminished APA, shortened  $\text{APD}_{50}$  and  $\text{APD}_{90}$ , and decreased contractile force in LA at a concentration of 5%. Similar to the effect with thrombin, the blood clot solution ( $n=6$ ) increased the diastolic tension of LA by  $294\pm 83$  mg ( $P=0.017$ ) and  $455\pm 78$  mg ( $P=0.002$ ). In the presence of L-NAME (100  $\mu\text{mol/L}$ ), the blood clot solution (0.5% and 5%) did not significantly change LA electrophysiological or mechanical characteristics (Figure 5B).



**Figure 5.** Effects of blood clot solution on the left atrium (LA) in the presence or absence of  $N^G$ -nitro-L-arginine methyl ester (L-NAME). **A**, Examples and average data of the blood clot solution (0.5% and 5%) on the action potential (AP) and contractile forces in LA ( $n=6$ ). **B**, Examples and average data of the blood clot solution (0.5% and 5%) on the AP and contractile forces in LA ( $n=6$ ) in the presences of L-NAME. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.005$  vs baseline.

## Effects of Thrombin in Dabigatran-Treated PVs and LA

Figure 6A shows the spontaneous activity in dabigatran-treated PVs, which had slower PV beating rates than control PVs. In dabigatran-treated PVs, thrombin (1 unit/mL) did not significantly change PV spontaneous activity or diastolic tension (Figure 6B). Similarly, the blood clot solution (5%) did not change PV spontaneous activity and diastolic tension (Figure 6C).

As shown in Figure 7A, the dabigatran-treated and control LA had similar RMP, APA,  $APD_{20}$ ,  $APD_{90}$ , diastolic tension, and contractile force. However, the dabigatran-treated LA had a longer  $APD_{50}$  than the control LA. As shown in Figure 7B, thrombin (1 unit/mL) did not change the AP morphology or diastolic tension in the dabigatran-treated LA. However, the 5% blood clot solution increased the APA and contractile forces in the dabigatran-treated LA.

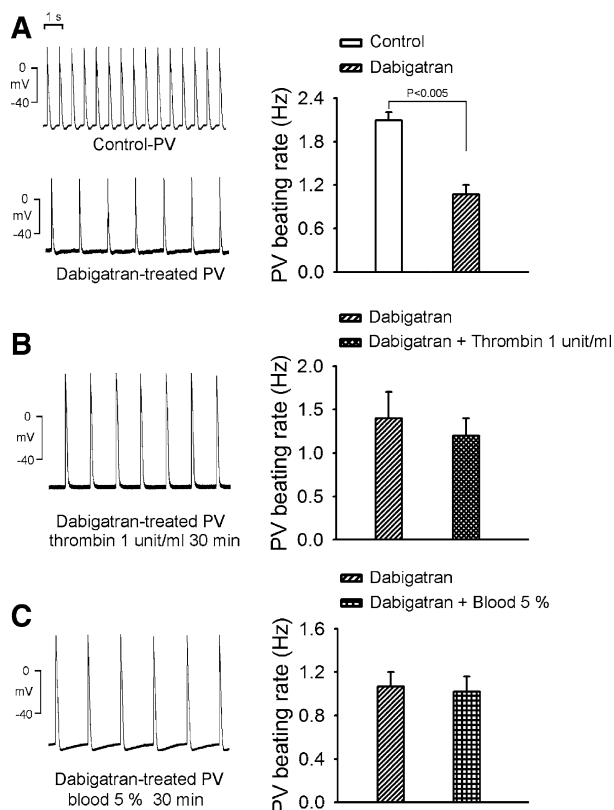
As shown in Figure 8, the dabigatran-treated PVs had lesser expressions of CaMKII, NCX, and  $Ca_v1.2$  than control PVs. However, control and dabigatran-treated PVs had similar expression of ryanodine receptor, sarcoendoplasmic

reticulum  $Ca^{2+}$ -ATPase, hyperpolarization-activated cyclic nucleotide-gated potassium channel 4, phospholamban, and phosphorylated phospholamban.

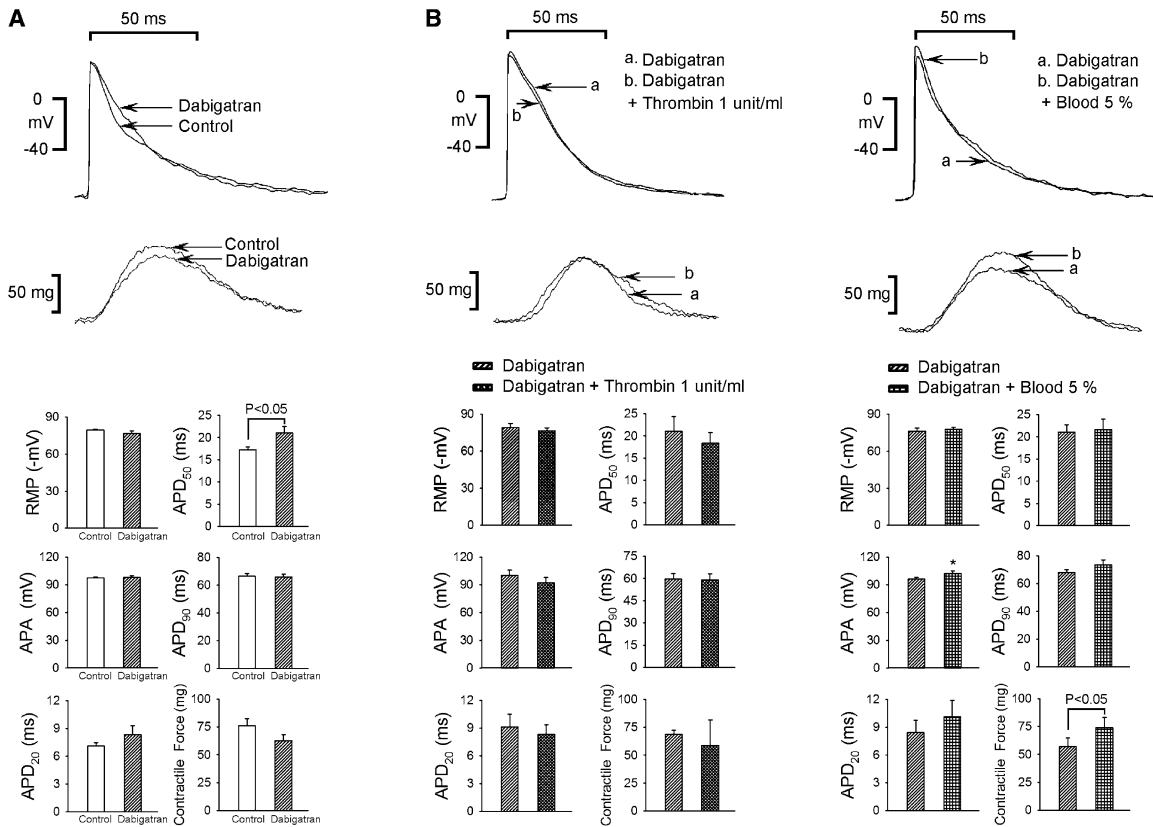
## Discussion

This study showed that thrombin reduced PV spontaneous activity but induced trigger activity and pause-dependent repetitive burst firing in PVs. Thrombin was shown to increase intracellular sodium and calcium ( $Ca^{2+}_i$ ) mediated by PAR1 activation, which may provoke the occurrence of afterdepolarization and trigger activity of PVs.<sup>26</sup> Thrombin increased  $Ca^{2+}_i$  of PV cardiomyocytes, which occurs at membrane voltages negative to the equilibrium potential for NCX, activating an inward NCX current, generating afterdepolarization, and reexcitation of the myocardium.<sup>6–8,26</sup> The pause-dependent robust accumulation of  $Ca^{2+}_i$  was demonstrated by the augmentation of contractility of beats after a long pause (Figures 1B and 4B). Thus, repetitive burst firings that exclusively occurred during slow PV activity or when the PV spontaneous activity ceased could be attributed to the pause-dependent higher concentration of  $Ca^{2+}_i$ . The thrombin-induced triggered beats and brady-tachyarrhythmic rhythm in PVs provided the arrhythmogenic triggers, which suggest a high arrhythmogenic potential of thrombin. In the presence of BMS 200261 (a PAR1 blocker), thrombin did not significantly change the PV electrophysiological or mechanical properties, which suggests that PAR1 plays a major role in the effects of thrombin in PVs. NO was previously shown to reduce the PV spontaneous electric activity.<sup>22</sup> In this study, we found that thrombin could relax PVs. This effect was caused by the activation of NOS through the PAR1 pathway, because thrombin did not change the PV electrophysiological or mechanical activity in the presence of L-NAME (the NOS inhibitor). Furthermore, thrombin has been shown to activate  $G_{\alpha q}$  and release ADP through PAR1 pathway.<sup>23</sup> Therefore, thrombin may also reduce PV spontaneous activity by an acetylcholine-like effect of ADP via its breakdown to adenosine. The synergistic effect of NO and ADP both mediated by thrombin would explain the marked attenuation or even pause in PV spontaneous activity. In vascular injury with thrombus formation, thrombin activity levels can reach as high as 10 to 30 units/mL.<sup>27</sup> Considering the thrombus formation and increased thrombin level in LA during AF, the nearby PV could be affected by the high thrombin level from LA. The dosage of thrombin used in this study was clinically relevant, and our findings may be applicable to the clinical presentation of AF.

The study also found that thrombin can elevate RMP, shorten APD, increase diastolic tension, and decrease contractile force in LA. These results can facilitate the genesis of AF and thrombosis in LA as a result of electric and mechanical changes.<sup>17,28</sup> Although the underlying molecular mechanisms for the effects of thrombin on LA were not fully elucidated in this study, the administration of BMS 200261 and L-NAME blocked the effects of thrombin, which suggests that PAR1's activation of NOS contributes to thrombin's effects on LA. Furthermore, stimulation of innervated vagal nerve sprouting around the atrium by thrombin may also contribute to the shortening of the APD and decreased contractility in LA.<sup>29</sup> However, different



**Figure 6.** Effects of thrombin and a blood clot solution on dabigatran-treated pulmonary veins (PVs). **A**, An example and average data show that dabigatran-treated PVs ( $n=10$ ) had slower beating rates than control PVs ( $n=30$ ) analyzed by unpaired *t* test. All baseline data (before drug or blood clot administration) of control and dabigatran-treated left atrium (LA) or PVs were collected. **B**, An example and average data show that thrombin did not change the spontaneous beating rates in dabigatran-treated PVs ( $n=4$ ) analyzed by 1-way repeated ANOVA. **C**, An example and average data show that the blood clot solution did not change the spontaneous beating rates in dabigatran-treated PVs ( $n=6$ ) analyzed by 1-way repeated ANOVA.



**Figure 7.** Effects of thrombin and a blood clot solution on the dabigatran-treated left atrium (LA). **A**, An example and average data show that the dabigatran-treated ( $n=10$ ) LA had a longer action potential duration (APD)<sub>50</sub> than the control ( $n=30$ ) LA analyzed by unpaired  $t$  test. **B**, Examples of the action potential amplitude (APA), APDs, and contractile force of the LA in rabbits treated with dabigatran insignificantly changed after the administration of 1 unit/mL thrombin ( $n=4$ ) or 5% blood clot solution ( $n=6$ ) analyzed by 1-way repeated ANOVA.

from those in PVs, thrombin increased LA diastolic tension. PAR1 agonists were also demonstrated to have inconsistent vessel effects with venous constriction and arterial dilation in humans.<sup>30</sup> Because distribution of NOS isoforms may differ between the atrium and PVs, it is possible that different activations of distinct cGMP-dependent and cGMP-independent signaling pathways may result in the different effects of thrombin on diastolic tensions between PV and LA.<sup>31</sup> Thrombin's reduction of LA contractile force may have resulted from mixed effects of vagal stimulation and the compartmentalized distribution of NOS isoforms and NO-related congeners.

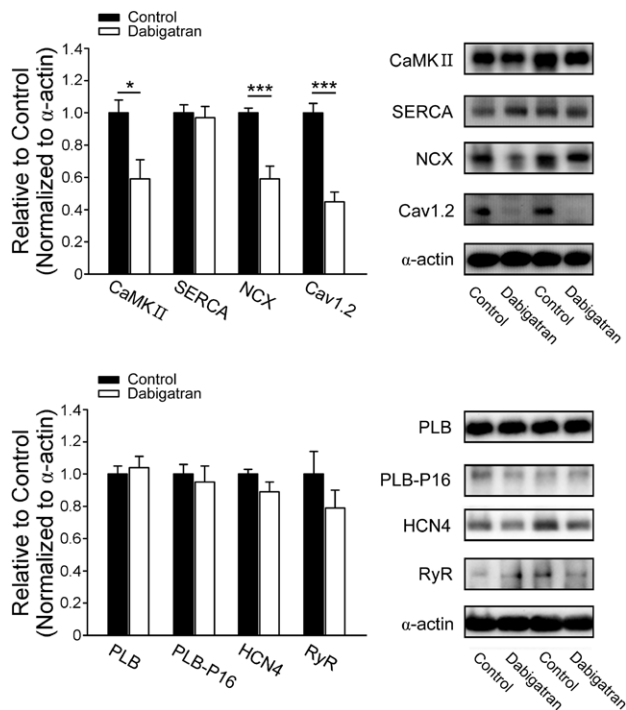
We found that the blood clot solution shared similar electrophysiological and mechanical effects to those of thrombin on PVs and LA, which were also attenuated by the presence of L-NAME. Therefore, blood clots may have significant cardiac effects mostly mediated by thrombin-activated NO production.

In this study, for the first time, we found that dabigatran may have electrophysiological effects by slowing PV spontaneous activity and prolonging LA APD<sub>50</sub>. However, we found a similar APD<sub>90</sub> in control and dabigatran-treated LA, which is comparable with findings in humans, whereas dabigatran did not change corrected QT interval.<sup>32</sup> The immunoblotting results showed lesser expressions of CaMKII, NCX, and Ca<sub>v</sub>1.2 on dabigatran-treated PVs. Thrombin has been shown to modulate CaMKII and activate NCX and L-type calcium current.<sup>7,33,34</sup> Therefore, dabigatran may reduce these calcium regulation proteins by inhibition of thrombin. Because CaMKII, NCX, and L-type calcium current

play an important role in PV electric activity,<sup>25,35</sup> these findings may contribute to the slower beating rates in dabigatran-treated PVs. However, it is not clear whether the effects of PV slowing and AP prolongation in LA by dabigatran may play a role in clinical applications. In addition, this study did not evaluate the changes of PAR1 expressions in dabigatran-treated PVs because the commercialized antibody cannot detect PAR1 in rabbits. After treatment with dabigatran, thrombin (1 unit/mL) had little electrophysiological effects on PVs and LA, which confirmed its thrombin-inhibitory effects. Interestingly, in the dabigatran-treated LA, the blood clot solution (5%) increased LA APA and contractility. Therefore, the blood solution may contain other factors that modulate LA electric and mechanical characteristics.

### Study Limitations

The data from this study should be interpreted with caution. First, microelectrode recordings showed electrophysiological heterogeneity within the PV with different diastolic depolarization. We may not directly record the APs from the ectopic activity. However, the microelectrode recordings can provide the information about the effects of thrombin on PV spontaneous activity because of overdrive suppressions from ectopy. Second, it would benefit from optical mapping of calcium and conduction in the tissue preparations. Dual imaging of transmembrane potential and intracellular calcium would be of great benefit to mechanistic dissection of the observed phenomena. Furthermore, we did not exclude the possibility



**Figure 8.** Expressions of ion channel proteins in control and dabigatran-treated pulmonary veins (PVs). The dabigatran-treated PVs ( $n=5$ ) had lesser expressions of Ca<sub>v</sub>1.2, sodium/calcium exchanger (NCX) and calmodulin kinase II (CaMKII) than control PVs ( $n=5$ ). The ryanodine receptor (RyR), sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA2a), hyperpolarization-activated cyclic nucleotide-gated potassium channel 4 (HCN4), phospholamban (PLB), and Ser16 phosphorylated phospholamban (p-PLB) were similar between control and dabigatran-treated PVs. Protein-band intensities normalized to  $\alpha$ -actin, relative to control. \* $P<0.05$ , \*\*\* $P<0.005$ .

that the thrombin effects may be attenuated by factors related to duration of the experiment (ie, edema, cellular uncoupling). Although thrombin or blood clot solution had significant effects on PVs and LA, the small sample size may limit the power of the study. Finally, we used normal rabbit PVs for this study. It is unclear whether the same applies to diseased PVs. Future studies in the intact atrium of an animal model of AF are needed to clarify whether dabigatran can be antiarrhythmic.

### Conclusions

Thrombin and blood clot solution regulated PV and LA arrhythmogenesis through electric and mechanical modifications by NO production and PAR1 activation. Dabigatran has electrophysiological effects and attenuated the effects of thrombin and blood clot solution on PV and LA electric and mechanical modulation.

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### Disclosures

None.

### References

- Ren JF, Marchlinski FE, Callans DJ, Gerstenfeld EP, Dixit S, Lin D, Nayak HM, Hsia HH. Increased intensity of anticoagulation may reduce risk of thrombus during atrial fibrillation ablation procedures in patients with spontaneous echo contrast. *J Cardiovasc Electrophysiol*. 2005;16:474–477.
- Zabalgaitia M, Halperin JL, Pearce LA, Blackshear JL, Asinger RW, Hart RG. Transesophageal echocardiographic correlates of clinical risk of thromboembolism in nonvalvular atrial fibrillation. Stroke Prevention in Atrial Fibrillation III Investigators. *J Am Coll Cardiol*. 1998;31:1622–1626.
- Choudhury A, Lip GY. Atrial fibrillation and the hypercoagulable state: from basic science to clinical practice. *Pathophysiol Haemost Thromb*. 2003;33:282–289.
- Akar JG, Jeske W, Wilber DJ. Acute onset human atrial fibrillation is associated with local cardiac platelet activation and endothelial dysfunction. *J Am Coll Cardiol*. 2008;118:476–481.
- Yan GX, Park TH, Corr PB. Activation of thrombin receptor increases intracellular Na<sup>+</sup> during myocardial ischemia. *Am J Physiol*. 1995; 268(4 pt 2):H1740–H1748.
- Chien WW, Mohabir R, Clusin WT. Effect of thrombin on calcium homeostasis in chick embryonic heart cells. Receptor-operated calcium entry with inositol trisphosphate and a pertussis toxin-sensitive G protein as second messengers. *J Clin Invest*. 1990;85:1436–1443.
- Albitz R, Droogmans G, Nilius B, Casteels R. Thrombin stimulates L-type calcium channels of guinea pig cardiomyocytes in cell-attached patches but not after intracellular dialysis. *Cell Calcium*. 1992;13:203–210.
- Jiang T, Danilo P Jr, Steinberg SF. The thrombin receptor elevates intracellular calcium in adult rat ventricular myocytes. *J Mol Cell Cardiol*. 1998;30:2193–2199.
- Pinet C, Le Grand B, John GW, Coulombe A. Thrombin facilitation of voltage-gated sodium channel activation in human cardiomyocytes: implications for ischemic sodium loading. *Circulation*. 2002;106:2098–2103.
- Avkiran M, Haworth RS. Regulatory effects of G protein-coupled receptors on cardiac sarcolemmal Na<sup>+</sup>/H<sup>+</sup> exchanger activity: signalling and significance. *Cardiovasc Res*. 2003;57:942–952.
- Pinet C, Algalarrondo V, Sablayrolles S, Le Grand B, Pignier C, Cussac D, Perez M, Hatem SN, Coulombe A. Protease-activated receptor-1 mediates thrombin-induced persistent sodium current in human cardiomyocytes. *Mol Pharmacol*. 2008;73:1622–1631.
- Steinberg SF, Robinson RB, Lieberman HB, Stern DM, Rosen MR. Thrombin modulates phosphoinositide metabolism, cytosolic calcium, and impulse initiation in the heart. *Circ Res*. 1991;68:1216–1229.
- Jacobsen AN, Du XJ, Lambert KA, Dart AM, Woodcock EA. Arrhythmogenic action of thrombin during myocardial reperfusion via release of inositol 1,4,5-trisphosphate. *Circulation*. 1996;93:23–26.
- Woodcock EA, Matkovich SJ, Binah O. Ins(1,4,5)P<sub>3</sub> and cardiac dysfunction. *Cardiovasc Res*. 1998;40:251–256.
- Yang Z, Arnet U, Bauer E, von Segesser L, Siebenmann R, Turina M, Lüscher TF. Thrombin-induced endothelium-dependent inhibition and direct activation of platelet-vessel wall interaction. Role of prostacyclin, nitric oxide, and thromboxane A<sub>2</sub>. *Circulation*. 1994;89:2266–2272.
- Kang KW, Choi SY, Cho MK, Lee CH, Kim SG. Thrombin induces nitric-oxide synthase via G $\alpha$ 12/13-coupled protein kinase C-dependent I-kappaB $\alpha$  phosphorylation and JNK-mediated I-kappaB $\alpha$  degradation. *J Biol Chem*. 2003;278:17368–17378.
- Chang SL, Chen YC, Chen YJ, Wangcharoen W, Lee SH, Lin CI, Chen SA. Mechano-electrical feedback regulates the arrhythmogenic activity of pulmonary veins. *Heart*. 2007;93:82–88.
- Haissaguerre M, Jaïs P, Shah DC, Takahashi A, Hocini M, Quiniou G, Garrigue S, Le Mouroux A, Le Métayer P, Clémenty J. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N Engl J Med*. 1998;339:659–666.
- Chen SA, Hsieh MH, Tai CT, Tsai CF, Prakash VS, Yu WC, Hsu TL, Ding YA, Chang MS. Initiation of atrial fibrillation by ectopic beats originating from the pulmonary veins: electrophysiological characteristics, pharmacological responses, and effects of radiofrequency ablation. *Circulation*. 1999;100:1879–1886.
- Chen YJ, Chen SA. Electrophysiology of pulmonary veins. *J Cardiovasc Electrophysiol*. 2006;17:220–224.

21. Chen YJ, Chen SA, Chang MS, Lin CI. Arrhythmogenic activity of cardiac muscle in pulmonary veins of the dog: implication for the genesis of atrial fibrillation. *Cardiovasc Res.* 2000;48:265–273.
22. Lin YK, Lu YY, Chen YC, Chen YJ, Chen SA. Nitroprusside modulates pulmonary vein arrhythmogenic activity. *J Biomed Sci.* 2010;17:20.
23. Tsai WC, Chen YC, Lin YK, Chen SA, Chen YJ. Sex differences in the electrophysiological characteristics of pulmonary veins and left atrium and their clinical implication in atrial fibrillation. *Circ Arrhythm Electrophysiol.* 2011;4:550–559.
24. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, Pogue J, Reilly PA, Themeles E, Varrone J, Wang S, Alings M, Xavier D, Zhu J, Diaz R, Lewis BS, Darius H, Diener HC, Joyner CD, Wallentin L; RE-LY Steering Committee and Investigators. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med.* 2009;361:1139–1151.
25. Lo LW, Chen YC, Chen YJ, Wongcharoen W, Lin CI, Chen SA. Calmodulin kinase II inhibition prevents arrhythmic activity induced by alpha and beta adrenergic agonists in rabbit pulmonary veins. *Eur J Pharmacol.* 2007;571:197–208.
26. Patterson E, Jackman WM, Beckman KJ, Lazzara R, Lockwood D, Scherlag BJ, Wu R, Po S. Spontaneous pulmonary vein firing in man: relationship to tachycardia-pause early afterdepolarizations and triggered arrhythmia in canine pulmonary veins *in vitro*. *J Cardiovasc Electrophysiol.* 2007;18:1067–1075.
27. Park TH, McHowat J, Wolf RA, Corr PB. Increased lysophosphatidylcholine content induced by thrombin receptor stimulation in adult rabbit cardiac ventricular myocytes. *Cardiovasc Res.* 1994;28:1263–1268.
28. Kalifa J, Jalife J, Zaitsev AV, Bagwe S, Warren M, Moreno J, Berenfeld O, Nattel S. Intra-atrial pressure increases rate and organization of waves emanating from the superior pulmonary veins during atrial fibrillation. *Circulation.* 2003;108:668–671.
29. Kwong K, Nassenstein C, de Garavilla L, Meeker S, Udem BJ. Thrombin and trypsin directly activate vagal C-fibres in mouse lung via protease-activated receptor-1. *J Physiol (Lond).* 2010;588(pt 7):1171–1177.
30. Gudmundsdóttir IJ, Megson IL, Kell JS, Ludlam CA, Fox KA, Webb DJ, Newby DE. Direct vascular effects of protease-activated receptor type 1 agonism *in vivo* in humans. *Circulation.* 2006;114:1625–1632.
31. Ziolo MT, Kohr MJ, Wang H. Nitric oxide signaling and the regulation of myocardial function. *J Mol Cell Cardiol.* 2008;45:625–632.
32. Ring A, Clemens A, Rathgen K, Stangier J, Feifel U, Reilly P, Friedman J. Dabigatran does not prolong the QTC interval with supratherapeutic exposure: a thorough QTC study. *J Am Coll Cardiol.* 2011;57:E56.
33. Wang Z, Ginnan R, Abdullaev IF, Trebak M, Vincent PA, Singer HA. Calcium/Calmodulin-dependent protein kinase II delta 6 (CaMKIIdelta6) and RhoA involvement in thrombin-induced endothelial barrier dysfunction. *J Biol Chem.* 2010;285:21303–21312.
34. Roberts DE, Matsuda T, Bose R. Molecular and functional characterization of the human platelet Na(+)/Ca(2+) exchangers. *Br J Pharmacol.* 2012;165:922–936.
35. Wongcharoen W, Chen YC, Chen YJ, Chang CM, Yeh HI, Lin CI, Chen SA. Effects of a Na+/Ca2+ exchanger inhibitor on pulmonary vein electrical activity and ouabain-induced arrhythmogenicity. *Cardiovasc Res.* 2006;70:497–508.

### CLINICAL PERSPECTIVE

Atrial fibrillation is associated with an increased risk of cardiac thrombin formation and stroke, which can be prevented by oral anticoagulant therapy, including the selective direct thrombin inhibitor dabigatran. Thrombin has electrophysiological effects, increasing intracellular calcium and sodium currents. In the present study, we demonstrated that thrombin and blood clot solution reduced the spontaneous beating rate and induced delayed afterdepolarization or burst firings in pulmonary veins (PVs) in rabbit atrial preparations. In addition, thrombin and blood clot solution enhanced the diastolic tension and decreased the action potential duration and contractility in left atrium. Treatment of N<sup>G</sup>-nitro-L-arginine methyl ester or a protease-activated receptor type 1 blocker (BMS 200261) attenuated the effects of thrombin and blood clot solution on PVs and left atrium. Dabigatran-treated PVs had slower spontaneous activity with decreased expression of calmodulin kinase II, the sodium calcium exchanger, and Ca<sub>v</sub>1.2 compared with control PVs. Thrombin and blood clot solution did not change the electric and mechanical properties in dabigatran-treated PVs or left atrium. These findings suggest that higher cardiac thrombin may increase arrhythmogenesis in PVs and left atrium. Furthermore, dabigatran may have anti-atrial fibrillation potential through reducing thrombin effects and PV arrhythmogenesis.



## Dabigatran and Thrombin Modulate Electrophysiological Characteristics of Pulmonary Vein and Left Atrium

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