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

Running title: Chang et al.; Dabigatran and Thrombin on Pulmonary Vein and LA

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Abstract:

Background - Dabigatran reduces stroke in atrial fibrillation (AF). Pulmonary veins (PVs) and left atrium (LA) play a critical role in the pathophysiology of AF. 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 (APs) 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±0.2 to 1.7±0.2, 1.6±0.2, and 1.4±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±0.4 to 1.8±0.4 and 1.3±0.3 Hz (P=0.044). Thrombin (0.01, 0.1, and 1.0 units/ml) and the blood clot solution (0.5% and 5.0%) increased LA diastolic tension and the resting membrane potential with decreased AP duration and contractility. Thrombin (0.01, 0.1 and 1 units/ml) and blood clot solution (0.5% and 5%) induced delayed afterdepolarization and burst firing in PVs, but not in LA. L-NAME (100 μM) or a protease-activated receptor type 1 (PAR1) blocker (BMS 200261, 1 μM) attenuated the effects of thrombin and the blood clot solution in PVs and LA. Dabigatran-treated PVs had slower spontaneous activity (1.1±0.1 Hz, n=10, P=0.0001 vs. control). There electrophysiological characteristics were not changed by thrombin (1 unit/ml) and blood clot solution (5%).

Conclusions - Thrombin modulates PV and LA electrical and mechanical characteristics, which were blocked by dabigatran.

Key words: atrial fibrillation; direct thrombin inhibitor; protease-activated receptor; pulmonary vein; thrombin
Introduction

Atrial fibrillation (AF) with spontaneous echo contrast or thrombus formation in LA and appendage were reported from 10 to 35%. An increased cardiac thrombin concentration associated with a prothrombotic situation was demonstrated during paroxysm of AF. Thrombin can modulate electrophysiological properties of cardiomyocyte and promotes the genesis of ventricular arrhythmia with haemostatic thrombosis. Thrombin affects spontaneous automaticity and elevates intracellular calcium and sodium in cardiomyocytes. Thrombin also increases cesium-induced early afterdepolarizations in isolated canine Purkinje fibers and induces proarrhythmic events during early repolarization in intact adult rat hearts. These findings indicate that thrombin has direct electrophysiological effects on cardiomyocytes. Thrombin activates protease-activated receptor type 1 (PAR1) and stimulates nitric oxide (NO) synthase (NOS) with NO production and vascular relaxation. Accordingly, thrombin may attenuate vascular stretching and modulate mechanoelectrical feedback, which may contribute to the electrophysiological effects of thrombin and blood clots.

Pulmonary veins (PVs) are important AF initiators, and left atrium (LA) is the main AF substrate for reentry. PVs contain a mixture of working myocardium and pacemaker cells that can induce atrial arrhythmias. Previous studies indicated that the NO donor has anti-AF effects and reduces PV arrhythmogenic activity. In addition, mechanoelectrical feedback plays
an important role in the pathophysiology of PV arrhythmogenesis.\textsuperscript{17,23} Therefore, thrombin or blood clots may modulate PV or LA arrhythmogenesis.

Dabigatran, a selective direct thrombin inhibitor, reversibly binds to thrombin reduces thrombus formation, and can reduce stroke in AF patients.\textsuperscript{24} 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 evaluate the potential modulatory roles of NO, PAR1, and a direct thrombin inhibitor.

Methods

Rabbit PV and LA Tissue Preparations

Experiments in this study conformed to the institutional \textit{Guide for the Care and Use of Laboratory Animals} and the \textit{Guide for the Care and Use of Laboratory Animals} published by the US National Institutes of Health. Control and dabigatran-treated (6 mg, twice/day for consecutive 3 days) male rabbits (that weighted 1–2 kg) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg kg\textsuperscript{-1}). A midline thoracotomy was then performed, and the heart and lungs were removed as described previously.\textsuperscript{25} 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’s solution with a composition (in mM) of 137 NaCl, 4 KCl, 15 NaHCO₃, 0.5 NaH₂PO₄, 0.5 mgCl₂, 2.7 CaCl₂, 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. The adventitial or epicardial side of the preparations faced upwards. The PV and LA tissue strips were superfused at a constant rate (3 ml/min) with Tyrode’s solution saturated with a 97% O₂ -3% CO₂ gas mixture. The temperature was maintained at 37°C, and the preparations were allowed to equilibrate for 1 h 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 M KCl before and after the administration of thrombin (0.01, 0.1, and 1 units/ml) or 0.5% and 5% blood clot solutions (blood clot from 0.5 ml or 5 ml rabbit blood, and administrated in 100 ml Tyrode’s solution for 30 min) in control and dabigatran-treated preparations. Only one PV or LA strip from each control or dabigatran-treated rabbit sequentially received different concentrations of thrombin (0.01, 0.1, and 1 units/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.23,25 The electrical 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 cut-off 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 electrical stimulus. In order to study the molecular mechanisms of thrombin, a BMS-200261 (PAR1 blocker, 1 μM, Sigma), or N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) solution (100 μM) was administrated to the PV or LA in the presence or absence of thrombin (1 unit/ml). L-NAME (100 μM) was administrated to the PV or LA with a blood clot solution to evaluated the mechanism of blood clots. The electrical 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 APD\textsubscript{90}, APD\textsubscript{50}, and APD\textsubscript{20}, respectively. The RMP, APA, APD\textsubscript{90}, APD\textsubscript{50}, APD\textsubscript{20}, and contractile forces were measured under 2-Hz pacing of the LA before and after drug administration.
Delayed afterdepolarizations (DADs) were defined as the presence of a spontaneous depolarization of the impulse after full repolarization.25

**Western blot analysis of ion channel proteins**

Control and dabigatran–treated PVs were washed with cold PBS and lysed on ice for 30 min in a radio-immuno-precipitation assay (RIPA) buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS and protease inhibitor cocktails (Sigma-Aldrich Corp., St Louis, MO, USA). 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 Cav1.2 (I\textsubscript{Ca-L} subunit, Alomone Labs, Jerusalem, Israel), ryanodine receptor (RyR, Thermo, Rockford, IL, USA), sarcoendoplasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA2a, Santa Cruz Biotechnology, Santa Cruz, CA, USA), calmodulin kinase II (CaMKII, Abcam, Cambridge, UK), sodium/calcium exchanger (NCX, Swant, Bellinzona, Switzerland), hyperpolarization activated cyclic nucleotide-gated potassium channel 4 (HCN4, Millipore, Billerica, MA, USA), phospholamban (PLB, Thermo, Rockford, IL, USA), Ser16 phosphorylated PLB (p-PLB, Badrilla, Leeds, UK) and secondary antibodies conjugated with horse radish peroxidase. Bound antibodies were detected with the ECL detection system (Millipore) and analyzed with ALPHAEASEFC software (Alpha Innotech, San Leandro, CA, USA). Targeted bands were normalized to cardiac
α-sarcomeric actin (Sigma-Aldrich Corp.) to confirm equal protein loading.

**Statistical Analysis**

All continuous variables are expressed as the mean ± SEM. One-way repeated measures analysis of variance (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, 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. McNemar's test was used to compare the incidences of a long pause, DAD, and burst firing before and after drug administration on PVs. A P value of <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 units/ml) concentration-dependently decreased PV beating rates. In addition, thrombin induced long pauses (≥3 s) in 2 of 6 PVs (33% vs. 0% at baseline, P=0.157) at 0.01 units/ml, and induced long pauses in 4 of 6 PVs (67% vs. baseline, P=0.045) at 0.1 and 1 units/ml, respectively (Figure 1B). As compared to baseline, thrombin (0.01, 0.1, and 1 units/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 DADs and triggered beats in 3 PVs (50% vs. 0% at baseline, P=0.083) at 0.01 units/ml, and 5 PVs (83%, P=0.025) at 0.1 and 1 units/ml (Figure 1C). Moreover, thrombin (0.01, 0.1, and 1 units/ml) concentration-dependently decreased the diastolic tension of PVs by 2±7 (P=0.452), 6±7 (P=0.265) and 48±25 mg (P=0.031, Figure 1D), respectively.

In the presence of L-NAME (100 μM), 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 μM), 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$_{20}$ and APD$_{90}$, but shortened APD$_{50}$ at 1 unit/ml, and decreased contractile force at 1 unit/ml in LA. Moreover, thrombin (0.01, 0.1, and 1 units/ml) respectively increased the LA diastolic tension by 477±160 (P=0.047), 543±185 (P=0.019), and 488±190 mg (P=0.034, Figure 3B). In the presence of L-NAME (100 μM) or BMS-200261 (1 μM), thrombin (1 unit/ml) did not significantly change LA electrophysiological or mechanical characteristics (Figure 3C).

**Effects of blood clot solution in PVs and LA**
As shown in Figure 4A, blood clot solutions (0.5% and 5%) concentration-dependently decreased PV spontaneous beating rate. Similar to those in thrombin, the blood clot solution induced a long pause in 3 of 5 PVs (60% vs. 0% at baseline, P=0.083) at a concentration of 0.5%, and 5 of 5 PVs (100% vs. baseline, P=0.025) at a concentration of 5%. Moreover, the blood clot solution (0.5% and 5%) induced repetitive PV burst firings in 60% (vs. 0% at baseline, P=0.083) and 80% of PVS (vs. baseline, P=0.045), respectively (Figure 4B). Compared to the baseline, the blood clot solution (0.5% and 5%) also induced the occurrence of DADs 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±13 mg (P=0.147), and 81±46 mg (P=0.04). In the presence of L-NAME (100 μM), 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 APD_{50} and 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±83 (P=0.017) and 455±78 mg (P=0.002). In the presence of L-NAME (100 μM), the blood clot solution (0.5% and 5%) did not significantly change LA electrophysiological or mechanical characteristics (Figure 5B).
**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 5% 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$_v$1.2 than control PVs. However, control and dabigatran-treated PVs had similar expression of RyR, SERCA2a, HCN4, PLB and p-PLB.

**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+}) mediated by PAR1 activation, which may provoke the occurrence of afterdepolarization and trigger activity of PVs\textsuperscript{26}. Thrombin increased Ca^{2+} of PV cardiomyocytes which occurs at membrane voltages negative to the equilibrium potential for sodium-calcium exchanger (NCX), activating an inward NCX current, generating afterdepolarization and re-excitation of the myocardium\textsuperscript{6-8,26}. The pause-dependent robust accumulation of Ca^{2+} was demonstrated by the augmentation of contractility of beats after a long-pause (Figure 1B, 4B). Thus repetitive burst firings that exclusively occurred during slowed or ceased of PV spontaneous activity could be attributed to the pause-dependent higher concentration of Ca^{2+}. 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 electrical activity\textsuperscript{22}. In this study, we found that thrombin could relax PVs. This effect was caused by the activation of NOS through the PAR1 pathway, since thrombin did not change the PV electrophysiological or mechanical activity in the presence of L-NAME (the NOS inhibitor). Moreover, thrombin has been shown to activate G_{eq} and release ADP through PAR1 pathway\textsuperscript{23}. 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 10–30 units/ml. Considering the thrombus formation and increased thrombin level in LA during atrial fibrillation, 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 due to electrical and mechanical changes. 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 thrombin’s effects, which suggests that PAR1’s activation of NOS contributes to thrombin’s effects on LA. Moreover, 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. However, different from those in PVs, thrombin increased LA diastolic tension. PAR-1 agonists were also demonstrated to have inconsistent vessel effects with venous constriction and arterial dilatation in humans. Since distribution of NOS isoforms may differ between the atrium and PVs, it is possible that different
activations of distinct cGMP-dependent and/or cGMP-independent signaling pathway may result in the different effects of thrombin on diastolic tensions between PV and LA. 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 the 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$_{50}$. However, we found a similar APD$_{90}$ in control and dabigatran-treated LA, which is comparable with findings in humans whereas dabigatran did not change corrected QT interval. The immunoblotting results showed lesser expressions of CaMKII, NCX and Ca$_v$1.2 on dabigatran-treated PVs. Thrombin has been shown to modulate CaMKII and activate NCX and L-type calcium current. Therefore, dabigatran may reduce these calcium regulation proteins by inhibition of thrombin. Since CaMKII, NCX and L-type calcium current play an important role in PV electrical activity, 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 during clinical applications. In addition, this study did not evaluate the changes of PAR1 expressions in dabigatran-treated PVs since the commercialized antibody can’t 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 which modulate LA electrical 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 the ectopy. Second, it will be 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. Moreover, we did not exclude the possibility that the thrombin effects may be attenuated by factors related to duration of the experiment (i.e. edema, cellular uncoupling, etc.). 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 if 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 a blood clot solution regulated PV and LA arrhythmogenesis through electrical and mechanical modifications by NO production and PAR1 activation. Dabigatran has electrophysiological effects and attenuated the effects of thrombin and a blood clot solution on PV and LA electrical and mechanical modulation.

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Conflict of Interest Disclosures: None.

References:


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**Figure Legends:**

**Figure 1.** Effects of thrombin on pulmonary veins (PVs). (A) Example and average data of thrombin (0.01, 0.1, and 1 units/ml) on PV beating rates (*n*=6). (B) Examples of thrombin-induced pauses (left panel), and pause-related burst firing in PVs (right panel) (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.

**Figure 2.** Effects of thrombin on pulmonary veins (PVs) in the presence of L-NAME or BMS-200261. Examples and average data show that thrombin (1 units/ml) did not change the PV spontaneous beating rates in the presence of L-NAME (100 µM, panel A, n=6) or BMS-200261 (1 µM, panel B, n=6).

**Figure 3.** Effects of thrombin on the left atrium (LA) in the presence or absence of L-NAME or BMS-200261. (A) Examples and average data of thrombin (0.01, 0.1, and 1 units/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 µM) or BMS-200261 (1 µM). *P< 0.05 vs. baseline.

**Figure 4.** Effects of a blood clot solution on pulmonary veins (PVs) before and after the presence of L-NAME. (A) Example and average data of a blood clot solution (0.5% and 5%) on PV beating rates (n=5) (B) Examples of blood clot solution-induced pauses (upper panel) and burst firings (lower panel) 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 µM,
n=6). *P< 0.05 vs. baseline.

**Figure 5.** Effects of a blood clot solution on the left atrium (LA) in the presence or absence of 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 presence of L-NAME. *P< 0.05, **P< 0.01, ***P< 0.005 vs. baseline.

**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 drugs or blood clot solution administration) of control and dabigatran-treated LA or PV 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 one-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 one-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
APD<sub>50</sub> than the control (n=30) LA analyzed by unpaired t-test. (B) Examples of the action potential amplitude (APA), action potential durations (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 one-way repeated ANOVA.

**Figure 8.** Expressions of ion-channel proteins in control and dabigatran-treated PVs. The dabigatran-treated (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), sarcoendoplasmic 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 α-actin, relative to control.

*P< 0.05, *** P<0.005.
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