International Journal of Cardiology 184 (2015) 559–567

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Selective and non-selective non-steroidal anti-inflammatory drugs differentially regulate pulmonary vein and atrial arrhythmogenesis

Chien-Jung Chang [a,b,1], Chen-Chuan Cheng [c,d,1], Ten-Fang Yang [a], Yao-Chang Chen [e], Yung-Kuo Lin [f], Shih-Ann Chen [g,h], Yi-Jen Chen [f,i,*]

[a] Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan
[b] Division of Cardiology, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan
[c] Division of Cardiology, Chi-Mei Medical Center, Tainan, Taiwan
[d] School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[e] Department of Biomedical Engineering, and Institute of Physiology, National Defense Medical Center, Taipei, Taiwan
[f] Division of Cardiovascular Medicine, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan
[g] National Yang-Ming University, School of Medicine, Taipei, Taiwan
[h] Division of Cardiology and Cardiovascular Research Center, Veterans General Hospital-Taipei, Taipei, Taiwan
[i] Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form 12 January 2015
Accepted 3 March 2015
Available online 5 March 2015

Keywords:
Celecoxib
Indomethacin
Atrial fibrillation
Pulmonary vein
Atrium
Ranolazine

ABSTRACT

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) increase the risk of atrial fibrillation (AF). This study investigated whether selective and non-selective NSAIDs differentially regulate the arrhythmogenesis of pulmonary veins and atria.

Methods: Conventional microelectrodes were used to record action potentials (APs) in isolated rabbit PVs, sino-atrial node (SAN), left atrium (LA), and right atrium (RA) preparations before and after celecoxib or indomethacin administration. A whole-cell patch clamp was used to record the sodium–calcium exchanger (NCX) current, L-type calcium current ($I_{Ca\text{-}L}$), and late sodium current ($I_{Na\text{-}late}$) before and after celecoxib administration in isolated PV cardiomyocytes.

Results: Celecoxib (0.3, 1, and 3 μM) reduced PV spontaneous beating rates, and induced delayed afterdepolarizations and burst firings in four of eight PV preparations (50%, $p < 0.05$). Celecoxib also reduced SAN beating rates and decreased AP durations (APDs) in RA and LA, but did not change the resting membrane potential. Indomethacin (0.3, 1, 3, and 10 μM) changed neither the PV or SAN beating rates nor RA APDs, but it reduced LA APDs. Celecoxib (3 μM) significantly increased the NCX current and decreased the $I_{Ca\text{-}L}$, but did not change the $I_{Na\text{-}late}$. Ranolazine (10 μM) suppressed celecoxib (3 μM)-induced PV burst firings in 6 (86%, $p < 0.05$) of 7 PVs. KB-R7943 (10 μM) suppressed celecoxib (3 μM)-induced PV burst firings in 5 (71%, $p < 0.05$) of 7 PVs.

Conclusions: Selective and non-selective NSAIDs differentially modulate PV and atrial electrophysiological characteristics. Celecoxib increased PV triggered activity through enhancement of the NCX current, which contributed to its arrhythmogenesis.

1. Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia observed in clinical practice [1–3]. There is accumulating evidence which suggests that inflammation is involved in AF pathogenesis [4–6]. The use of non-steroidal anti-inflammatory drugs (NSAIDs) was logically expected to be antiarrhythmic; however, their use was reported to increase the risk of AF [7–9]. The mechanisms through which NSAIDs increase AF occurrences are still not fully elucidated; in addition, the use of selective cyclooxygenase (COX)-2 inhibitors was found to produce a remarkably higher risk of AF (with an adjusted incidence ratio of up to 1.71) [9].

Celecoxib is a widely used selective COX-2 inhibitor, and the only remaining one in US market [10], but it was shown to reduce the heart rate and induce arrhythmia in *Drosophila* and rat cardiac cells [11–13]. Selective COX-2 inhibition disturbs the balance between pro-aggregatory thromboxane and anti-aggregatory prostacyclin, which can enhance atherosclerosis and coronary heart disease [14]. Prostacyclin, a major COX-2-derived prostaglandin, was demonstrated to act as an endogenous antiarrhythmic by directly inhibiting epicardial sympathetic nerve activity [15].

* Corresponding author at: Division of Cardiovascular Medicine, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, 111 Hsin-Lung Road, Sec. 3, Taipei 110, Taiwan.
E-mail address: a9900112@ms15.hinet.net (Y.-J. Chen).
[1] CJ Chang and CC Cheng contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.ijcard.2015.03.066
0167-5273/

In addition, selective deletion of COX-2 in mice was reported to induce interstitial and perivascular fibrosis with an enhanced susceptibility to induced arrhythmias [16]. On the other hand, celecoxib has several ionic channel effects and interferes with the cardiovascular electrophysiology [12,17]. Therefore, COX-2 inhibitors can directly affect ion channels and intracellular signaling pathways, which may contribute to arrhythmogenesis in addition to the consequence of COX-2 inhibition.

Pulmonary veins (PVs) and left atrium (LA) are the most important AF trigger and substrate, respectively. PVs contain both cardiomyocytes and vascular structures; accordingly, vascular mechanoelectrical feedback shares an important role in PV arrhythmogenesis. Since selective and non-selective NSAIDs have significant cardiovascular effects, it is not clear whether selective and non-selective COX-2 inhibitors differentially regulate PV or atrial electromechanical characteristics and lead to different risks of AF genesis. In addition, AF is known to induce electrical remodeling with abnormal calcium handling and several types of ionic dysregulation in PVs and atrial substrates. As the sodium calcium exchanger (NCX) and late sodium current ($I_{Na\text{-}late}$) play pivotal roles in the pathophysiology of AF, both of them are also targets for AF therapy [18–21].

Ranolazine was developed as an antianginal agent with an additional antiarrhythmic potential. Ranolazine can inhibit both the $I_{Na\text{-}late}$ and NCX channel, which improves diastolic compliance and thereby increases coronary artery blood flow [22–24]. COX-2 inhibitors are documented to increase coronary vasculopathy and arrhythmia, both of which are potentially treated by ranolazine. Therefore, the purposes of this study were to evaluate the electrical effects of selective and non-selective NSAIDs on PVs and the atria, and additionally to elucidate the potential mechanisms. Furthermore, we also evaluated whether ranolazine can modulate the electrophysiological effects of NSAIDs.

2. Methods

2.1. Rabbit PV and LA tissue preparations

The investigation was approved by a local ethics review board (IACUC-14-187) and 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 (NIH publication NO. 85-23, revised 1996). Male rabbits (weighing 1.5–2 kg) were anesthetized with an intraperitoneal injection of sodium pentobarbital (100 mg kg^{-1}). A midline thoracotomy was then performed, and the heart and lungs were removed as described previously [25]. The PVs were separated from LA 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 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $mgCl_2$, 2.7 $CaCl_2$, and 11 dextrose (the pH was adjusted to 7.4 by titration with NaOH). One end of a preparation, 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 silk thread. For atrial experiments, the right atrium (RA) and LA were isolated and prepared as described previously [26]. The adventitial or epicardial side of the preparations faced upwards. PVs, RA, and LA tissue strips were superfused at a constant rate (3 ml/min) with Tyrode's solution saturated with a 97% O_2–3% CO_2 gas mixture. The sinoatrial node (SAN) behaves spontaneous beating and is located near the junction between the RA and superior vena cava. The temperature was maintained at 37 °C, and preparations were allowed to equilibrate for 1 h before the electrophysiology assessment.

2.2. Electrophysiological and pharmacological studies of the PV and atrial preparations

Transmembrane action potentials (APs) of the PVs, RA, and LA were recorded using machine-pulled glass capillary microelectrodes filled with 3 M KCl before and after administration of celecoxib (0.03, 0.1, 0.3, 1, and 3 μM) or indomethacin (0.3, 1, 3, and 10 μM). Preparations were connected to a WPI model FD223 electrometer under a tension of 150 mg as described previously [25,26]. The electrical and mechanical events (diastolic tension) were simultaneously displayed and recorded on a Gould 4072 oscilloscope and a Gould TA11 recorder. 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. The electrical and mechanical events (diastolic tension) were continuously and simultaneously displayed and recorded during all of 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 (APDs) at repolarization rates of 90%, 50%, and 20% of the APA were respectively measured as the APD_{90}, APD_{50}, and APD_{20}. The RMP, APA, and APDs were measured under 2-Hz pacing of the RA and LA before and after drug administration. Delayed afterdepolarizations (DADs) were defined as the presence of spontaneous depolarization of an impulse after full repolarization. As described previously, burst firing was defined as the occurrence of an accelerated spontaneous action potential (faster than the basal rate) with sudden onset and termination [25,26]. Ranolazine (10 μM) or KB-R7943 (2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl] isothiourea methane sulfonate, 10 μM) was administered in the PVs with celecoxib (3 μM)-induced burst firings.

2.3. Ionic currents in isolated PV cardiomyocytes

Single PV cardiomyocytes were enzymatically dissociated with the same procedure as described previously [27]. A whole-cell patch clamp was used on single isolated cardiomyocytes before and after administration of celecoxib (3 μM) using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA, USA) at 35 ± 1 °C [28]. Borosilicate glass electrodes (o.d., 1.8 mm) with tip resistances of 3–5 MΩ were used. Before the formation of the membrane-pipette seal, the tip potentials were zeroed in Tyrode's solution containing (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 10, and glucose 11 (adjusted to pH 7.4 with NaOH). Ionic currents were recorded in the voltage-clamp mode. A small hyperpolarizing step from a holding potential of −50 mV to a test potential of −55 mV for 80 ms was delivered at the beginning of each experiment. The area under the capacitative current was divided by the applied voltage step to obtain the total cell capacitance. Normally, 60%–80% series resistance (Rs) was electronically compensated for.

The $I_{Ca\text{-}L}$ was measured as an inward current during depolarization from a holding potential of −50 mV to test potentials that ranged −40 to +60 mV in 10-mV steps for 300 ms at a frequency of 0.1 Hz by means of a perforated patch clamp. The NaCl and KCl in the external solution were respectively replaced with TEACl and CsCl. Micropipettes were filled with a solution containing (in mM) CsCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, NaGTP 0.1, and Na_2 phosphocreatine 5 (adjusted to a pH of 7.2 with CsOH).

The $I_{Na\text{-}Late}$ included a step/ramp protocol (−100 mV stepping to +20 mV for 100 ms, then ramping back to −100 mV over 100 ms) at room temperature with an external solution containing (in mM): NaCl 130, CsCl 5, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 10, and glucose 10 (at pH 7.4 adjusted with NaOH). Micropipettes were filled with a solution containing (in mM): CsCl 130, Na_2ATP 4, $MgCl_2$ 1, EGTA 10, and HEPES 5 (at pH 7.3 adjusted with NaOH). An equilibration period (5–10 min) of dialysis was allowed to adequately clamp the cell currents. The $I_{Na\text{-}Late}$ was measured as the tetrodotoxin (TTX, 30 μM)-sensitive portion of the current traces obtained during voltage ramping back to −100 mV [29].

The NCX current was elicited by depolarizing pulses between −100 and +100 mV from a holding potential of −40 mV for 300 ms at a frequency of 0.1 Hz. The amplitudes of the NCX current were measured as 10-mM nickel-sensitive currents [30]. The external solution (in mM) consisted of NaCl 140, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 5, and glucose 10 at