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Comparison of the Effects of a Transient Outward Potassium Channel Activator on Currents Recorded from Atrial and Ventricular Cardiomyocytes

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Abstract

Introduction—NS5806 activates the transient outward potassium current (I_{to}) in canine ventricular cells. We compared the effects of NS5806 on canine atrial versus ventricular tissues and myocytes.

Methods and Results—NS5806 (10 μM) was evaluated in arterially-perfused canine right atrial and right ventricular wedge preparations. In ventricular wedges NS5806 (10 μM) accentuated phase 1 in epicardium (Epi), with little effect in endocardium (Endo), resulting in augmented J-waves on the ECG. In contrast, application of NS5806 (10 μM) to atrial preparations had no effect on phase 1 repolarization but significantly decreased upstroke velocity (dV/dt) and depressed excitability, consistent with sodium channel block. Current and voltage-clamp recordings were made in the absence and presence of NS5806 in (10 μM) enzymatically dissociated atrial and ventricular myocytes. In ventricular myocytes, NS5806 increased I_{to} magnitude by 80% and 16% in Epi and Endo, respectively (at +40 mV). In atrial myocytes, NS5806 increased peak I_{to} by 25% and had no effect on the sustained current, I_{Kur}. Under control conditions, I_{Na} density in atrial myocytes was nearly double that in ventricular myocytes. NS5806 caused a shift in steady-state mid-inactivation ($V_{½}$) from −73.9±0.27 to −77.3±0.21 mV in ventricular and from −82.6±0.12 to −85.1±0.11 mV in atrial cells, resulting in reduction of I_{Na} in both cell types. Expression of mRNA encoding putative I_{Na} and I_{to} channel subunits was evaluated by qPCR.

Conclusion—NS5806 produces a prominent augmentation of I_{to} with little effect on I_{Na} in the ventricles, but a potent inhibition of I_{Na} with little augmentation of I_{to} in atria.

Keywords

Transient outward potassium current; sodium current; atria; ventricle; NS5806

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AUTHOR CONTRIBUTIONS

Conception and design of study; JMC and KC. Collection, analysis and interpretation of data; JMC, KC, NC, EN, TJ, JMD. Drafting the article or revising it critically; JMC, KC, SPO, CA. All authors approved the final version of the manuscript.

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INTRODUCTION

The action potential (AP) in cardiac muscle is an important physiological parameter: 1) it is responsible for electrical conduction, 2) it modulates the refractory period and 3) associated with each action potential is a contraction via the process of excitation-contraction coupling. Marked differences in the shape and duration of the cardiac action potential have been described in different regions of the mammalian heart, reflecting differences in the underlying ionic currents. For example, comparative studies of K^+ currents in atrial and ventricular tissue have shown that the inward rectifier K^+ current is large in ventricle versus atria[1,2] and the ultra rapid delayed rectifier K current, I_{Kur}, is large in atria but small[3] or virtually absent in the ventricle[4]. These differences in the complement of K^+ currents between the 2 tissue types have been ascribed to variations in the molecular subunits[5–7]. Marked differences in the kinetics and properties of Na^+ current have also been described in the 2 tissue types[8–10]. Na^+ current in atrial tissue has a larger current density and more negative steady-state inactivation compared to ventricular tissue; these differences are thought to contribute to the atrial-selective anti-arrhythmic effects of many compounds.

In atrial and ventricular tissue from dog, the action potential exhibits a large, rapid phase 1 repolarization, suggesting the presence of a Ca^{2+}-independent transient outward K^+ current (I_{to}). I_{to} is thought to be mediated by $K_v4.3$[11,12] together with K^+ Channel Interacting Protein (KChIP2)[13]. The expression of KChIP2 is high in the epicardium (Epi) and midmyocardium (Mid), and low in the endocardium (Endo) which is believed to account for the transmural gradient of I_{to}[14,15] but other proteins, such as the dipeptidyl aminopeptidase-like protein (DPP6)[15,16] and KCNEs[17,18] may also associate with $K_v4.3$ and affect current. It has been suggested that $K_v1.4$ contributes to canine ventricular I_{to} and although $K_v1.4$ is uniformly expressed across the ventricular wall[15,19], it may have a relatively larger contribution to Endo I_{to}, where KChIP2 expression is lower, resulting in smaller $K_v4.3$/KChIP2 current compared to the Epi- and Mid. The slow recovery from inactivation of Endo I_{to} compared to Epi- and Mid[15] provide further support for a role of $K_v1.4$. The molecular composition of the channels contributing to phase 1 repolarization in the canine atria is also debated. Besides I_{to}, a slower inactivating, ultra rapid delayed rectifier current (I_{Kur}) is present. It is believed that $K_v1.5$ mediates canine I_{Kur} similar to human atrial I_{Kur}[6].

We have recently identified a compound, NS5806 that produces an increase in the size of current carried through $K_v4.3$/KChIP2 channels expressed in both *Xenopus laevis* oocytes and CHO-K1 cells. Similarly, this compound activates I_{to} in canine ventricular Epi- and Mid cells, whereas minimal effects on Endo I_{to} were observed. In this study, we investigated the effect of NS5806 on canine atrial currents compared to effects on ventricular currents. Application of NS5806 resulted in about a 30% increase in the magnitude of I_{to} in atria, a 80% increase in Epi I_{to}, and no significant effect on Endo I_{to} and. Surprisingly, application of NS5806 caused a marked reduction in the magnitude of Na^+ current in atrial cells compared to ventricular cells. Preliminary results have been presented in abstract form[20].

METHODS

NS5806

NS5806 was synthesized at NeuroSearch A/S, Ballerup, Denmark by reaction of 6-cyano-2,4-dibromoaniline with sodium azide to form the respective 2,4-dibromo-6-tetrazolylaniline, which was condensed with 3,5-bis-trifluoromethyl-phenylisocyanate to provide the target 1-[2,4-dibromo-6-(1H-tetrazol-5-yl)-phenyl]-3-(3,5-bis-trifluoromethyl-phenyl)-urea (NS5806). NS5806 was dissolved in DMSO (20 mM stock).

Experimental animals

This investigation conforms to the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No 85–23, Revised 1996) and was approved by the Animal Care and Use Committee of the Masonic Medical Research Laboratory. Adult mongrel dogs of either sex were used to perform all experimental series. The animals were anticoagulated with heparin and anesthetized with pentobarbital (35mg/kg, i.v.). Their hearts were rapidly removed and placed in cold (4°C) cardioplegic solution (in mM): NaCl 129, KCl 12, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, glucose 5.5.

Isolated ventricular and atrial preparations

Experiments were performed using isolated arterially perfused canine right atrial (RA) preparations or right ventricular (RV) wedge preparations. The RV wedges (2 × 1.5 × 1 cm) were excised from the base of the right ventricular free wall. A descending branch of the right coronary artery (RCA) was cannulated to deliver the perfusate. The isolated atrial preparations were perfused through the ostium of the RCA. During the cannulation procedure, the preparations were perfused with cardioplegic solution (4° C Tyrode's containing 12 mM KCl) and arterial branches were ligated using silk ligatures. Subsequently, the preparations were placed in a tissue bath and perfused with Tyrode's solution of the following composition (mM): NaCl 129, KCl 4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, glucose 5.5, bubbled with 95% O_2 and 5% CO_2 (37±0.5°C). The perfusate was delivered at a constant flow. Pacing stimuli was delivered to the Endo surface at 2x the diastolic excitability at a basic cycle length (BCL) of 1 s. Control recordings were obtained 1–1.5 hours after mounting; the compounds were applied for 30 min before recordings. A pseudo-ECG was recorded with 2 electrodes consisting of Ag/AgCl half-cells placed 1.0 to 1.2 cm from 2 opposite sides of the atrial preparation. In the RV ventricular wedge, the electrodes were position facing the Endo and Epi surfaces (Epi, positive pole). Intracellular recordings were obtained using floating glass microelectrodes (Endo, approx. 2–3 mm from the endocardial surface, Epi, approx 0–3 mm from the Epi surface, atria, from the pectinate muscle (PM) and the crista terminalis (CT)). ECG and AP signals were amplified and digitized and analyzed using Spike 2 for Windows (Cambridge Electronic Design [CED], Cambridge, UK).

Isolation of adult canine cardiomyocytes

Myocytes from the Epi and Endo region were prepared from canine ventricles or atria. Left ventricular or right atrial preparations were dissected as described above. The preparation was initially perfused with nominally Ca^{2+}-free solution (mM): NaCl 129, KCl 5.4, $MgSO_4$ 2.0, NaH_2PO_4 0.9, glucose 5.5, $NaHCO_3$ 20, bubbled with 95% O_2/5% CO_2 containing 0.1% BSA for a period of about 5 minutes. The preparation was then subjected to enzyme digestion with the nominally Ca^{2+}-free solution supplemented with 0.5 mg/ml collagenase (Type II, Worthington), 0.1 mg/ml protease (Type XIV, Sigma) and 1 mg/ml BSA for 8–12 minutes. For isolation of ventricular cells, thin slices of tissue from the Epi and Endo were shaved from the wedge using a dermatome. For atrial cells, the pectinate muscle was isolated. The tissue was then placed in separate beakers minced and incubated in fresh buffer containing 0.5 mg/ml collagenase, 1 mg/ml BSA and agitated. The supernatant was filtered, centrifuged at 200 rpm for 2 minutes and the myocyte containing pellet was stored in 0.5 mM Ca^{2+} HEPES buffer at room temperature.

Voltage Clamp Recordings

Cells were placed in a temperature controlled chamber (PDMI-2, Medical Systems Corp.) mounted on the stage of an inverted microscope (Nikon TE300). Voltage-clamp and current-

clamp recordings were made using a MultiClamp 700A amplifier and MultiClamp Commander (Axon Instruments). Patch pipettes were fabricated from borosilicate glass capillaries (1.5 mm O.D., Fisher Scientific, Pittsburgh, PA). The pipettes were pulled using a gravity puller (Model PP-830, Narishige, Tokyo, Japan) and the pipette resistance ranged from 1–3 MΩ. Initially the cardiomyocytes were superfused with a HEPES buffer of the following composition (mM): NaCl 126, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 2.0, HEPES 10, and glucose 11. pH adjusted to 7.4 with NaOH. The patch pipette solution had the following composition (mM): K-aspartate 90, KCl 30, glucose 5.5, $MgCl_2$ 1.0, EGTA 5, MgATP 5, HEPES 5, NaCl 10, pH=7.2 with KOH and APs were recorded in this solution. For I_{to} recordings the HEPES buffer was supplemented with 300 μM cadmium to block I_{Ca} as previously described[21]. Sodium currents were recorded as previously described[22] in a low Na^+ extracellular solution (in mM): $CaCl_2$ 0.5, Glucose 10, $MgCl_2$ 1.5, Choline-Cl 120, NaCl 5, HEPES 10, KCl 4, Na acetate 2.8, $CoCl_2$ 1, $BaCl_2$ 0.1, pH=7.4. The pipette solution consisted of: $MgCl_2$ 1, NaCl 15, KCl 5, CsF 120, HEPES 10, EGTA 10, Na_2ATP 4, and the pH was adjusted to 7.2. All experiments were performed at 37° C except for the sodium current recordings which were performed at room temperature. After a whole cell patch was established, cell capacitance was measured by applying −5 mV voltage steps. Electronic compensation of series resistance to 60–70% was applied to minimize voltage errors. All analog signals (cell current and voltage) were acquired at 10–50 kHz, filtered at 4–6 kHz, digitized with a Digidata 1322 converter (Axon Instruments) and stored using pClamp9 software

Quantitative Real Time PCR

mRNA extraction and real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was performed as previously reported[15]. The pre-designed gene expression assay from Applied Biosystems was, in addition to the previously reported, cf02625015 (SCN5A). The primers and probes targeting *SCN1B, SCN2B*, *SCN3B*, and *SCN4B* were designed and synthesized by Applied Biosystems, following submission of sequences including intron-exon boundaries using Primer Express 3.0 software.

Statistical Analysis

Data are presented as Mean±SEM. Statistical comparisons were made using paired or unpaired Student's t-test or one way ANOVA followed by Student Newman-Keuls post test for multiple comparison. $p < 0.05$ was considered statistically significant (*).

RESULTS

As an initial basis of comparison, we tested the effect of NS5806 in arterially perfused right atrial preparations (Figure 1A). Application of 10 μM NS5806 had no significant effect on phase 1 repolarization (from 5.1±2.1% in control to 6.3±3.7% of phase 2 amplitude, n=4). Action potential duration (APD) was virtually unchanged following 10 μM NS5806 (APD_{90}=223±9.7 ms before and 247±13.8 ms after application of NS5806, n=4, p=N.S.) and the effective refractory period was158±11 ms in control and 183±10 ms in presence of NS5806 (n=4, p=N.S.). At 15 μM NS5806, the atrial action potential showed a depressed phase 0 depolarization and in 2 out of 4 preparations, the depressed phase 0 depolarization deteriorated into complete loss of excitability suggesting block of sodium channels. Similar effects were found on action potentials recorded from the crista terminalis (data not shown).

In right ventricular preparations, the Epi APs have a prominent phase 1 repolarization and this was further increased by NS5806 (10 μM). In contrast, Endo APs have a small phase 1 repolarization and the APs were unaffected by 10 μM NS5806. The increase in the Epi notch size was accompanied by an accentuation of the J-wave on the ECG (Figure 1B). At 15 μM

NS5806, there were minimal effects on the Endo action potential and a prominent enhancement of the spike and dome morphology of the Epi action potential was still present. In addition, there was no significant effect on transmural conduction time, as previously described [22], suggesting the effect of 15 μM NS5806 on ventricular I_{Na} is minor.

To further compare the effect of NS5806 on canine atrial and ventricular cells, we characterized the effect of NS5806 on APs recorded from isolated cardiomyocytes. Figure 1C shows APs and corresponding upstroke velocity (dV/dt) in the absence and presence of NS5806. Application of 10 μM NS5806 to isolated right atrial cells (Figure 1C, left panel) dramatically reduced upstroke velocity (dV/dt) by 71±7% (n=7) resulting in a decrease in phase 0 amplitude and in APD_{90} (Table 1). For Epi cells, application of NS5806 resulted in a dramatic increase in phase 1 repolarization leading to loss of the AP dome, which was accompanied by a significant abbreviation of the APD_{90}. The dV/dt was decreased by 9.8±4.8% (n=5) and phase 0 amplitude unaffected (Figure 1C, middle panel). NS5806 did not have any significant effect on the waveform of Endo APs and dV/dt was reduced by 4.9±2.8% (n=4, p=N.S.) (Figure 1C, right panel). The effect on the different electrophysiological parameters is summarized in Table 1.

To address the differences in the effect of NS5806 (10 μM) on phase 1 repolarization in atrial and ventricular cells, the density of I_{to} was measured in isolated canine atrial cells and ventricular Endo and Epi cells. I_{to} was recorded using whole-cell patch-clamp techniques and Cd^{2+} (300 μM) was added to the extracellular solution to block I_{CaL}. Following a brief step to −50 mV to discharge sodium channels, voltage steps from −40 to +50 mV were applied and elicited fast activating and rapidly inactivating currents as well as a sustained pedestal current component (Figure 2A). Application of NS5806 significantly increased the peak current density from 15.5±1.6 to 18.9±2.3 pA/pF (at +50 mV, n=14) whereas NS5806 did not affect the sustained current component (from 3.8±0.3 to 3.7±0.3 pA/pF) (Figure 2B). Mono-exponential equations were fit to the current traces and the time constants of decay (τ) following application of NS5806 were significantly slower (from τ=20.0±0.7 to 26.5±1.5 ms at +50 mV) (Figure 2C). For comparison, currents recorded from ventricular Epi and Endo cells using identical experimental conditions are shown in Figure 2D-I.

The steady-state inactivation of atrial I_{to} was evaluated using a prepulse-test pulse voltage-clamp protocol in the presence of Cd^{2+} and the effect of 10 μM NS5806 was evaluated (Figure 3A and B). The peak current following a 2 s prepulse was normalized to the maximum current and plotted as a function of the prepulse voltage to obtain the availability of the channels. A Boltzmann function was fit to the data (Figure 3C). In the absence of drug, the mid-inactivation voltage was $V_{½}$=−33.8±0.46 mV and the slope factor k= −7.15±0.40. Application of NS5806 caused a significant negative shift in $V_{½}$ to −41.1±0.50 mV and k=−7.35±0.42, (n=8). By comparison, the mid-inactivation voltage of I_{to} was −46.0±1.0 mV, k=−4.75±0.44 for LV Epi cells and −52.9±1.1, k=−8.45±1.34 for LV Endo cells. Application of NS5806 (10 μM) significantly shifted $V_{½}$ to −52.0±0.9 mV, k= −4.18±0.37 for Epi and −56.3±0.8 mV, k=−7.73±1.07 for Endo cells.

We next tested if recovery from inactivation of atrial I_{to} was affected by10 μM NS5806. Currents were elicited by a double pulse protocol, with increasing interpulse intervals. In absence of NS5806, reactivation of I_{to} showed a fast and a slow phase, with τ_{fast} of 55±1.97 ms and a τ_{slow} of 2368±327 ms. In the presence of NS5806, the reactivation of atrial I_{to} conformed to a single exponential equation and was markedly faster with τ=69.3±1.0 ms (n=5). The results are summarized in Figure 3D.

In contrast to Epi cells, atrial cells have a prominent sustained current, I_{Kur}. To address the effect of NS5806 on the sustained current, a two-pulse protocol was used to separate I_{Kur}

and I_{to}. From a holding of −80 mV, I_{to} was activated and inactivated by a step to 0 mV for 80 ms. The second pulse was preceded by a 5 ms step to −80 mV and I_{Kur} was elicited by a series of voltages ranging from −30 to 40 mV in absence and presence of 10 μM NS5806[23]. Application of NS5806 did not alter peak or sustained I_{Kur} (data not shown).

Application of NS5806 to atrial cells resulted in a decrease in dV/dt (Figure 1C and Table 1) suggesting an inhibition of atrial sodium channels. We next recorded I_{Na} from cells isolated from canine atrial or ventricular Endo. Endo cells were chosen to minimize the impact of I_{to}. Representative I_{Na} recordings are shown in Figure 4. The current-voltage relationship shows a two-fold higher I_{Na} density in atrial cells compared to Endo cells. Steady-state gating parameters were addressed using a standard prepulse-testpulse voltage protocol and mid-inactivation potential ($V_{½}$) of −82.6±0.12 mV (n=8) for atrial and −73.9±0.27 mV (n=10) for endocardial I_{Na} was obtained.

As demonstrated in Figure 4C-D, NS5806 induced a highly significant left-shift in mid-inactivation potential $V_{½}$ for I_{Na}. For atrial I_{Na}, from $V_{½}$= −82.6±0.1 mV to −85.1±0.1 mV (n=8) and for Endo I_{Na} from $V_{½}$= −73.9±0.27 mV to −77.3±0.21 mV (n=9). As atrial cells have a more negative $V_{½}$ compared to Endo cells, this suggest that as cells depolarize, atrial cells would lose excitability at less negative voltages than Endo cells. This tendency would be further enhanced by NS5806 suggesting that the effect of NS5806 on I_{Na} could dependent on resting membrane potential.

We next tested the effect of 10 μM NS5806 on ventricular and atrial peak I_{Na} using holding potentials ranging from −80 to −120 mV as illustrated by the representative atrial I_{Na} recordings (Figure 5A). The current-voltage relationships for atrial (Figure 5B) and Endo I_{Na} (Figure 5C) recorded at different holding potentials. For both cells types, I_{Na} peak currents were smaller when the holding potential was −80 mV versus −120 mV. Application of NS5806 caused a progressive inhibition on I_{Na} and the effect was more pronounced in atrial cells.

The differential effects of NS5806 on atrial and ventricular I_{to} and I_{Na} suggest different macromolecular ion channel complexes. To determine the expression level of mRNA encoding proteins thought to mediate I_{Na} or I_{to} as well as other K^+ currents, we performed qPCR experiments on mRNA isolated from Epi, Mid and Endo as well as from PM and right atrial appendage (App) (Figure 6). We observed a KChIP2 gradient across the right ventricle but no *KCND3* gradient (encoding K_v4.3). These observations suggest an I_{to} gradient is present in the right ventricle, similar to what has been observed in the left ventricle. The relative mRNA expression levels of *KCND3*, showed an almost 4-fold higher expression in RV compared to RA. In contrast *KCNA4* and *KCNA5* encoding K_v1.4 and K_v1.5, respectively had a 2 fold higher expression in the atria. *KCNH2* encoding K_v11.1 and *KCNQ1* encoding K_v7.1 was expressed at similar levels. We also tested the expression of several ancillary subunits and found a high expression of *KCNIP2* encoding KChIP2, and a moderate expression of *KCNE1, KCNE3 and KCNE4. For KCNIP2*, the expression level in the atria was more than 2-fold higher than in ventricle and similar was found for *KCNE4*. A transmural expression gradient in the RV was also found for *KCNE1*. We observed no differences in any mRNA levels between PM and App. However, previous studies have shown that the electrophysiology is not uniform throughout the atria[8,24] suggesting some differences in ion channel expression throughout the atria. The expression level of the genes encoding proteins suspected to mediate I_{Na} was not significantly different between atria and ventricle, nor was any transmural gradient in expression found (Figure 6B)

DISCUSSION

In the present work, we compared the effect of NS5806 on I_{to} and I_{Na} in canine atria and ventricle. In canine right ventricular wedges, phase 1 repolarization was increased in Epi while repolarization of Endo was largely unaffected. These changes resulted in an augmented J-wave on the ECG. In contrast, in atrial preparations, phase 1 repolarization was unaffected by NS5806 but excitability was reduced suggesting Na^+ channel block.

Effect of NS5806 on I_{to}

It is believed that $K_v4.3$ channels comprise the majority of transient outward K^+ channels in canine ventricle[11,12]. Several β-subunits can modify $IK_v4.3$, such as KChIP2 that increases peak $IK_v4.3$ density, slows decay of the current and accelerates recovery from inactivation[13]. In the left and right ventricle, previous qPCR studies have shown that the I_{to} gradient across the ventricles is due to a KChIP2 gradient[12]. This gradient in mRNA was also confirmed at the protein level[15]. In the present study, qPCR analysis revealed a 3-fold lower expression of $K_v4.3$ mRNA in atria compared to ventricle; interestingly, the expression of KChIP2 mRNA was 2-fold greater than RV Epi levels. Voltage clamp analysis of I_{to} showed that I_{to} was smaller in atria, in agreement with the qPCR data. However, this study raises an important question, namely what is the functional role of excess KChIP2 in canine atria? KChIP2 was initially identified as a β-subunit for K_v4 channels; however, KChIP2 has been found to associate with I_{CaL}[25] and I_{Na}[26] suggesting it may alter the biophysical properties of many ion channels.

Epi I_{to} was larger than atrial I_{to} (Figure 2), however atrial cells had a prominent sustained current, I_{Kur} that was not observed in Epi cells. NS5806 induced an increase in I_{to} in both cells types, but the increase was more pronounced for Epi than atrial I_{to}. The sustained current was unaffected by NS5806. Using qPCR, we found higher expression of $K_v1.4$ and $K_v1.5$ mRNA in the atria compared to ventricle. $K_v1.4$ is thought to mediate a slowly recovering I_{to}, $I_{to,\ slow}$ and $K_v1.5$ is thought to mediate I_{Kur}. For heterologously expressed $K_v1.5$, 10 μM NS5806 induced a minor block[27] consistent with a lack of effect on canine atrial sustained current. For heterologously expressed $K_v1.4$, application of 10 μM NS5806 resulted in an almost complete block [27]. The higher expression of $K_v1.4$ and $K_v1.5$ in the atria compared to the ventricle could explain why the relative increase in peak current induced by NS5806 was smaller in the atria than in Epi as NS5806 would enhance a $K_v4.3$ mediated current component, block a $K_v1.4$ component of I_{to}, and leave a Kv1.5 mediated sustained current component unaffected.

The effect on different kinetic parameters of atrial I_{to} was addressed. Compared to ventricular I_{to}, atrial I_{to} had a slower current decay (Figure 2C,F and I). Application of NS5806 further slowed the current decay, similarly to the effects on ventricular I_{to}. In a previous study, we found that NS5806 only slowed current decay of $K_v4.3$ channels when co-expressed with KChIP2[22,27], suggesting that at least part of the atrial I_{to} is mediated by $K_v4.3$ in complex with KChIP2. The steady-state mid-inactivation potential for atrial I_{to} was shifted to more negative potentials by NS5806 similar to results seen in ventricular cells[15].

Effect of NS5806 on I_{Na}

Canine I_{Na} is functionally distinct in different areas of the heart. In the ventricles, Epi I_{Na} has a significant more negative steady-state mid-inactivation potential compared to Endo[28]. In agreement with other studies[8,10,29,30] we found that atrial I_{Na} has a higher current density and a more negative steady-state mid-inactivation potential compared to ventricular Endo I_{Na}. The negative steady-state mid-inactivation potential will tend to leave a larger fraction of atrial I_{Na} channels in an inactivated state. NS5806 produced a significant shift of steady-

state inactivation to more negative potentials for both atrial and ventricular cells; this would be expected to lead to a larger pool of inactivated channels. The more negative mid-inactivation potential and more positive resting membrane potential in the atria (Table 1), can account for the greater depression of I_{Na} in atria vs. ventricles. We investigated the level of expression of $Na_v1.5$ subunits in the canine atria and in the three ventricular layers but did not detect significant differences in expression level of mRNA encoding $Na_v1.5$ or $Na_v\beta1–4$ suggesting mRNA levels measured by qPCR may not reflect protein levels in the membrane, or other proteins and regulatory factors account for the distinct behaviour of the channels.

Effect on action potential waveform

In RV ventricular wedges NS5806 (10 μM) increased phase 1 repolarization in the Epi with no effect on repolarization of Endo as previously described[22]. In contrast there was no effect on phase 1 repolarization in atrial APs. Surprisingly, NS5806 reduced the AP amplitude, dV/dt and significantly depressed excitability in atrial tissue. NS5806 exerted similar effects in isolated atrial myocytes, whereas the effect on upstroke velocity of APs recoded from isolated ventricular Epi and Endo cells was minor. This disparate effect in atria vs. ventricle is likely due to the greater effect of NS5806 to depress I_{Na} for reasons discussed above.

Physiological implications of I_{to} activation and I_{Na} block by NS5806

Many antiarrhythmic agents are effective in terminating atrial fibrillation in both the experimental and clinical setting due to their I_{Na} blocking ability. A major limitation of some of these agents is their potential proarrhythmic actions in the ventricle. Previously, it was shown that the antianginal agent, ranolazine was effective at suppressing atrial fibrillation with little effect in the ventricle[8]. This atrial effectiveness of ranolazine was due to intrinsic differences in sodium channels between atria and ventricle, namely the more negative steady-state inactivation curve[8]. In the present study, we also found intrinsic differences in sodium channels between atria and ventricle which resulted in greater sodium current inhibition by NS5806 in atria than in ventricle. While the effectiveness of NS5806 in terminating atrial fibrillation was not addressed in the present study, it is clear that NS5806 also exhibits atrial selectiveness in Na^+ channel blockade.

Of interest is the mechanism by which NS5806 activates I_{to}. We have previously shown this compound works through KChIP to cause an increase in the size of I_{to}[15]. In the canine ventricle where $K_v4.3$ levels are equivalent in Epi, Mid and Endo layers, the I_{to} enhancement was greatest in Epi and Mid cells where KChIP levels are highest. Although atrial tissue has high KChIP levels, $K_v4.3$ levels were 3 times lower which may explain the modest increase in I_{to} following application of NS5806 in this tissue type, together with a potential block of atrial $IK_v1.4$ and $IK_v1.5$. In the uncompromised myocardium, application of this compound to ventricular wedges resulted in loss of the epicardial action potential dome and a Brugada-like ECG[22]. However, in pathophysiological condition such as heart failure, a reduction in I_{to} due to loss of both $K_v4.3$ and KChIP levels has been observed[31,32]. If $K_v4.3$ and KChIP levels are not severely diminished in heart failure, application of NS5806 may restore I_{to} and restore the spike and dome morphology.

The influence of action potential waveform and duration on excitation-contraction coupling is well known (for review see Sah et al., 2003[33]). The presence of a spike and dome morphology results in a significant increase in peak I_{CaL} as well as total charge when compared to a cell type where I_{to} and spike and dome morphology is absent[34,35]. These observations suggest the spike and dome morphology of the canine Epi action potential is a more efficient releaser of Ca^{2+} from the SR. In the setting of heart failure, where I_{to} is known to be reduced, this reduction in I_{to} would result in reduced Ca^{2+} influx into the cell via I_{CaL}. Augmentation of I_{to} in the setting of heart failure may have a beneficial effect on

cardiac output by restoring the spike-and-dome morphology resulting in increased Ca^{2+} influx during the course of an action potential.

In summary, NS5806 produces a prominent augmentation of I_{to} with little effect on I_{Na} in canine ventricles, but a potent inhibition of I_{Na} with little augmentation of I_{to} in canine atria. Molecular analysis of mRNA for atria and ventricle revealed more KChIP2, $K_v1.5$ and $K_v1.4$ and less $K_v4.3$ in atria versus ventricle

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Abbreviations

I_{to}	transient outward current
AP	action potential
Epi	epicardium
Endo	endocardium
KChIP2	K Channel Interacting Protein 2
DPP6	Dipeptidyl aminopeptidase-like protein 6
PM	pectinate muscle
CT	crista terminalis
RV	right ventricle
LV	left ventricle
RA	Right Atrial

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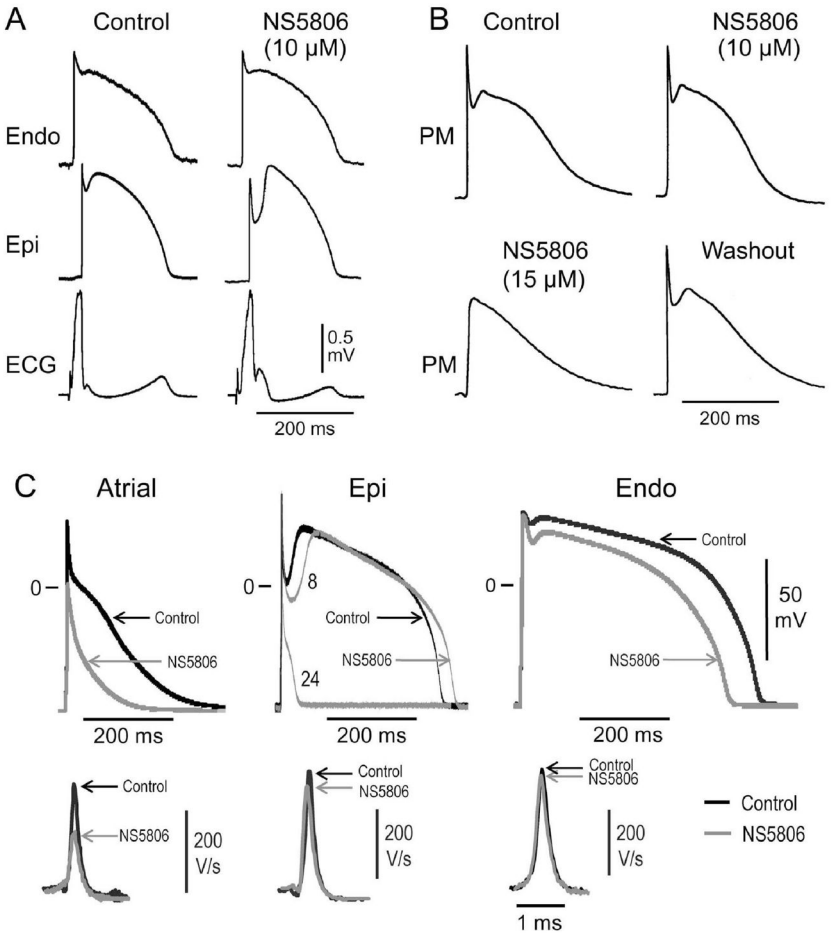

Figure 1.

Effect of NS5806 on arterially perfused atrial and ventricular preparations paced at BCL=1 sec. **Panel A)** Representative action potentials (AP) recordings from right atrial pectinate muscle (PM) preparation in presence of 0–15 μM NS5806 (n=4). **Panel B)** Representative AP recordings and corresponding ECG from a right ventricular wedge in absence and presence of 10 μM NS5806 (n=5). **Panel C)** Representative APs and corresponding dV/dt recorded from isolated canine cardiomyocytes at a BCL of 1 sec. APs recorded from a atrial cell (left panel), LV Epi cell (middle panel), and LV Endo cell (left panel) in absence (black traces) and presence of 10 μM NS5806 (gray traces). The APs recorded from the LV Epi cell were 8 and 24 sec after application of 10 μM NS5806. The results are summarized in Table 1.

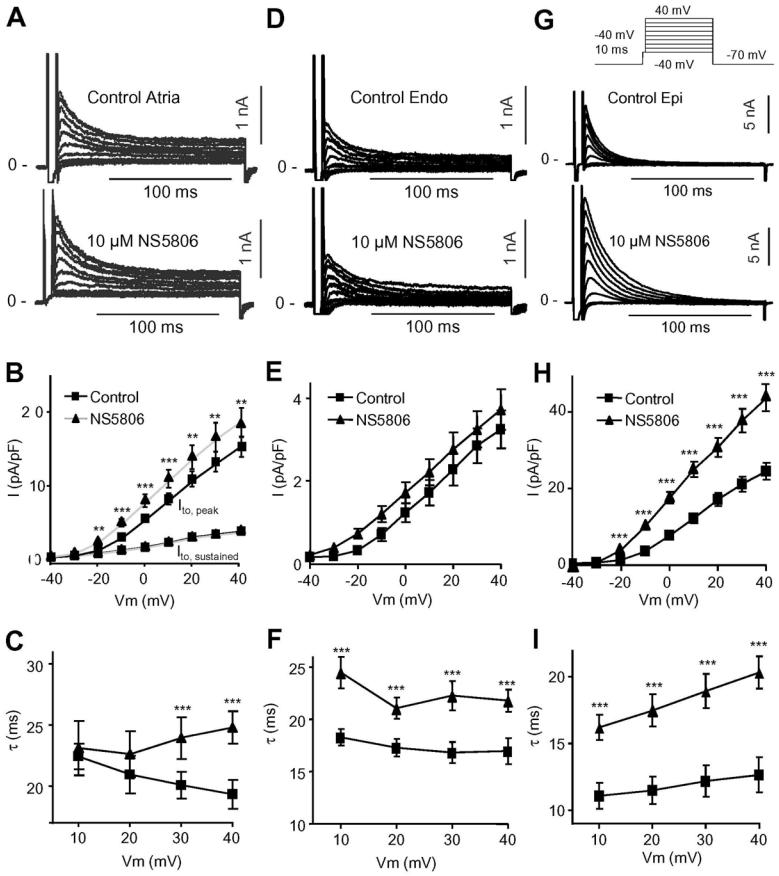

Figure 2.

I_{to} in canine atrial and ventricular cells in absence and presence of NS5806 (10 μM). Currents were activated by the voltage clamp protocol (top of figure) and Cd^{2+} was present to block I_{CaL}. **Panel A)** Representative I_{to} traces recorded from canine atrial cells. **Panel B)** Mean I–V relation for atrial peak I_{to} as well as for the sustained component **Panel C)** I_{to} decay as a function of voltage under control (n=14). **Panel D)** Representative I_{to} traces recorded from canine LV Endo cells. **Panel E)** Mean I–V relation for peak I_{to}. **Panel F)** I_{to} decay as a function of voltage (n=9). **Panel G)** Representative I_{to} traces recorded from canine LV Epi cells. **Panel H)** Mean I–V relation for peak I_{to}. **Panel I)** I_{to} decay as a function of voltage (n=10).

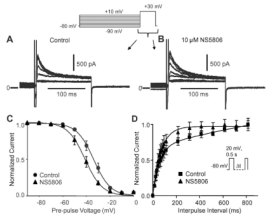

Figure 3.

Voltage dependence of inactivation of atrial I_{to}. Representative traces recorded under control conditions (**Panel A**) and after application of 10 μM NS5806 (**Panel B**) showing voltage dependence of inactivation of I_{to}. **Panel C)** The currents were normalized to max-current and plotted as a function of the conditioning pulse and Boltzmann curves were fit to the data. In the absence of drug, the mid-inactivation voltage was $V_{1/2}$=−33.8±0.46 mV and the slope factor k=−7.15±0.40. In presence of NS5806 $V_{1/2}$ was −41.1±0.50 mV and k= −7.35±0.42 (n=8). **Panel D)** Time-dependence of recovery from inactivation of atrial I_{to}. Currents were elicited by the depicted protocol and normalized recovered currents were plotted as a function of the interpulse interval (n=6).

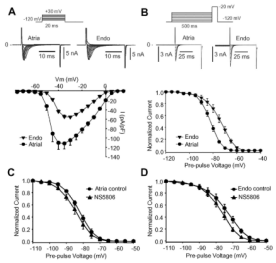

Figure 4.

I_{Na} recorded from canine atrial cells or endocardial cells. Cd^{2+} was present to block I_{CaL}. **Panel A)** Representative I_{Na} traces recorded from atrial (n=8) and Endo cells (n=9) and corresponding mean I–V relation for peak current is shown. **Panel B)** Representative steady-state inactivation recordings from an atrial or Endo cell. The peak-current following a 500 ms prepulse was normalized to the maximum current and plotted against the conditioning potential to obtain the availability of channels. A Boltzmann equation was fit to the data resulting in a mid-inactivation ($V_{½}$) of −82.6±0.12 mV (n=8) for atrial and −73.9±0.27 mV (n=9) for Endo I_{Na}. **Panel C)** Steady-state inactivation for I_{Na} recorded from atrial cells in absence and presence of 10 μM NS5806. $V_{½}$= −82.6±0.1 mV in control and −85.1±0.1 mV in presence of NS5806 (n=8). **Panel D)** Steady-state inactivation for I_{Na} recorded from isolated LV Endo cells in absence and presence of 10 μM NS5806. $V_{½}$= −73.9±0.27 mV in control and −77.3±0.21 mV in presence of NS5806 (n=9).

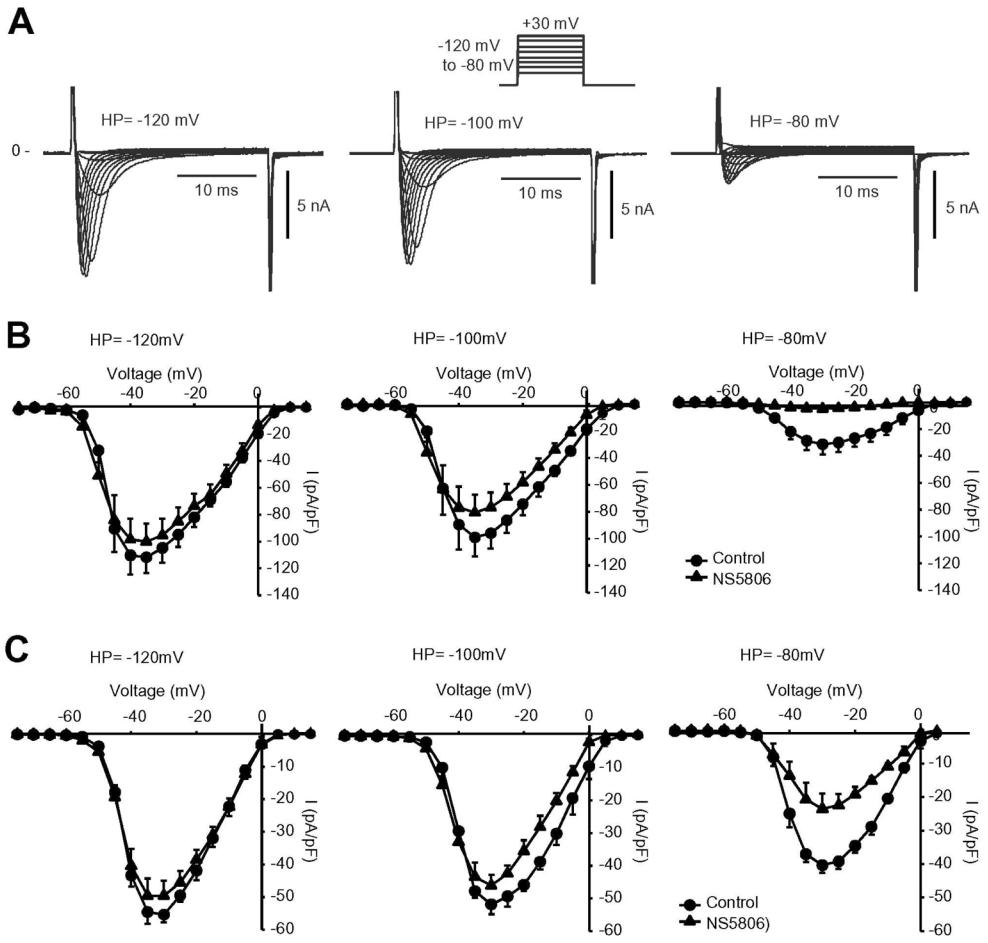

Figure 5.

The effect of different holding potentials on atrial and ventricular I_{Na} in absence and presence of 10 μM NS5806. The voltage clamp protocol is shown at the top of the figure and Cd^{2+} was present to block I_{CaL}. **Panel A)** Representative I_{Na} traces recorded from canine right atrial cells using holding potentials of either −80 mV, −100 mV or −120 mV. **Panel B)** Mean I–V relation for atrial I_{Na} peak current recorded using different holding potentials in absence and presence of 10 μM NS5806 (n=5). **Panel C)** Mean I–V relation for LV Endo I_{Na} peak current recorded using different holding potentials in absence and presence of 10 μM NS5806 (n=5).

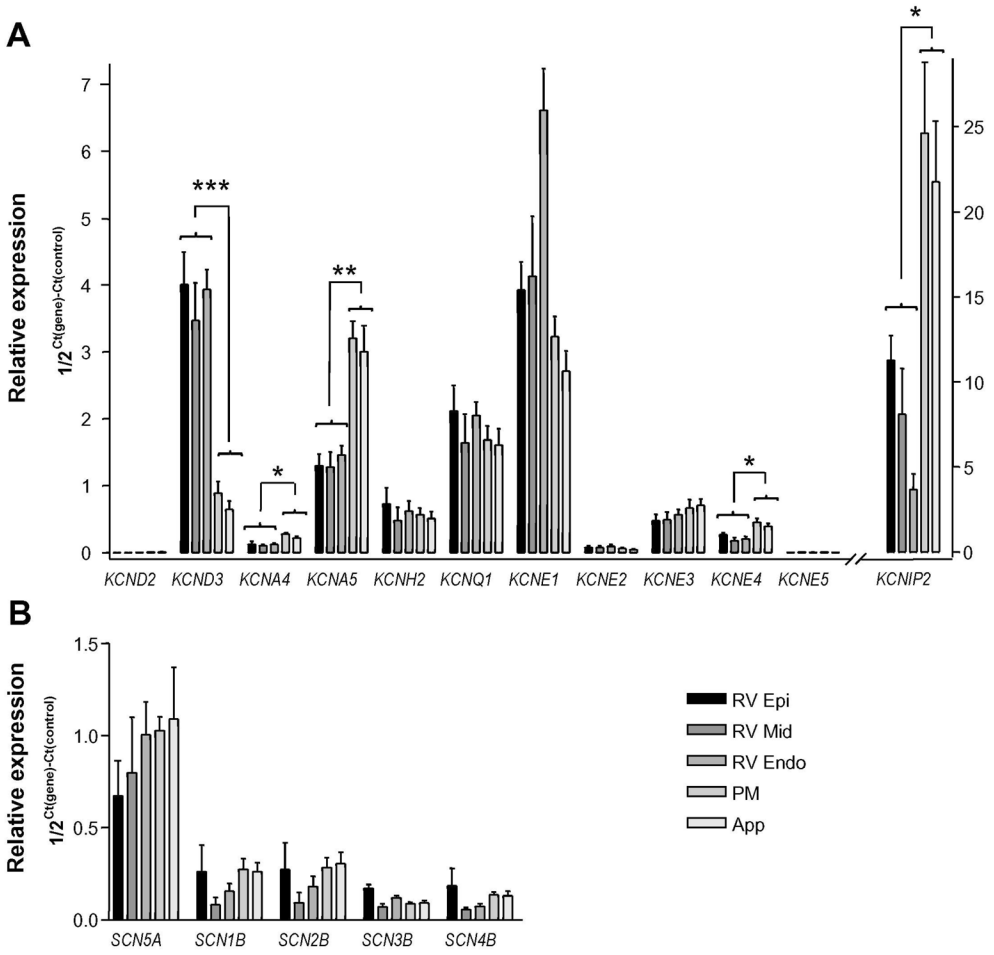

Figure 6.

qPCR of canine right ventricular (Epi, Mid, and Endo) and right atrial pectinate muscle (PM) and appendage (App) tissue isolated from 5 canine hearts. Taqman based assays (Applied Biosystems) were used to quantify mRNA of interest which was normalized to *cyclophilin B* expression. **Panel A)** Expression of K^+ channel subunit mRNA. **Panel B)** Expression of Na^+ channel subunit mRNA.

Table 1

Action potentials were recorded from isolated canine atrial or left ventricular Epi or Endo cells at a basic cycle length (BCL) of 1 sec in the absence or presence of 10 μM NS5806. The effects of NS5806 on upstroke velocity (dV/dt), on phase 0 (Ph0), phase 1(Ph1) and phase 2 (Ph2) as well as on action potential duration (ADP) and on resting membrane potential (E_{rest}) of different cell types are summarized.

	Atria	**Epi**	**Endo**
ΔdV/dt	−71±7%, (n=6)***	−9.8±4.8%, (n=5) *	−4.9±2. 8%, (n=4) *
Ph0 Amplitude + NS5806	108±4 mV 72±5 mV, (n=6) **	124±3 mV 124±3 mV, (n=5), NS	126±8 mV 125±7, (n=4), NS
Notch, Ph1% of Ph2 + NS5806	-	28.2±11.1%, (n=5) no Ph2 in 5/5	5.5±1.0% 5.1±1.8%, (n=4), NS
APD90 + NS5806	294±28 ms 187±24 ms, (n=6) ***	527±46 mS 59±6 mS, (n=5) ***	535±56 mS 450±28 mS, (n=4), NS
E_{rest} + NS5806	−71.9±0.3 mV −72.6±0.6 mV, (n=6), NS	−87.1±5 mV −87.4±3 mV, (n=5), NS	−86.0±6 mV −84.9±6 mV, (n=4), NS