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Original article

Differential effects of the transient outward K^+ current activator NS5806 in the canine left ventricle

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ABSTRACT

To examine the electrophysiological and molecular properties of the transient outward current (I_{to}) in canine left ventricle using a novel I_{to} activator, NS5806, I_{to} was measured in isolated epicardial (Epi), midmyocardial (Mid) and endocardial (Endo) cells using whole-cell patch-clamp techniques. NS5806 activation of $K_v4.3$ current was also studied in CHO-K1 cells and *Xenopus laevis* oocytes. In CHO-K1 cells co-transfected with $K_v4.3$ and KChIP2, NS5806 (10 μM) caused a 35% increase in current amplitude and a marked slowing of current decay with τ increasing from 7.0 ± 0.4 to 10.2 ± 0.3 ms. In the absence of KChIP2, current decay was unaffected by NS5806. In ventricular myocytes, NS5806 increased I_{to} density by 80%, 82%, and 16% in Epi, Mid, and Endo myocytes, respectively (at +40 mV) and shifted steady-state inactivation to negative potentials. NS5806 also significantly slowed decay of I_{to}, increasing total charge to 227%, 192% and 83% of control in Epi, Mid and Endo cells, respectively (+40 mV, $p<0.05$). Quantification of $K_v4.3$ and KChIP2 mRNA in the 3 ventricular cell types revealed that levels of $K_v4.3$ message was uniform but those of KChIP2 were significantly greater in Epi and Mid cells. The KChIP2 gradient was confirmed at the protein level by Western blot. Our results suggest that NS5806 augments I_{to} by increasing current density and slowing decay and that both depend on the presence of KChIP2. I_{to} and its augmentation by NS5806 are greatest in Epi and Mid cells because KChIP2 levels are highest in these cell types.

1. Introduction

Repolarization of the cardiac action potential is initiated and controlled by activation of a number of time- and voltage-dependent K^+ currents. In dog heart at least four K^+ currents play important roles in regulating the cardiac action potential duration: (i) a Ca^{2+}-independent transient outward K^+ current (I_{to}); (ii) an inwardly rectifying K^+ current (I_{K1}) and (iii) the rapid and slow forms of the delayed rectifier K^+ current (I_{Kr} and I_{Ks}, respectively). An I_{to} has been identified in the myocardium of most mammalian species (for review see [1]). Ventricular epicardial (Epi) tissue has a more prominent I_{to} compared to endocardial (Endo) tissue [2–5]. Recently, it has been demonstrated that I_{to} can be modulated by several proteins such as K^+-channel interacting protein (KChIP) [6,7], IRX [8], calcineurin/NFAT [9], DPP's [10] and various KCNE subunits [11,12].

Although an I_{to} gradient between Epi and Endo has been identified, the precise molecular identity of I_{to} in the canine ventricular myocardium remains unclear. It is generally believed that $K_v4.3$ channels comprise the majority of transient outward K^+ channels in canine heart [13] but previous studies have also identified *$K_v1.4$* and *$K_v1.5$* gene products in ventricular tissue [13,14]. However, the precise role of these alpha subunits and their contribution to canine I_{to} remain to be determined. Recent evidence also suggests that several β-subunits including KChIP2 can alter peak $I_{Kv4.3}$ density, slow decay of the current and accelerate recovery from inactivation [7]. However, the relative abundance of $K_v4.3$ and KChIP2 in canine ventricle remains controversial. Several studies suggest that $K_v4.3$ levels are uniform throughout the canine left ventricle and the gradient in I_{to} expression is due to a gradient in KChIP2 [15,16]. In contrast, another study found that KChIP2 protein was uniform throughout the left ventricle suggesting that I_{to} gradient in ventricle is not due to a gradient in KChIP2 levels [7]. Finally, Zicha et al. [17] found that both $K_v4.3$ and KChIP2 exhibit a transmural gradient, with Epi expression being greater than Endo expression in canine ventricle.

The present study compares the electrophysiological and molecular properties of the Ca^{2+}-independent transient outward K^+ currents in single myocytes isolated from the canine left ventricle. Results of our study indicate that the biophysical and molecular properties of I_{to} differ significantly in endocardial cells compared to midmyocardial (Mid) or epicardial cells. Analysis of the molecular subunits revealed that KChIP2 mRNA levels are lower in the

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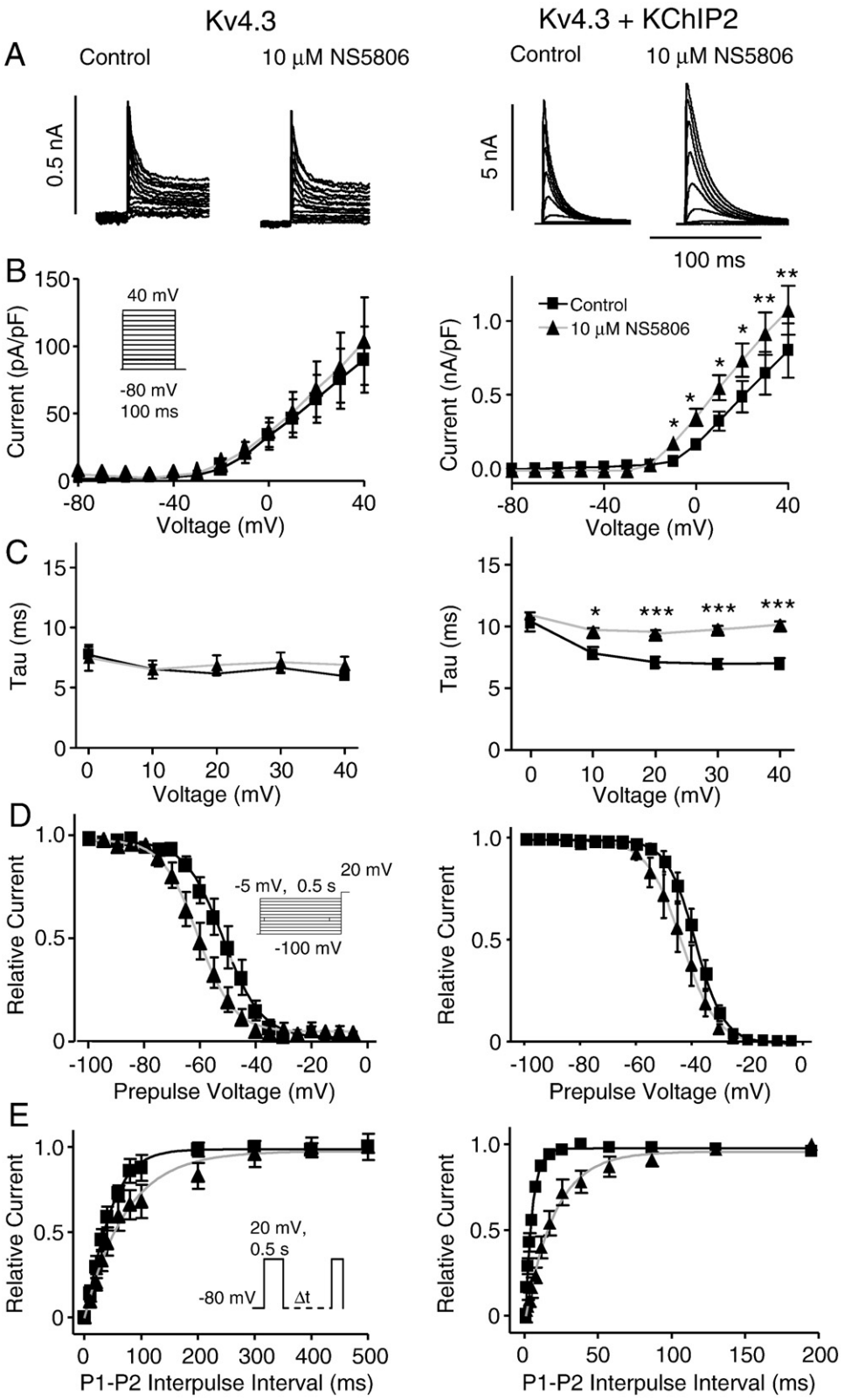

Fig. 1. (A) Representative traces of $K_v4.3$ currents recorded in the absence (left, $n=6$) and presence of KChIP2 (right, $n=5$–10) under control conditions and in the presence of 10 μM NS5806. From a holding potential of −80 mV, cells were stepped to +40 mV in 10 mV increments. (B) Mean I–V relations for peak current density. (C) Mean τ's in the absence and presence of NS5806. (D) Voltage dependence of inactivation and Boltzmann curves showing mid-inactivation. (E) Time-dependent recovery from inactivation was evaluated using a standard double-pulse protocol from a holding potential of −80 mV.