

Full Paper

Direct Effects of Esmolol and Landiolol on Cardiac Function, Coronary Vasoactivity, and Ventricular Electrophysiology in Guinea-Pig Hearts

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Abstract. The ultra-short acting, selective β_1 -adrenergic antagonists landiolol and esmolol are widely used perioperatively; however, little is known about their acute direct actions on the heart. The current study utilized the Langendorff perfused heart system to measure changes in cardiac function and hemodynamics in response to each drug. Furthermore, electrophysiological analysis was performed on isolated ventricular myocytes. Direct application of esmolol significantly decreased systolic left ventricular pressure and heart rate at concentrations $> 10 \mu\text{M}$, while it dose-dependently increased coronary perfusion pressure. Esmolol also shortened the action potential duration (APD) in a concentration-dependent manner, an action maintained even when the delayed rectifier K^+ current or ATP sensitive K^+ current was blocked. Moreover, esmolol inhibited both the inward rectifier K^+ current (I_{K1}) and the L-type Ca^{2+} current (I_{CaL}) and increased the outward current dose-dependently. In contrast, landiolol had minimal cardiac effects. In the Kyoto Model computer simulation, inhibition of either I_{K1} or I_{CaL} alone failed to shorten the APD; however, an additional increase in the time-independent outward current caused shortening of the APD, equal to that induced by esmolol. In conclusion, esmolol directly inhibits cardiac performance significantly more so than landiolol, an effect revealed to be at least in part mediated by esmolol-induced APD shortening.

Keywords: esmolol, landiolol, Langendorff perfusion, patch clamp, coronary perfusion pressure

Introduction

β -Adrenoceptor blockers are widely used for perioperative control of tachyarrhythmia. Esmolol and landiolol are two commonly used ultra-short-acting intravenous β -blockers, with a β_1/β_2 selectivity ratio of 33 and 255 for esmolol and landiolol, respectively (1). Because of their ultra-short-acting properties and high β_1 -selectivity, both drugs are particularly suited to the perioperative period and have proven to be safe in patients with airway hyper-reactivity (1 – 3). Moreover, β_1 -adrenoceptor blockers have been administered to septic patients as a therapy to treat sepsis-induced immune, cardiovascular, and coagulation dysfunctions (4). Despite the usefulness of β_1 -adrenoceptor blockers in these settings, application of these drugs to septic patients or those in the perioperative

period who are already hypotensive can exacerbate a further drop in blood pressure via direct actions on the cardiovascular system in addition to their β_1 -blocking effects. For example, esmolol has been shown to exert a potent negative inotropic action in both in vivo and in vitro studies, as well as inducing excessive hypotension in patients with poor cardiac function (5, 6). Several independent studies comparing the negative chronotropic and inotropic effects of esmolol and landiolol revealed that esmolol had greater negative inotropic potency than landiolol (7 – 9); however, the mechanisms underlying the disparity in negative inotropic potency between the two drugs are currently unknown. The majority of these comparative studies were conducted with sympathetic stimulation (9, 10), and hence the direct effects of these drugs on cardiac function are undefined. Ikeshita et al. (9) showed that when esmolol was applied cumulatively to isolated rabbit heart in the presence of isoproterenol, it produced significant negative inotropic action. They also noted that both left ventricular developed pressure

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and the maximal rate of left ventricular force development were reduced to levels less than the baseline (in the absence of isoproterenol and esmolol) value. The finding strongly suggests additional mechanisms of negative inotropic action of esmolol. In order to analyze the direct action of β antagonists on the heart independently from their β -blocking actions, experimental protocols without sympathetic stimulation are prerequisite. The current study sought to compare the direct effects of landiolol and esmolol on cardiac contractility and coronary perfusion pressure in isolated guinea-pig hearts. Moreover, ventricular cells were isolated to enable the measurement of action potentials and ionic currents. Together, these methods provide a comprehensive and comparative investigation into the direct effects of esmolol and landiolol on cardiac function, as well as cellular electrophysiology.

Materials and Methods

Measurement of left ventricular function

All animal experiments were approved by The Animal Ethics Committee of Akita University School of Medicine. Guinea pigs weighing 200–300 g were anesthetized with pentobarbital (50 mg/kg, i.p.). The heart was rapidly excised and the ascending aorta cannulated to enable retrograde coronary perfusion with control Tyrode solution. The excised heart was then mounted on a Langendorff perfusion device. A fluid-filled balloon connected to a pressure transducer was inserted into the left ventricle (LV) via the left atrium to measure left ventricular pressure (LVP). The balloon volume was adjusted to maintain the end-diastolic pressure at 10 mmHg. The heart was then immersed in a chamber filled with Tyrode solution at 37°C. The heart was continuously perfused with oxygenated Tyrode solution with 5.5 μ M atropine (37°C) at a constant flow of 6–8 ml/min using a peristaltic pump. LVP and coronary perfusion pressure (CPP) were recorded using a PowerLab (AD Instruments, Colorado Springs, CO, USA) recording at 1 kHz.

Isolation of ventricular myocytes and patch clamping

Ventricular myocytes were isolated from guinea-pig hearts using enzymatic dissociation as previously described (11). Briefly, the heart was perfused with Ca^{2+} -free Tyrode solution for 5 min using a Langendorff perfusion system. The perfusate was then switched to a Ca^{2+} -free Tyrode solution containing 0.02% collagenase (Wako, Osaka) and the heart digested for approximately 30 min. The heart was then rinsed in a high K^+ , low Cl^- solution before the left ventricle was dissected from the digested heart and stored in the same solution at 4°C until

required. A small piece of ventricular tissue was dissected and gently agitated in the recording chamber (0.5 ml in volume), containing normal Tyrode solution. Once the cells had settled on the floor of the recording chamber, they were perfused with normal Tyrode solution at 2–3 ml/min. Experiments were performed at 36°C–37°C on rod-shaped quiescent single cells that had clear sarcomere striations.

Cells were patch-clamped to record whole-cell currents using a patch-clamp amplifier (EPC-7; List, Darmstadt, Germany). Patch pipettes were pulled with a micropipette puller (Model P-97; Sutter Instrument Co., Novato, CA, USA) from glass capillaries (Warner Instrument Co., Hamden, CT, USA). Electrode resistance ranged from 3 to 5 M Ω . Data were recorded at 2–10 kHz on an IBM PC using pClamp software (Axon Instruments, Foster City, CA, USA).

Solutions and drugs

Tyrode solution comprised 136.9 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl_2 , 0.53 mM MgCl_2 , 0.33 mM NaH_2PO_4 , 5.0 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 5.5 mM glucose (pH 7.4, adjusted with NaOH). Cs-Tyrode solution used when recording the L-type Ca^{2+} current (I_{CaL}) was prepared by replacing KCl in the Tyrode solution with equimolar CsCl. The external solution used to record the K^+ current was produced by adding 0.3 μ M nisoldipine to Tyrode solution to block the I_{CaL} . To record the esmolol-induced current, 0.3 μ M nisoldipine and 2 mM BaCl_2 were added to the Tyrode solution (Fig. 7A). Nisoldipine and glibenclamide were dissolved in DMSO as a 10 mM stock solution and added to the external solution where required. The high K^+ , low Cl^- solution to store the heart tissue comprised 10 mM taurine, 10 mM oxalic acid, 70 mM L-glutamic acid, 25 mM KCl, 10 mM KH_2PO_4 , 0.5 mM EGTA, 11 mM glucose, and 10 mM HEPES (pH 7.4, adjusted with KOH).

The pipette solution for recording action potentials and K^+ currents and for simultaneous recording of the action potential and I_{CaL} comprised 110 mM K-aspartate, 20 mM KCl, 4 mM K_2ATP , 2 mM MgCl_2 , 5 mM HEPES, and 10 mM EGTA (pH 7.0, adjusted with KOH). In some experiments, EGTA was replaced with 10 mM BAPTA. A K^+ -free and Cs^+ -rich pipette solution for recording I_{CaL} comprised 130 mM CsOH, 30 mM TEA-Cl, 5 mM MgATP, 10.0 mM EGTA, 10 mM HEPES, and 0.1 mM GTP- Na_2 (pH 7.2, adjusted with aspartic acid). The K^+ -free, Cs^+ -rich solution was also used to examine the ion selectivity of the esmolol-induced current (Fig. 7B).

Statistical analysis

Results are expressed as the mean \pm S.E.M. Compari-

son of more than three groups was conducted by one-way repeated measures analysis of variance (ANOVA) with Dunnett's or Bonferroni's multiple comparison tests post hoc. Comparison of two groups was conducted by the paired *t*-test. $P < 0.05$ was considered to indicate a statistical significance. Statistical analysis was performed by using Dr. SPSS II software (SPSS, Chicago, IL, USA).

Results

Effects of esmolol and landiolol on isolated guinea-pig hearts

Once hearts were attached and the CPP, LVP, and heart rate (HR) stabilized for > 20 min, esmolol (Fig. 1A) or landiolol (Fig. 1B) was administered cumulatively. Esmolol dose-dependently increased CPP at all doses administered from 3–30 μM ($P < 0.05$, 3 μM esmolol vs. control; $P < 0.01$, 10 and 30 μM esmolol vs. control), while significantly decreasing systolic LVP (sLVP) in a dose-dependent manner ($P < 0.05$, 10 μM esmolol vs. control; $P < 0.01$, 30 μM esmolol vs. control). In contrast, landiolol was found to have no significant effect on CPP (Fig. 1C) and only induced a minor effect on sLVP at 10–100 μM (Fig. 1D). Esmolol and landiolol both caused minor, but dose-dependent, reductions in HR;

esmolol: 176.6 ± 4.8 , 177.3 ± 5.4 , 175.3 ± 4.4 , 170.8 ± 4.2 , and 159.3 ± 6.3 bpm at 0, 1, 3, 10, and 30 μM , respectively ($P < 0.01$, 30 μM esmolol vs. control); landiolol: 191.9 ± 3.0 , 182.8 ± 3.0 , 177.1 ± 2.8 , and 169.9 ± 2.6 bpm at 0, 10, 30 and 100 μM , respectively ($P < 0.01$, 10, 30, and 100 μM landiolol vs. control; Fig. 1E).

Effects of esmolol and landiolol on myocyte action potential

Action potentials were elicited every 10 s using conventional whole-cell clamp conditions, with representative traces illustrated in Fig. 2A. Esmolol (at 3–100 μM) dose-dependently shortened the action potential duration (APD), significantly at concentrations above 10 μM ($P < 0.05$ vs. control). The APD₉₀ values for esmolol were 221.6 ± 14.6 , 209.6 ± 37.9 , 178.8 ± 29.4 , and 155.4 ± 27.6 ms at 0, 3, 10, and 30 μM , respectively. In contrast, landiolol had minimal effects on the APD (Fig. 2B). Both esmolol and landiolol had no effect on the amplitude of action potentials and resting potentials (Fig. 2C).

Effects of esmolol and landiolol on ion currents

Effects of β -blockers on I_{CaL} : To specifically evaluate I_{CaL} , an internal K^+ -free solution containing Cs, was used

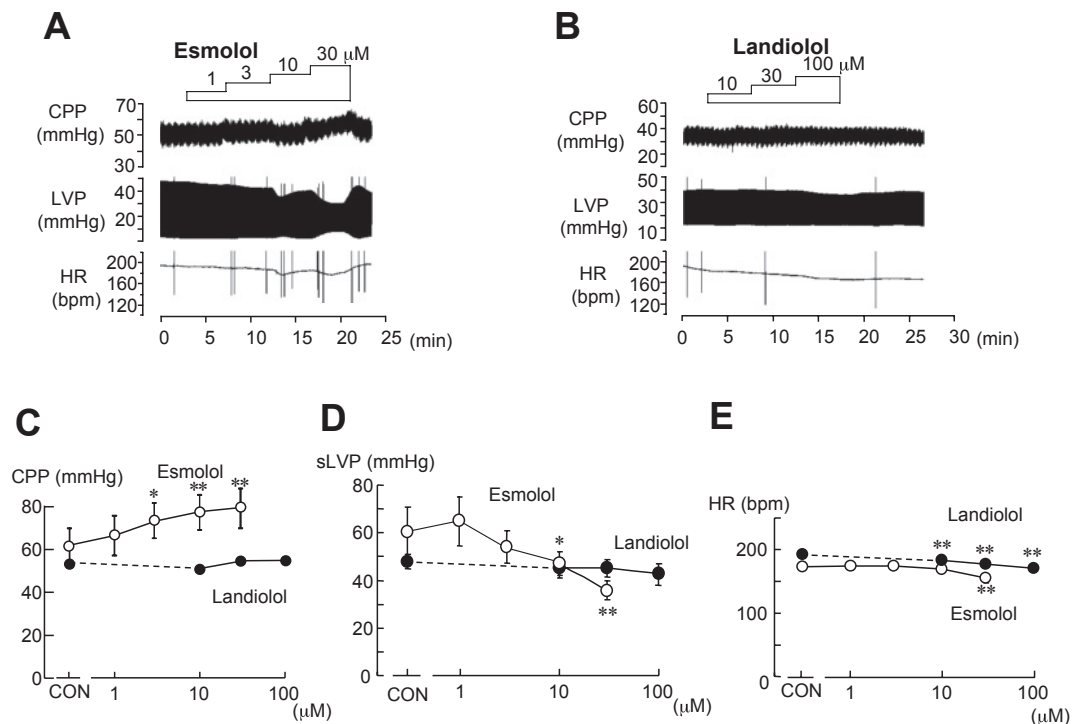


Fig. 1. Effect of esmolol and landiolol on isolated guinea-pig hearts. A and B: Representative traces of the effects of esmolol (left panel) and landiolol (right panel) on coronary perfusion pressure (CPP, upper trace), left ventricular pressure (LVP, middle trace), heart rate (HR, lower trace). C – E: Concentration-dependent effects of esmolol (open circles, $n = 6$) and landiolol (filled circles, $n = 5$) on CPP (C), sLVP (D), and HR (E). * $P < 0.05$, ** $P < 0.01$.

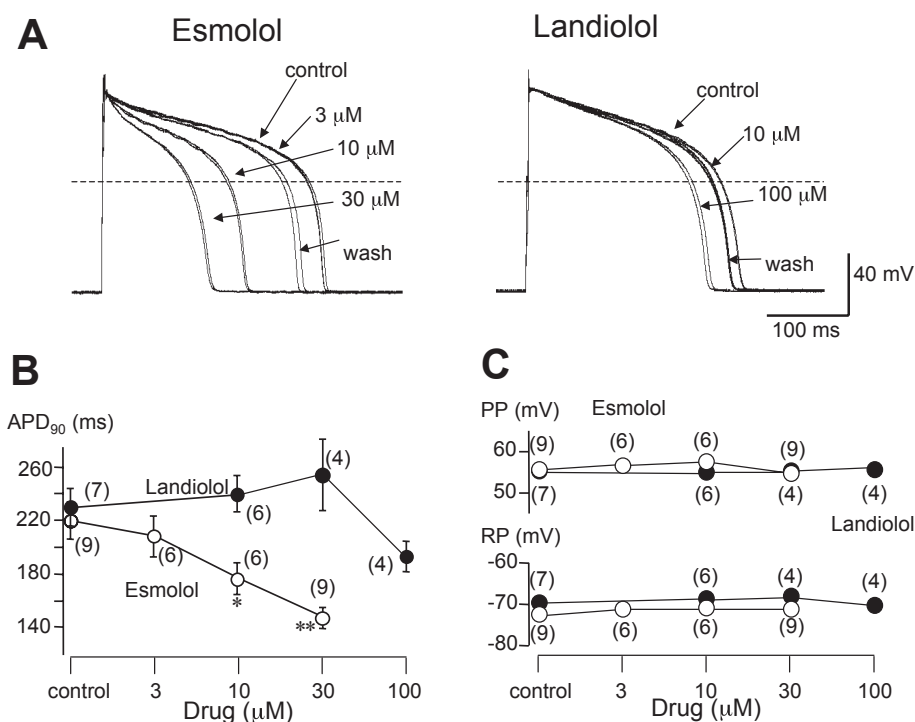


Fig. 2. Effect of esmolol and landiolol on action potentials. A: Representative recordings of action potentials obtained with esmolol (left panel) and landiolol (right panel); drug concentration is indicated near each trace. B: Plots of the association between drug concentrations and action potential duration for esmolol (open circles) and landiolol (filled circles). The number of experiments for each treatment is indicated in parentheses. * $P < 0.05$, ** $P < 0.01$. C: Correlation between drug concentrations and the peaks of action potentials (PP, upper panel) and resting potentials (RP, lower panel) in the presence of esmolol (open circles) and landiolol (filled circles).

with an external Cs-Tyrode solution. The holding potential was set to -40 mV and a 300-ms depolarizing pulse was applied to produce 0 mV to evoke I_{CaL} . Esmolol had minimal effects on the I_{CaL} at concentrations of < 30 μ M, but significantly inhibited the I_{CaL} at 100 μ M ($P < 0.01$ vs. control; Fig. 3: A and B). The kinetics of the I_{CaL} showed little change in response to esmolol. Landiolol had little effect on the I_{CaL} (data not shown), as expected following the lack of change in the action potential (Fig. 2). To examine whether the APD shortening induced by esmolol is due to inhibition of the I_{CaL} , the action potential and I_{CaL} were simultaneously recorded from the same cell (Fig. 3C). The internal solution was K^+ -rich, with the external solution being normal Tyrode solution. After the I_{CaL} was recorded in the voltage clamp mode, action potentials were elicited every 10 s using the current clamp mode and 10 μ M esmolol was applied. Application of 10 μ M esmolol caused approximately 20% shortening of the APD, while the peak I_{CaL} decreased only slightly. Upon wash-out of esmolol, the APD partially recovered, but I_{CaL} remained unchanged (Fig. 3C). Together, these findings indicate that APD shortening by esmolol is not regulated by the I_{CaL} alone. A similar inconsistency between the shortening of the APD and the peaks of Ca currents was observed in three other cells. Furthermore, chelating intracellular Ca^{2+} with BAPTA revealed that the esmolol-induced shortening of the APD was not dependent on intracellular Ca^{2+} (data not shown).

Effects of β -blockers on the delayed rectifier and ATP-

sensitive K^+ currents: The delayed rectifier K^+ current (I_K) in cardiac myocytes consists of fast and slow components: rapidly activating I_K (I_{Kr}) and slowly activating I_K (I_{Ks}). To examine the relative contribution of I_{Kr} and I_{Ks} upon APD shortening induced by esmolol, selective antagonists for I_{Kr} (E4031) and I_{Ks} (chromanol 293B) were administered (Fig. 4). Blockade of the I_{Kr} caused the APD to become prolonged, but failed to inhibit esmolol-induced shortening of the APD (at 10 μ M esmolol, $P < 0.05$, Fig. 4A). Averaging 5 independent experiments, the APD₉₀ was: 261.9 ± 14.9 , 306.4 ± 22.0 , and 271.9 ± 29.9 ms for the control, 5 μ M E4031, and 5 μ M E4031 plus 10 μ M esmolol, respectively. Inhibiting the I_{Ks} with 50 μ M chromanol 293B also had no influence on abrogating the reduction in APD observed following application of 10 μ M esmolol (Fig. 4B), with APD₉₀ of 276.1 ± 18.1 , 381.2 ± 29.4 , and 271.1 ± 31.3 ms for the control, 50 μ M chromanol 293B, and 50 μ M chromanol 293B plus 10 μ M esmolol, respectively ($n = 5$). Together, these results suggest that esmolol-induced APD shortening is not dependent upon either I_{Kr} or I_{Ks} . Furthermore, application of 3 μ M glibenclamide also had no antagonistic effect on APD shortening in response to 10 μ M esmolol (data not shown), indicating that the ATP-sensitive K^+ current is also unlikely to be involved in mediating esmolol activity.

Effects of β -blockers on the inward rectifier K^+ current (I_{K1}): The I_{K1} was recorded using a K^+ -rich internal solution surrounded by normal Tyrode solution containing

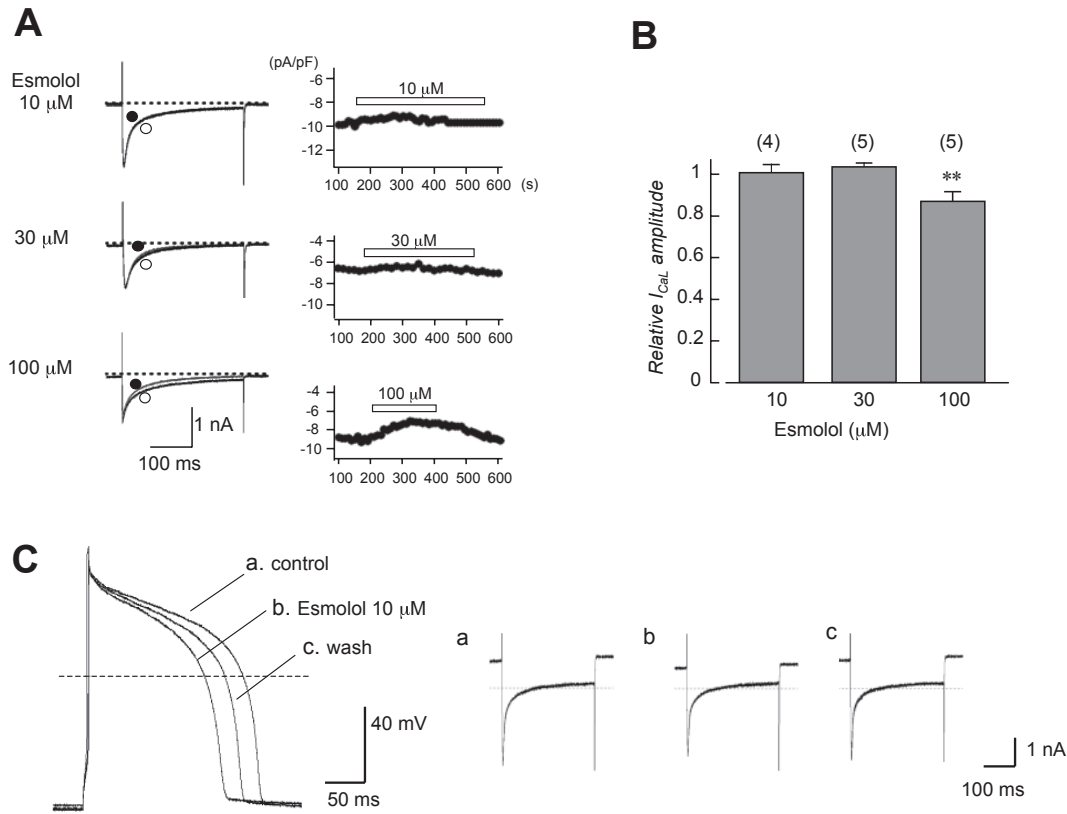


Fig. 3. Effect of esmolol on I_{CaL} . **A:** Representative current traces recorded before and during application of 10 μ M (upper), 30 μ M (middle), and 100 μ M (lower) esmolol. The time course of the effect of esmolol on the peak I_{CaL} is shown on the right of each trace. **B:** Summary of results obtained from 4–5 cells. The number of experiments is indicated in parentheses. **** P < 0.01.** **C:** Simultaneous recordings of the L-type Ca^{2+} current and action potential. Representative recordings of the action potential (left panel) and I_{CaL} (right panel) are shown from the same cell. The action potential was recorded using the current-clamp mode, with the I_{CaL} recorded using the voltage clamp method. To record the I_{CaL} , the holding potential was set to -40 mV, with a 300-ms depolarizing pulse down to 0 mV applied to evoke I_{CaL} . Dotted lines indicate a current level of zero.

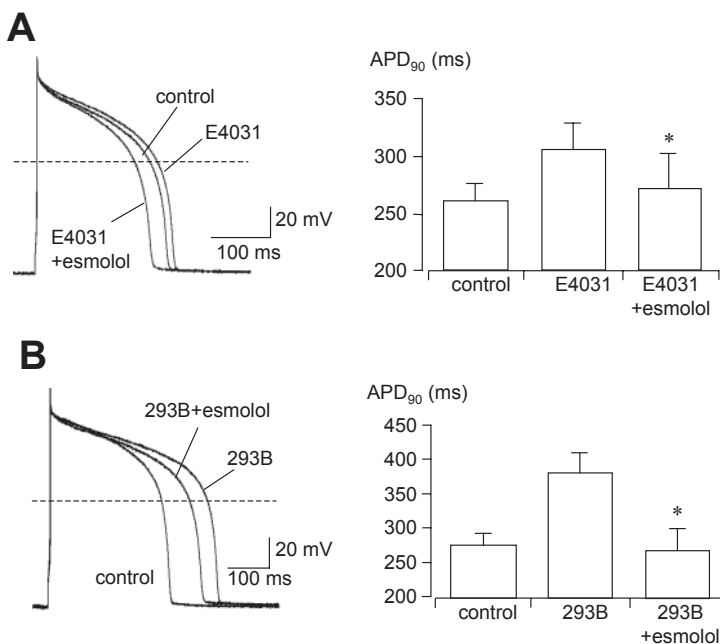


Fig. 4. Contributions of I_{Kr} and I_{Ks} in mediating the effect of esmolol on the action potential. **A:** Raw action potential traces from cells treated with vehicle (control), 5 μ M E-4031, or 10 μ M esmolol (left panel) and their average APD₉₀ (right panel). The dotted line indicates 0 mV. *** P < 0.05 vs. 5 μ M E-4031, n = 5.** **B:** Raw action potential traces (left panel) from cells treated with 50 μ M 293B or 10 μ M esmolol, with their average APD₉₀ displayed in the right panel. *** P < 0.05 vs. 50 μ M 293B, n = 5.**

0.3 μM nisoldipine, 5 μM E4031, or 50 μM 293B. Currents were obtained using square pulses of 500 ms, from a holding potential of -40 mV to several potentials in 10-mV steps (from -110 mV to $+80$ mV, Fig. 5A). The current–voltage (I–V) relationships obtained before (open circles) and during (filled circles) the application of 10 μM esmolol are displayed in Fig. 5C, revealing that esmolol inhibited the I_{K1} and increased an outward current at positive potentials. Figure 5B shows superimposed current traces at the test potential of $+60$ mV, recorded before and during the exposure to esmolol, demonstrating that the current increased by esmolol was time-independent. Both the inhibition of I_{K1} and the increase in the time-independent outward current caused by esmolol were confirmed using a ramp pulse protocol (Fig. 5D). The effect of esmolol was found to be reversible upon wash-out of esmolol (not illustrated). In contrast to esmolol, landiolol had little or no effect on the I_{K1} or outward current at positive potentials (data not illustrated).

Concentration-dependent effects of esmolol on I_{K1} and time-independent outward currents: A triangular ramp pulse ranging from $+80$ mV to -120 mV with a speed of $1 \text{ V}\cdot\text{s}^{-1}$ was applied every 10 s from an initial holding potential of -40 mV. After confirming the stability of the I–V association, 10, 30, and 100 μM esmolol were applied cumulatively, and the I–V curves displayed in Fig. 6A. Inhibition of the I_{K1} by esmolol was more pronounced

at -40 mV than at -100 mV (Fig. 6B, $n = 6$), suggesting the inhibitory effect of esmolol on I_{K1} to be voltage-dependent. As mentioned above, esmolol also increased the outward current at membrane potentials greater than -10 mV. The amplitude of the esmolol-induced outward current was measured at $+50$ mV, normalized to the cell capacitance, revealing esmolol to increase the outward current dose-dependently, and this was independent of time (Fig. 6C, $n = 6$).

We next examined the effect of esmolol on ionic changes when the I_{K1} was suppressed. Ba^{2+} (2 mM) and nisoldipine (0.3 μM) were added to the normal Tyrode solution to suppress the I_{K1} , producing a linear I–V curve (Fig. 7A). Application of 30 μM esmolol increased the outward current (esmolol in Fig. 7A) and had a reverse potential of -82.8 ± 8.6 mV ($n = 4$), indicating that the esmolol-induced current is carried by K^+ . The effect of esmolol was reversible upon wash-out. When the concentration of Ba^{2+} was increased to 5 mM, esmolol could no longer activate the outward current. To confirm that the esmolol-induced outward current was a K^+ current, experiments were repeated using a K^+ -free and Cs^+ -rich internal solution, with an external Cs^+ -Tyrode solution containing 2 mM BaCl_2 . Under these conditions, esmolol failed to increase the outward current, supporting the finding that the esmolol-induced outward current was a K^+ current (Fig. 7B).

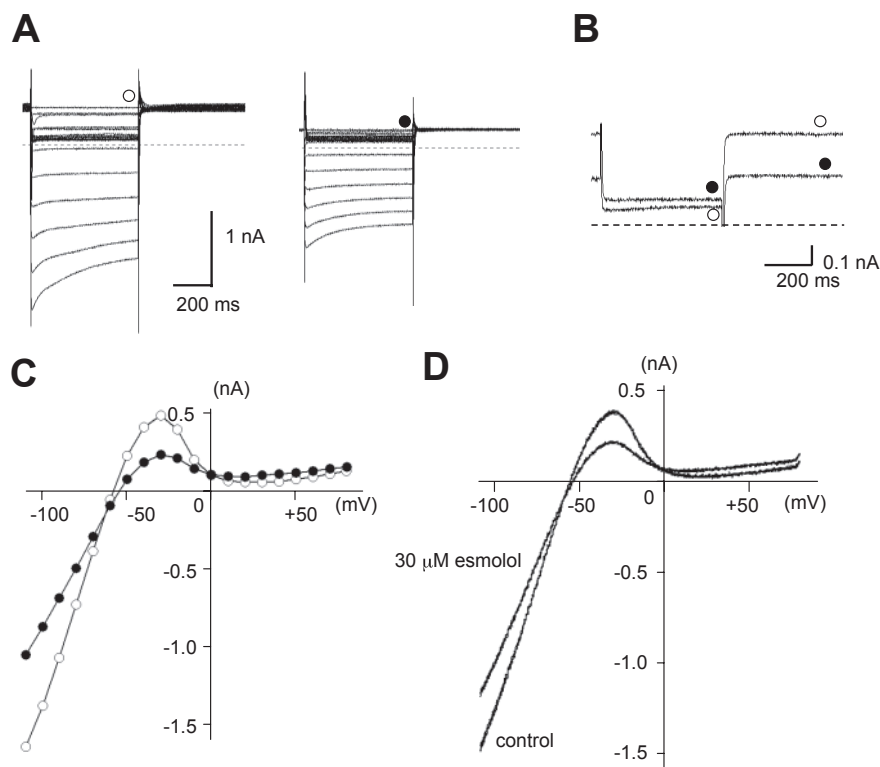


Fig. 5. Inhibitory effects of esmolol on the I_{K1} . A: Raw current traces using square pulses (-110 mV to $+80$ mV) recorded before (control, left panel) and during application of 30 μM esmolol (right panel). B: Current traces at $+60$ mV. Open and filled circles indicate the trace recorded in the absence and presence of esmolol, respectively. C: The current–voltage (I–V) relationship measured using square pulses, where the open circles denote the control and filled circles denote 30 μM esmolol. D: The I–V relationships measured by the ramp pulse protocol. A triangular ramp pulse ranging from $+80$ mV to -120 mV with a speed of $1 \text{ V}\cdot\text{s}^{-1}$ was applied every 10 s from an initial holding potential of -40 mV.

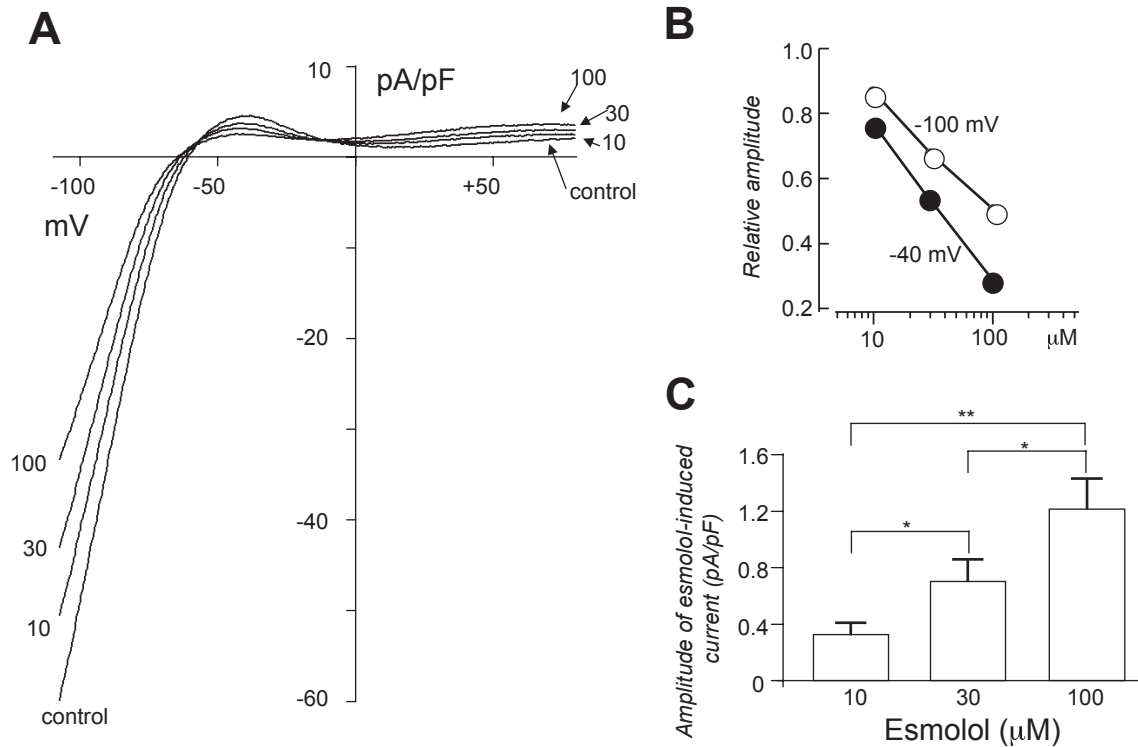


Fig. 6. Effects of esmolol on current–voltage (I–V) relationships. A: The I–V relationship before (control) and during application of 10, 30, and 100 μ M esmolol. B: Relationship between the concentration and relative amplitude of I_{K1} at -100 mV (open circles) and -40 mV (filled circles), $n = 6$. C: Concentration-dependent effect of esmolol on the time-independent outward current. The amplitude of the esmolol-induced current was measured at $+50$ mV and normalized to the cell capacitance, $n = 6$. * $P < 0.05$, ** $P < 0.01$.

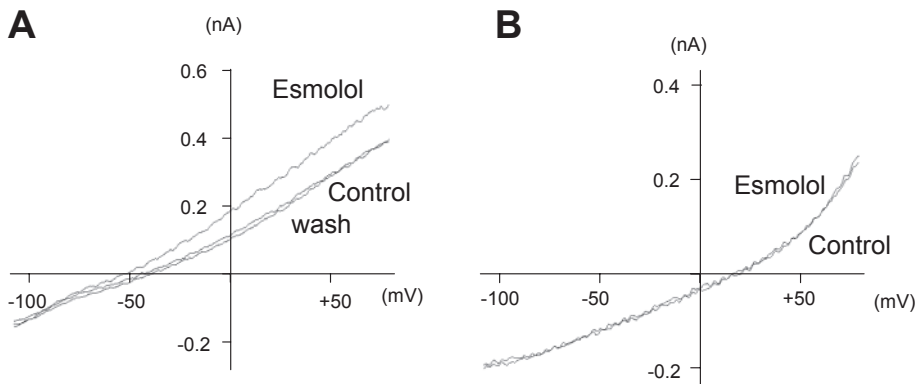


Fig. 7. Effect of esmolol on the esmolol-induced outward current. A: The current–voltage (I–V) relationship before (control) and during application of 30 μ M esmolol in the presence of 2 mM $BaCl_2$. B: The I–V relationship before (control) and during application of 30 μ M esmolol with an internal solution containing Cs and no K^+ , with an external Cs-Tyrode solution.

Discussion

The direct actions of esmolol and landiolol on cardiac function and electrophysiology were investigated. Esmolol was found to elicit significant negative inotropic and chronotropic effects on isolated guinea-pig hearts, while landiolol had very minor actions. Naturally, the negative inotropic and chronotropic actions of esmolol are likely to be due to its β_1 -blocking actions, assuming

that the β_1 -adrenoceptors expressed on cardiac myocytes were activated by residual sympathetic nerves *ex vivo*. The slight decrease in the heart rate, observed in the Langendorff perfused hearts (Fig. 1E), might be caused by this β_1 -adrenoceptor-blocking action of landiolol or esmolol. It should be noted, however, that landiolol, which is 10 times more selective for β_1 -adrenoceptors than esmolol, had negligible effects on altering LV contractility or HR. Furthermore, the shortening of the APD

observed in single cells in response to esmolol cannot be explained by β_1 -blocking activity. Changes in the duration of the APD are positively correlated with myocyte contractility, as the strength of the contraction is governed by the amount of Ca^{2+} released from intracellular storage sites (12). Therefore, it is postulated that the negative inotropic effect (NIE) of esmolol is, at least in part, the result of shortening of the action potential. In contrast to esmolol, landiolol had no apparent effects on the action potential or ionic currents of ventricular myocytes, consistent with the findings of previous studies (7, 9, 10, 13). Furthermore, the NIE of esmolol, but not of landiolol, has also been reported in previous studies; for example, Ikeshita et al. (9) reported that esmolol induces strikingly dose-dependent negative inotropic effects, at 1–10 μM , and almost completely abolished contraction in the rabbit heart at 100 μM . They suggested the actions of esmolol are largely due to its β -blocking effects (9). Further increasing the dose of esmolol to 1.0 mM has previously been shown to induce cardiac arrest (14), indicating its potency. We speculate that the NIE of esmolol accounts for the excessive hypotension observed clinically (6), particularly in patients with poor cardiac function. Furthermore, the increase in CPP reduces coronary blood flow, resulting in decreased myocardial oxygen supply and likely exacerbating esmolol's NIE.

Pharmacokinetic parameters for esmolol have been studied in healthy subjects and in patients with various diseases, and it is now established that its half-life and volume of distribution are approximately 10 min and 2 l/kg, respectively (6, 15, 16). Clinical application of esmolol is maintained in the low micromolar range in plasma, and Sintetos et al. (17) reported that a bolus injection of 30–150 mg esmolol resulted in a blood concentration of 0.8–1.5 $\mu\text{g/ml}$ (2.4–4.5 μM). It should be noted, however, that blood esmolol concentrations are 3- to 7-fold higher in arterial blood than in peripheral venous blood (6, 16, 18). In fact, Adamson et al. (16) reported that the arterial concentration of esmolol could range from 0.1 to 8 $\mu\text{g/ml}$ (0.3–24 μM), with a median value of 2.276 $\mu\text{g/ml}$ (6.8 μM), as the result of a bolus injection of 1 mg/kg esmolol. Thus, the concentration of esmolol reaching cardiac myocytes could be expected to be greater than initially anticipated *in vivo*.

Esmolol is also identified to affect various ionic current systems, in contrast to landiolol. For example, esmolol has been shown to inhibit the I_{CaL} with an IC_{50} value of 0.45 mM (14) and block the voltage-dependent Na^+ current in rat myocytes [IC_{50} : 0.17 mM (14) or 74.2 μM (19)]. Furthermore, esmolol, but not landiolol, can block tetrodotoxin (TTX)-resistant Na^+ currents in rat sensory neurons (13). Esmolol also has inhibitory actions on lipid metabolism, free radical-mediated reactions,

and the arachidonic acid cascade, all of which influence pathologic cellular interactions (20, 21). These actions might be related to the influence of esmolol on core components of anesthesia such as analgesia, hypnosis, and memory function (22).

The current study found that, despite a substantial shortening of APD, esmolol had negligible effects on the repolarizing currents I_{Kr} and I_{Ks} , but inhibited the I_{K1} by 20% (at 10 μM esmolol). The possible involvement of the I_{Kr} or I_{Ks} in mediating the effects of esmolol on the action potential was examined using inhibitors specific for each channel. Esmolol shortened the APD irrespective of the presence of E4031 or chromanol 293B (Fig. 4), indicating that esmolol does not elicit its APD-shortening actions via the I_{Kr} or I_{Ks} . In contrast, the effects of esmolol on the I_{CaL} require further study, as 10 μM esmolol prolonged the APD_{90} by 20%, while inhibiting the I_{CaL} by < 5%. Because of this discrepancy, it is unclear whether esmolol-induced APD shortening is entirely due to inhibition of I_{CaL} or whether other ion channels are also involved. To help solve this query we employed a computer simulation model—the Kyoto Model (23)—to examine the effects of esmolol on membrane currents, based on our existing quantitative data. It was determined that when 10 μM esmolol was present, the density of the I_{K1} and I_{CaL} currents were 0.8- and 1.0-fold of control expression, respectively. The simulation also indicated that inhibition of the I_{K1} alone does not shorten, but rather prolongs the APD (Fig. 8A). Significantly, the model also revealed that in order to obtain a change in the action potential equivalent to that produced by 10 μM esmolol, the I_{CaL} would need to be inhibited by > 50% (Fig. 8: A, B). Taken together, these results suggest another mechanism is likely responsible for esmolol's actions on the APD. One further mechanism potentially involved in the APD shortening induced by 10–30 μM esmolol is the outward current. In fact, incorporating the activation of time-independent outward current successfully reconstructed the shortening of APD by esmolol (Fig. 8C). This current was identified to be time-independent, carried by K^+ , and relatively insensitive to sub-millimolar $[\text{Ba}^{2+}]$. It should be noted, however that the APD is determined by a long-lasting depolarization that is controlled by a fine balance between small inward and outward ionic currents. It is thus possible that the window current of the voltage-dependent Na^+ channels that flows during the terminal phase of repolarization may also affect APD. When the amplitude of voltage-dependent Na^+ current was decreased to a half of the control in the Kyoto Model, the maximum rate of rise of the action potential was decreased from 152.5 to 90.6 V/s, whereas the APD was shortened only slightly (from 148 to 146 ms). The IC_{50} value to inhibit the TTX-resistant Na^+ current was

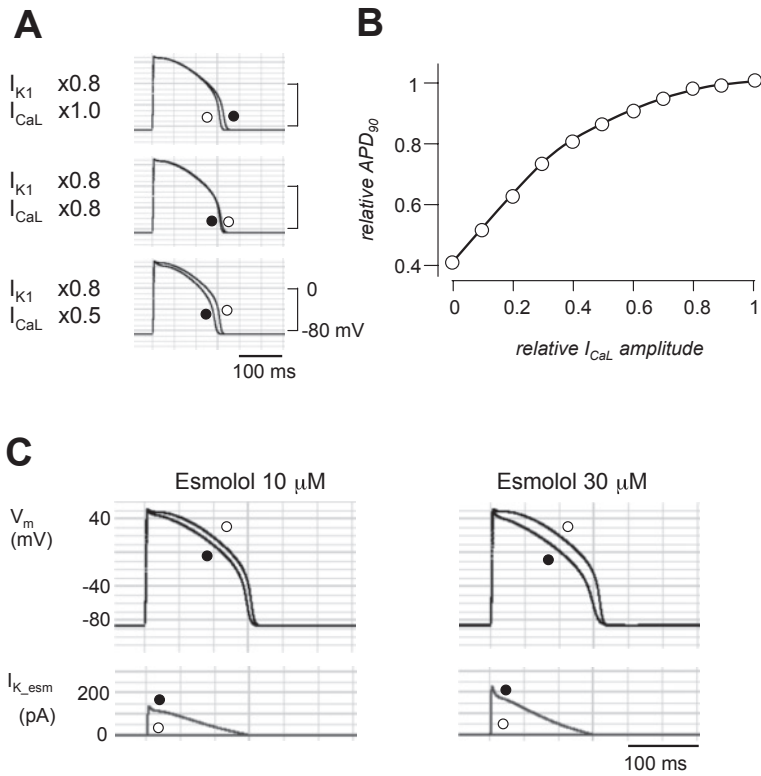


Fig. 8. Reconstruction of the action potential of guinea-pig ventricular cells using the Kyoto Model. **A:** The action potentials at control (open circles) and various amplitudes of I_{K1} and I_{CaL} (filled circles). To simulate the effect of 10 μ M esmolol, the I_{K1} density was maintained at 80% of control expression, and the I_{CaL} density was altered. Inhibition of the I_{K1} alone prolonged the APD, and in order to produce APD shortening comparable to that observed with 10 μ M esmolol, the I_{CaL} had to be inhibited by greater than 50%. **B:** Relationship between the current density of I_{CaL} and the APD using the Kyoto Model. **C:** Computer simulation of the effects of esmolol on the action potential displaying action potential traces (upper traces) and the esmolol-induced outward current (I_{K_eshm}) (lower traces). Open and filled circles indicate recordings from control and esmolol-treated cells, respectively (10 μ M on left, 30 μ M on right). Current densities of the I_{K1} and the I_{CaL} were adjusted to 0.8- and 1.0-fold or 0.55- and 0.9-fold of the control, to mimic the effects of 10 and 30 μ M esmolol, respectively. A time-independent outward current (I_{K_eshm}) was incorporated in the model. The cell was stimulated every 10 s.

reported to be 0.17 mM (14) or 74.2 μ M (19) for esmolol. Thus, the contribution of inhibition of the Na^+ current is unlikely involved in the APD shortening by esmolol.

The molecular basis for the time-independent outward current in the present study is unclear; however, similar electrophysiological characteristics have been reported following administration of drugs in previous studies. For example, it has been reported that bimoclolmol, a cytoprotective hydroxylamine derivative (which has a wide variety of beneficial effects for diabetic dysfunction, coronary vasospasm, and arrhythmias), activated an outward current in canine ventricular myocytes (24). The bimoclolmol-induced outward current was found to be time-independent, with a reversal potential of approximately -90 mV and was insensitive to 0.3 mM Ba^{2+} .

Further channels potentially mediating esmolol's electrophysiological effects include tandem pore K^+ channels. These channels have recently been identified and are composed of at least 15 different members (25), some of which are expressed in cardiac myocytes (26). The channels are time- and voltage-independent and carry leak or "background" currents that are regulated by many different signaling molecules and other factors, including stretch, polyunsaturated fatty acids, pH, inhalational anesthetics, and neurotransmitters, and can regulate the action potential of cardiac myocytes (26). Further studies are required to determine whether these new

channels also mediate the actions of esmolol.

A recent study by Fallouh et al. (14) in rat ventricular myocytes determined that the NIE of esmolol occurred in parallel with a reduction in the Ca^{2+} transient and that neither sarcoplasmic reticulum (SR) function nor myofibrillar Ca^{2+} sensitivity was affected by esmolol. Fallouh et al. (14) attributed the suppression of the Ca^{2+} transient chiefly to inhibition of the L-type Ca^{2+} channel, whereas our study indicated that the suppression of the Ca^{2+} transient is likely caused by the APD shortening due to activation of a time-independent outward current. One possible explanation for the discrepancies between studies is that the IC_{50} value for the I_{CaL} was 0.45 mM in Fallouh et al.'s report (14), a value relatively larger than those stimulating APD shortening in the present study. Furthermore, Fallouh et al. (14) only investigated the I_{CaL} and voltage-dependent Na^+ current, while the present study explored several other potential channels, revealing esmolol to activate a time-independent outward current. We suggest that it is this outward current that is predominantly responsible for the NIEs of esmolol at concentrations of < 0.1 mM, while inhibition of the I_{CaL} predominates at concentrations higher than 0.1 mM. Further studies are clearly required to clarify the functional role of the esmolol-induced outward current with regard to cardiac function.

In the current study, esmolol increased coronary perfu-

sion pressure in a concentration-dependent manner (Fig. 1). A similar finding was reported in a swine model of cardiopulmonary resuscitation (27), where esmolol improved the outcome of cardiopulmonary resuscitation while augmenting coronary perfusion pressure. Usually an increase in vascular resistance is described as one of adverse effects of β -blockers and attributed to their β_2 -blocking action. The β_1/β_2 selectivity ratio for esmolol, is reported to be approximately 33 (1), and coronary vasoconstriction occurred at concentrations greater than $3 \mu\text{M}$ (Fig. 1A). Provided that the β_1 -blocking effect emerges at submicromolar concentrations of esmolol, we speculate that β_2 -blocking action is involved in the coronary vasoconstriction in the present study. This view might be supported by a clinical study where the pulmonary artery pressure and pulmonary vascular resistance were also increased in patients with coronary artery diseases (28). Alternatively, esmolol may affect vascular smooth muscle directly to induce contraction, although systematic analysis has not been reported so far. Nevertheless, the elevations in coronary vascular resistance would require caution if used in patients with left ventricular dysfunction.

In conclusion, the current study confirmed esmolol to be a potent NIE, significantly more so when compared to landiolol. It was then identified that esmolol's NIEs were, at least in part, due to shortening of the APD. Further investigation determined that the inhibition of cardiac contractility and APD shortening by esmolol was not due to inhibition of the I_{CaL} , but rather may involve induction of an outward current. In addition, an increase in coronary resistance in response to esmolol may also further facilitate negative inotropy in vivo. In contrast to esmolol, landiolol had minimal effects on cardiac ionic currents, action potential, cardiac contractility, or cardiac coronary perfusion pressure.

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