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A pharmacologic activator of endothelial KCa channels increases systemic conductance and reduces arterial pressure in an anesthetized pig model

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ABSTRACT

SKA-31, an activator of endothelial KCa2.3 and KCa3.1 channels, reduces systemic blood pressure in mice and dogs, however, its effects in larger mammals are not well known. We therefore examined the hemodynamic effects of SKA-31, along with sodium nitroprusside (SNP), in anesthetized, juvenile male domestic pigs. Experimentally, continuous measurements of left ventricular (LV), aortic and inferior vena cava (IVC) pressures, along with flows in the ascending aorta, carotid artery, left anterior descending coronary artery and renal artery, were performed during acute administration of SKA-31 (0.1, 0.3, 1.0, 3.0 and 5.0 mg/ml/kg) and a single dose of SNP (5.0 μg/ml/kg). SKA-31 dose-dependently reduced mean aortic pressure (mP_{AO}), with the highest dose decreasing mP_{AO} to a similar extent as SNP (-23 ± 3 and -28 ± 4 mm Hg, respectively). IVC pressure did not change. Systemic conductance and conductance in coronary and carotid arteries increased in response to SKA-31 and SNP, but renal artery conductance was unaffected. There was no change in either LV stroke volume (SV) or heart rate (versus the preceding control) for any infusion. With no change in SV, drug-evoked decreases in LV stroke work (SW) were attributed to reductions in mP_{AO} (SW vs. mP_{AO}, $r^2 = 0.82$, $P < 0.001$). In summary, SKA-31 dose-dependently reduced mP_{AO} by increasing systemic and arterial conductances. Primary reductions in mP_{AO} by SKA-31 largely account for associated decreases in SW, implying that SKA-31 does not directly impair cardiac contractility.

1. Introduction

The vascular endothelium plays a critical role in the regulation of blood pressure and blood flow distribution by controlling the intraluminal diameter of conduit and small resistance arteries. This dynamic regulation occurs via the activation of distinct vasodilatory mechanisms in the endothelium that reduce contractile tone in the surrounding vascular smooth muscle, leading to increased intraluminal diameter, arterial conductance and blood flow. Major pathways contributing to endothelium-dependent vasodilation include the de novo synthesis of nitric oxide, prostacyclin and the generation of a hyperpolarizing electrical signal that acts on vascular smooth muscle. Endothelium-dependent hyperpolarization (EDH) is generated primarily via the activation of endothelial small- and intermediate-conductance, Ca^{2+}-activated K^+ channels (KCa2.3 and KCa3.1 channels, respectively) and is transmitted via myoendothelial gap junction connections to the adjacent smooth muscle, where it causes membrane hyperpolarization and reduced Ca^{2+} influx via voltage-gated Ca^{2+} channels. Small-molecule activators of KCa2.3 and KCa3.1 channels evoke direct hyperpolarization of endothelial cells [1–5], relax myogenically active resistance arteries [1,6] increase coronary flow in isolated heart preparations [7], and lower blood pressure in normo- and hypertensive mice [2,5]. In conscious dogs, bolus administration of a KCa channel activator transiently lowers

Abbreviations: G, conductance; HR, heart rate; IVC, inferior vena cava; KCa channel, calcium-activated K^+ channel; mPAO, mean aortic pressure; mP_{IVC}, mean inferior vena caval pressure; PBS, phosphate-buffered saline; P_{LVED}, left ventricular end-diastolic pressure; SKA-31, naphtho[1,2-*d*]thiazol-2-ylamine; SNP, sodium nitroprusside; SV, stroke volume; SVR, systemic vascular resistance; SW, stroke work; Vol_D, volume of distribution.

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systemic blood pressure [4]. In contrast, genetic knockout of endothelial KCa channels in mice leads to elevated systemic blood pressure and impairs or abolishes stimulus-evoked vasodilatory processes in isolated arteries and tissues [8]. Endothelial KCa channel activity may also be important in disease settings, since KCa channel activation is able to restore agonist-evoked vasodilatory responses in the coronary circulation of a rodent model of type II diabetes exhibiting endothelial dysfunction [9].

To advance our knowledge of the in vivo cardiovascular effects of endothelial KCa channel activators, the goal of the present study was to investigate the systemic hemodynamic effects of SKA-31, a recently described, second-generation KCa channel activator [2], in a large animal model, the anesthetized, instrumented pig. Our results demonstrate that bolus intravenous injections of SKA-31 dose-dependently lower mean aortic pressure and increase systemic conductance to levels comparable to those elicited by the nitrovasodilator sodium nitroprusside (SNP). SKA-31 increased arterial conductance in coronary and carotid arteries, indicating that SKA-31 may have broad vasodilatory action in the vasculature. Neither SKA-31 nor SNP appeared to directly alter myocardial contractility. In summary, our data demonstrate that SKA-31 effectively lowers systemic blood pressure and increases arterial conductance in the peripheral circulation of the anesthetized pig. These observations suggest that SKA-31 may also be an effective vasodilator in the human vasculature.

2. Methods and materials

The experimental protocols used in this study were approved by the University of Calgary Animal Care Committee, and conform to the NIH-published Guide for the Care and Use of Laboratory Animals (8th edition, 2011), and are further consistent with those of the American Physiological Society.

2.1. Animal preparation

Domestic pigs (25–30 kg body weight, average weight 27 kg, 16–18 weeks of age) were obtained from a local supplier. As female pigs were typically retained by the supplier for breeding purposes, we chose to utilize male pigs for our study to preserve homogeneity of the study population, given the modest sample size. Pigs were premedicated with an intramuscular injection of ketamine hydrochloride (600 mg), fentanyl citrate (2 mg), and midazolam (10 mg). A 20-gauge catheter was inserted into an ear vein and anesthesia was induced with sodium thiopental (25 mg/kg). Anesthesia (level 3) was maintained with a continuous intravenous (I.V.) infusion containing a mixture of fentanyl citrate (0.04 mg/ml), midazolam (0.025 mg/ml) and ketamine hydrochloride (0.3 mg/ml) at a rate of 100 ml/h. Both isoflurane (less than 1% in the ventilator) and lidocaine (3 bolus intravenous administrations, 1 mg/kg, 5 min apart, followed by an I.V. infusion of 0.75–1.0 mg/min) were used as required. The drug infusion rates were adjusted as necessary to ensure deep sedation without spontaneous respiratory effort. The animals were intubated with a cuffed endotracheal tube and ventilated with constant-volume ventilator (Harvard Apparatus, Millis, MA) with a 50% oxygen–50% nitrous oxide mixture. Tidal volume and respiratory rate were adjusted to maintain physiological values of blood gases and pH in accordance with recommended ventilation parameters for large animals [10]. $PaCO_2$ was maintained between 35 and 45 mm Hg. This ventilation procedure also resulted in an arterial pO2 of ~100 mm Hg, which was verified at the start of the experiment.

A median sternotomy was performed and the hearts were delivered from the pericardium through a base-to-apex incision. Sonomicrometry crystals (Sonometrics, London, ON) were implanted in the left ventricular endocardium and mid-wall of the septum to measure the minor-axis septum-to-left ventricular free wall and left ventricular antero-posterior dimensions [11–13]. Ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed on the ascending aorta, descending aorta (just above diaphragm), inferior vena cava (IVC) (just above the diaphragm), right carotid artery, left renal artery, and left anterior descending coronary artery. Thin walled 7-French fluid-filled catheters connected to pressure transducers (model P23 ID; Statham Gould, Oxnard, CA) were inserted into the left ventricle (LV) (P_{LV}; retrograde through the left carotid artery), aorta (P_{AO}; retrograde through the right femoral artery) and IVC (P_{IVC}; through the right jugular vein). An intravenous line was placed in the left external jugular vein for volume loading (Pentaspan™, 10% pentastarch in 0.9% NaCl) to replenish fluid loss during surgery. A thin-walled catheter was connected to the intravenous line for bolus infusions. Arterial samples for blood-gas analysis were obtained from a side-port on the aortic catheter. Body temperature was monitored with a rectal thermometer. After instrumentation, the heart was returned to the pericardium, which was closed with individual sutures, taking care not to compromise pericardial volume [14]. A single-lead electrocardiogram (ECG) was recorded.

2.2. Experimental protocol

Simultaneous pressure, dimension and flow measurements were recorded at baseline and during each intervention. After stabilization at an LV end-diastolic pressure (P_{LVED}) of ~10 mm Hg (11 ± 1 mm Hg), control data were collected for 60 s, immediately preceding a 5-min recording period, during and after drug infusion. Each 20 ml infusion was delivered over a 60 s period and proceeded in ascending order of SKA-31 dosage (0.1, 0.3, 1.0, 3.0, and 5.0 mg/ml/kg) followed by a single dosage of sodium nitroprusside (SNP; 5.0 μg/ml/kg). Washout and recovery periods of 15–20 min were interposed between drug infusions. At the end of the experiment, animals were sacrificed with an intracardiac bolus injection of concentrated KCl (25 mmol/kg) while under deep anesthesia; this procedure meets and exceeds the 2013 American Veterinary Medical Association guidelines for euthanasia. Following sacrifice, the positions of the sonomicrometry crystals within the myocardium were verified. Note that a total of 10 pigs were utilized in our study, however, 3 animals did not yield useful data, due to cardiovascular instability that developed part way through the experiment. In addition, SNP data are only included from 5 animals, as procedural complications were encountered in the 2 remaining animals. This difference in sample size is reiterated in the figure legends.

SKA-31 was synthesized and tested for identity and purity (NMR and HPLC/MS) as previously described [2]. SKA-31 was dissolved in a vehicle solution comprised of Cremophor EL (10% v/v) and phosphate-buffered saline (PBS) (90% v/v). Briefly, an aliquot of Cremophor EL was first heated in a beaker on a magnetic stir plate to a temperature of ~60 °C. The desired amount of solid SKA-31 was then added to the heated Cremophor EL liquid as it was being stirred. Once the added SKA-31 had dissolved completely, heating was stopped and stirring was maintained. The first few milliliters of PBS were then added slowly to the SKA-31/Cremophor EL solution and the remaining amount was added more quickly. The final SKA-31 solution was allowed to cool to room temperature with continuous stirring and appeared slightly yellowish. Solutions of SKA-31 in Cremophor-EL/PBS were freshly prepared for each experiment.

2.3. Data analysis

The conditioned signals were passed through a low-pass filter (100 Hz) and were digitized and recorded at 100 Hz (Sonometrics Corp. Acquisition System, London, ON). The digitized data were analyzed on a personal computer using custom software (CV Works, Calgary, AB) developed in our laboratory. Baseline and control data are expressed as mean values for the 60-s period immediately preceding each infusion event. All data associated with administration of drug or control solutions were extracted at the time of greatest decrease in mP_{AO}. If mP_{AO} did not change by at least 5 mm Hg during a given