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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

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intervention, acquired data points were averaged for the first 60 s of that period.

Systemic conductance ($G_{systemic}$, the reciprocal of systemic vascular resistance, SVR) was calculated as mean aortic flow / ($mP_{AO} - mP_{IVC}$) and expressed as a percent change from the preceding control value. Carotid conductance ($G_{carotid}$), renal conductance (G_{renal}) and coronary conductance ($G_{coronary}$) were expressed similarly and calculated by respectively substituting mean carotid, renal and coronary flow for mean aortic flow. LV stroke work (SW) was calculated as LV stroke volume (SV) × [mean P_{LV} (systolic) − P_{LVED}], where mean P_{LV} (systolic) was calculated as P_{AO} (diastolic) + 2/3 [P_{AO} (systolic) − P_{AO} (diastolic)]. As an index of LV end-diastolic volume, LV area (A_{LVED}), was calculated as the product of the 2 minor-axis LV dimensions [15,16]. SW and A_{LVED} values following drug infusions are expressed as the percent change from the preceding control values determined using the same calculations.

2.4. Measurements of SKA-31 concentration in plasma

Blood samples (~2 ml) were taken via a catheter inserted into the left external jugular vein at various intervals following drug infusion at the same site. Samples were collected in heparinized tubes to prevent coagulation and centrifuged at 400 ×*g* for 20 min at 4 °C. The resulting supernatants were then stored at −80°C prior to analysis. Plasma samples were then processed and analyzed in duplicate by HPLC/MS as recently described [4] and SKA-31 concentrations were determined from a standard curve. A semi-logarithmic plot of SKA-31 plasma concentrations vs. time was fitted with the following equation:

$$\text{SKA-31 Plasma Concent.} = C_0 * exp(-k_e t).$$

Where C_0 = the maximal initial concentration of SKA-31 in the plasma calculated from the y-intercept of the fitted line, k_e is the rate constant and *t* is the time interval following SKA-31 infusion. The volume of SKA-31 distribution (Vol_D) was calculated as follows:

$$Vol_D = \text{SKA-31 dosage}/C_0.$$

2.5. Statistical analysis

Statistical comparisons were performed using SigmaPlot (Systat Software, Inc. 2012). In Fig. 8, a linear correlation was calculated for the percentage changes for mP_{AO} and stroke work during saline, vehicle, and all drug infusions ($y = y_0 + a * x$). The Student's paired *t*-test was used to test for the significance of changes between a given infusion (i.e. vehicle or drug) and the preceding control period. Repeated-measures ANOVA (Holm–Sidak method) were used to test for the significance of differences between vehicle/SKA-31 infusions and SNP. A *P* value <0.05 was considered statistically significant. Except where noted, data are presented as mean ± SEM. Given the mean differences and levels of variance observed in hemodynamic parameters, the calculated level of power for our statistical analyses was found to be minimally 80% for the given sample sizes of 7 animals for SKA-31-mediated changes and 5 animals for SNP-related data.

3. Results

Seven anesthetized, juvenile pigs were acutely implanted with blood pressure transducers and Doppler flow probes that allowed us to measure mean aortic and inferior vena cava pressures, systemic conductance and regional conductance in carotid, renal and coronary arteries. Myocardial performance was monitored via a single lead electrocardiogram and implanted sonomicrometry crystals in the myocardium to assess LV dimensions. Average hemodynamic parameters (± SEM) in all 7 animals measured at baseline, following instrumentation and recovery, were as follows: heart rate, 122 ± 9 beats per min; mean aortic pressure, 71 ± 6 mm Hg; mean inferior vena cava pressure, 7 ± 1 mm Hg; LV end diastolic pressure, 11 ± 1 mm Hg and LV stroke volume, 23 ± 3 ml.

3.1. SKA-31 dose response

Following surgical interventions, animals were allowed to recover until steady-state basal levels of mean aortic pressure and heart rate were achieved. After a minimum 10 min period of steady-state baseline recording, we commenced with the first (saline) infusion. Fig. 1 displays representative tracings of the effect of individual bolus administrations of saline, drug vehicle, or SKA-31 (0.1–5.0 mg/ml/kg) on mean aortic pressure (mP_{AO}, panel A), systemic conductance (panel B), measured conductance in carotid, coronary and renal arteries (panel C) and heart rate (panel D). While SKA-31 infusions had clear effects on these hemodynamic parameters, neither saline nor vehicle infusions had any observable effects. In an effort to benchmark the effects of SKA-31 on the measured parameters, we infused a single dose of the well characterized nitrovasodilator sodium nitroprusside (SNP) following recovery from the SKA-31 evoked hemodynamic changes. As displayed on the right hand side of Fig. 1A–D, SNP infusion (5.0 μg/ml/kg) produced qualitatively similar changes in mP_{AO}, systemic conductance, carotid, coronary and renal artery conductances and heart rate when compared with SKA-31. In the case of mP_{AO} (Fig. 1A), intravenous infusion of SKA-31 significantly decreased mP_{AO} in a dose-dependent manner versus each preceding control period, with the greatest decrease occurring after the highest dose (5.0 mg/ml/kg) (Fig. 2). SNP infusion also significantly decreased mP_{AO} and this change was comparable to that measured following infusion of 5.0 mg/ml/kg SKA-31. The question of whether SKA-31 administration may potentially affect either the pharmacokinetics or pharmacodynamics of subsequent SNP infusion was not directly addressed in our study, although we would anticipate minimal interactions, based on the different chemical structures of these two drugs and their different mechanisms of action as vasodilators.

As quantified in Fig. 3, the time to peak response for the SKA-31 induced decrease in mP_{AO} was slowest at 0.1 mg/ml/kg drug administration and became faster with increasing dosages. At a dosage of 5.0 mg/ml/kg, SKA-31 infusion resulted in a significantly faster decline in mP_{AO} compared with SNP.

In contrast to the observed decreases in mP_{AO}, SKA-31 did not significantly alter mean inferior vena cava pressure (mP_{IVC}), compared with preceding control values (Fig. 4). In the case of SNP, we did observe a trend toward lower mP_{IVC}, although this change did not reach statistical significance (P = 0.052).

3.2. Conductance and resistance

Fig. 5 shows the effect of SKA-31 and SNP administration on absolute changes in systemic vascular resistance (SVR) (Fig. 5A), along with the calculated percent changes in systemic conductance (Fig. 5B). We observed no changes in SVR versus the preceding control values following infusions of saline, drug vehicle or lower dosages of SKA-31 (0.1 and 0.3 mg/ml/kg), whereas dosages of 1.0, 3.0 and 5.0 mg/ml/kg each significantly decreased systemic resistance. SKA-31 at the highest dosage decreased SVR to a level comparable to that evoked by 5.0 μg/ml/kg SNP. Predictably, the inverse relationships were observed for drug-induced changes in systemic conductance (Fig. 5B).

In addition to its impact on systemic conductance, we also examined the effect of SKA-31 on blood flow in select vascular regions. As shown in Figs. 1C and 6, SKA-31 and SNP infusions produced qualitatively similar effects on conductance in the right carotid artery ($G_{carotid}$), left anterior descending coronary artery ($G_{coronary}$), and left renal artery (G_{renal}). SKA-31 increased $G_{carotid}$ at dosages of 3.0 and 5.0 mg/ml/kg and produced a maximal change in conductance similar to that observed with 5.0 μg/ml/kg SNP. In the left anterior descending coronary artery, SKA-

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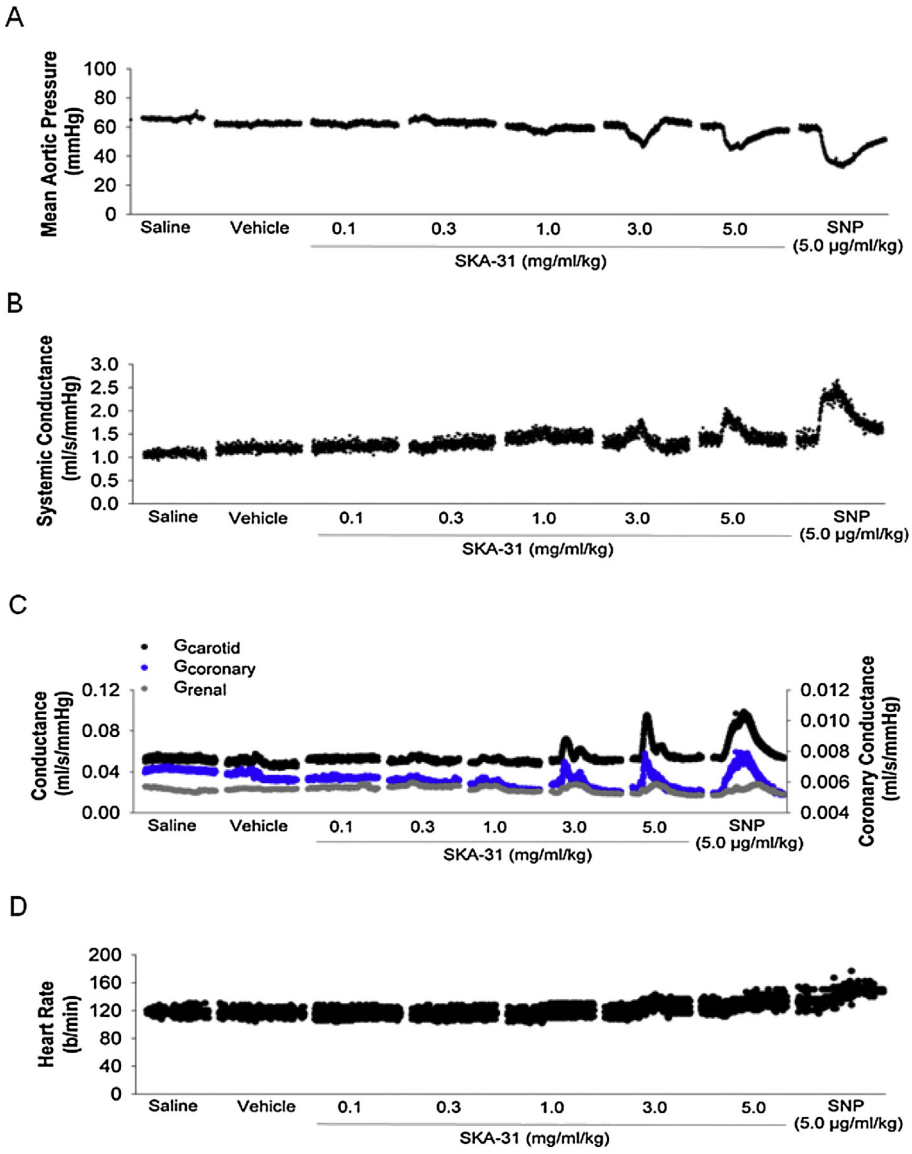

Fig. 1. Representative data from one pig demonstrating the rapid and reversible effects of SKA-31 and sodium nitroprusside (SNP) following acute intravenous infusion on mean aortic pressure (mP_{AO}) (panel A), systemic vascular conductance (panel B), measured conductance in coronary, carotid and renal arteries (panel C) and heart rate (panel D). In each panel, the sections of continuous data points displayed represent 5-min epochs that were extracted from the master data record and illustrate the basal levels and evoked changes in the measured parameters in response to the infusions. The horizontal bars and labels provided beneath each panel specify the experimental infusion for the 5-min section of data appearing immediately above each description. Note that all displayed data were acquired simultaneously during the experiment. Individual infusions were separated by a 15–20 min recovery period (indicated by the breaks between the sections of data points) and control hemodynamic data were acquired for the first 1–2 min period immediately prior to a given infusion, once a steady baseline was clearly apparent (not shown).

31 also significantly increased $G_{coronary}$ at doses of 3.0 and 5.0 mg/ml; the increase evoked by the latter dose approximated that observed with SNP. Interestingly, neither SKA-31 nor SNP significantly increased blood flow in the renal artery (compared with the preceding control conductance values) and vasodilatory responses in this artery were generally blunted compared with carotid and coronary vessels (Fig. 1C).

3.3. Cardiac function

As depicted in Fig. 7A, infusions of SKA-31 and SNP did not produce significant changes in either cardiac stroke volume (SV) or heart rate (HR) under our experimental conditions. We further examined potential drug-induced changes in the left ventricular end diastolic area (A_{LVED}), as measured by sonomicrometry crystals implanted in the septal wall and LV endocardium, and the calculated LV stroke work (SW_{LV}); both of these parameters are expressed as the percent change from the respective preceding control value (Fig. 7B). Over the dosage range of 1.0 to 5.0 mg/ml/kg, SKA-31 produced very modest decreases in A_{LVED} (<5% below control), whereas SNP reduced A_{LVED} by an average of 8% compared with control. In contrast to the slight decreases observed in A_{LVED}, SKA-31 evoked a clear, dose-dependent reduction in SW_{LV} over the range of 0.1 to 5.0 mg/ml/kg and produced a similar maximal decrease at a dosage of 5.0 mg/ml/kg (−29%) as that observed following SNP infusion (−32%).

To evaluate the observed decreases in SW_{LV} following SKA-31 and SNP administrations in greater detail, we plotted the calculated percent changes in SW_{LV} versus the observed percent changes in mean aortic pressure (mP_{AO}). The scatter plot in Fig. 8 shows the relation between SW_{LV} and mP_{AO}, based on the pooled data derived from all infusions of saline, vehicle, SKA-31 and SNP. Importantly, the calculated r^2 value of 0.82 for the linear regression line indicates that more than 80% of the variance in SW_{LV} can be explained by the variance in mP_{AO}. Using a similar approach, we also plotted the percent changes in A_{LVED} versus mP_{AO} for all animals and infusions (i.e. saline, vehicle, drug) examined.

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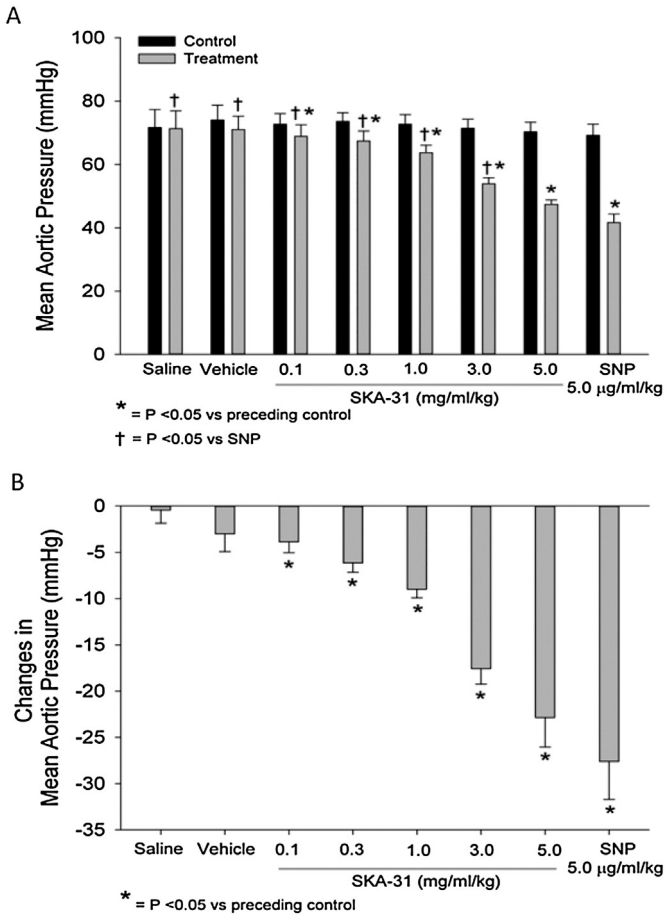

Fig. 2. Quantification of mean aortic pressure (mP_{AO}) under control conditions and following acute infusion of SKA-31 (0.1–5.0 mg/ml/kg) and SNP (5.0 μg/ml/kg) (panel A). Panel B quantifies the drug-evoked changes in mP_{AO} relative to the preceding control value for each experimental condition. For the SKA-31 related data in panels A and B, N = 7 animals; for the SNP-related data, N = 5 animals.

Linear regression analysis of this relation yielded a r^2 value of only 0.24, indicating that no more than 25% of the variance in A_{LVED} can be explained by the variance in mP_{AO} ($P < 0.001$; data not shown). Based

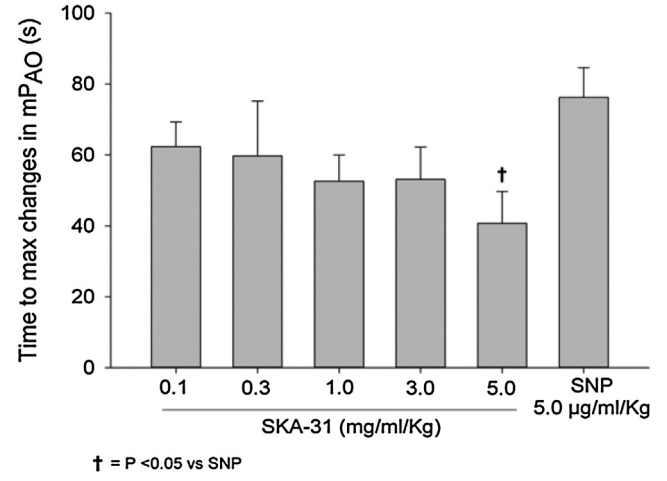

Fig. 3. Quantification of the time to maximal change in mean aortic pressure (mP_{AO}) following intravenous infusion of either SKA-31 (0.1–5.0 mg/ml/kg) or SNP (5.0 μg/ml/kg). Administration of either saline or drug vehicle did not evoke measurable changes in mP_{AO}. The response evoked by 5.0 mg/ml/kg SKA-31 was significantly faster than that elicited by SNP, as determined by two-way ANOVA; $P < 0.05$. Note that for the SKA-31 related data, N = 7 animals; for the SNP-related data, N = 5 animals.

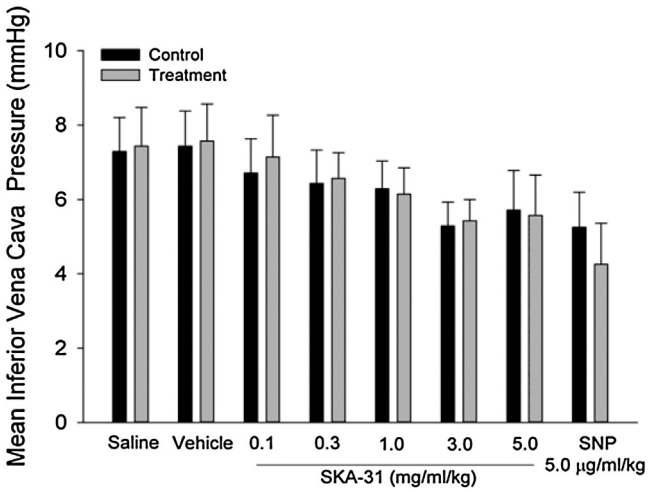

Fig. 4. Lack of effect of SKA-31 (0.1–5.0 mg/ml/kg) on mean inferior vena cava pressure (mP_{IVC}) following acute administration. Histogram displays mP_{IVC} values recorded in response to infusions of saline, drug vehicle and the indicated dosages of SKA-31 and SNP. Values for baseline mP_{IVC} (control) immediately preceding each infusion are designated by the black bars. For the SKA-31 related data, N = 7 animals; for the SNP-related data, N = 5 animals.

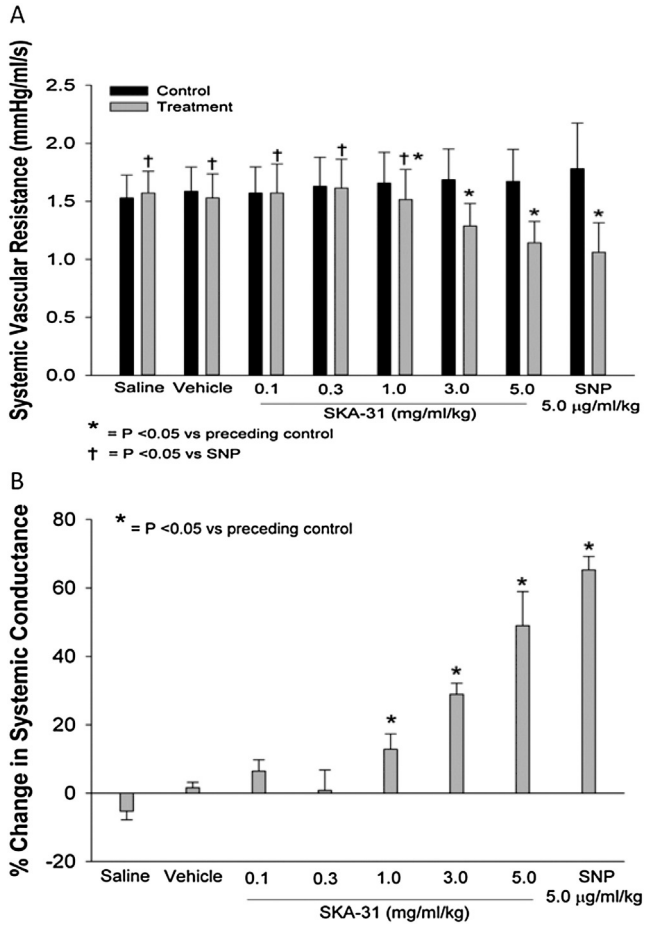

Fig. 5. Acute administration of SKA-31 and sodium nitroprusside (SNP) reduce systemic vascular resistance (SVR). Panel A displays absolute values for SVR recorded prior to a given drug infusion and following SKA-31 and SNP infusions at the indicated dosages. For the latter data, measurements were taken during the peak change in SVR. Panel B displays the calculated percentage change in systemic vascular resistance under each infusion condition compared with the preceding control. For the SKA-31 related data in panels A and B, N = 7 animals; for the SNP-related data, N = 5 animals.

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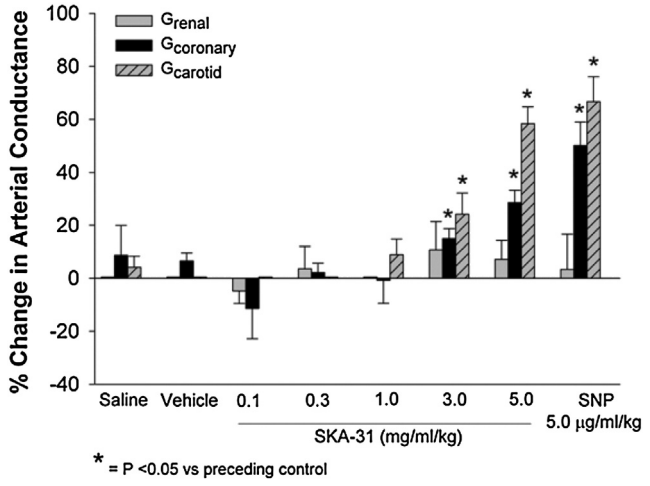

Fig. 6. Quantification of evoked changes in arterial conductance calculated for the carotid, coronary and renal arteries in response to infusions of saline, drug vehicle, SKA-31 (0.1–5.0 mg/ml/kg) and SNP (5.0 μg/ml/kg). Histogram displays the percentage change in conductance in each artery evoked by administered drugs relative to the preceding control value for each infusion. Asterisks indicate a statistically significant difference compared with the baseline conductance value preceding a given infusion. For the SKA-31 related data, N = 7 animals; for the SNP-related data, N = 5 animals.

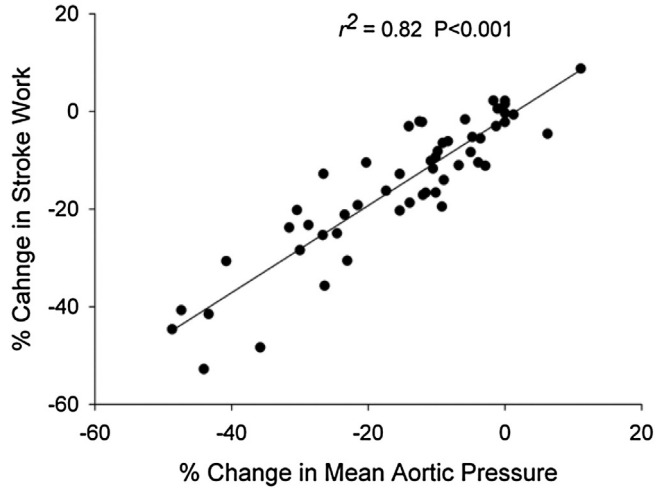

Fig. 8. Scatter plot displaying the relation between observed changes in left ventricular stroke work (SW) and mean aortic pressure (mP_{AO}) following infusions of saline, vehicle, SKA-31 and SNP. Percent changes in mP_{AO}, along with accompanying percent changes in SW, were first calculated in response to each infusion utilized in a given experiment. Data points from all 7 animals were then plotted against each other in a pair-wise fashion, as depicted by the individual symbols on the graph. The straight line through the symbols represents a linear regression fit to the pooled data points (r^2 value = 0.82; P < 0.001).

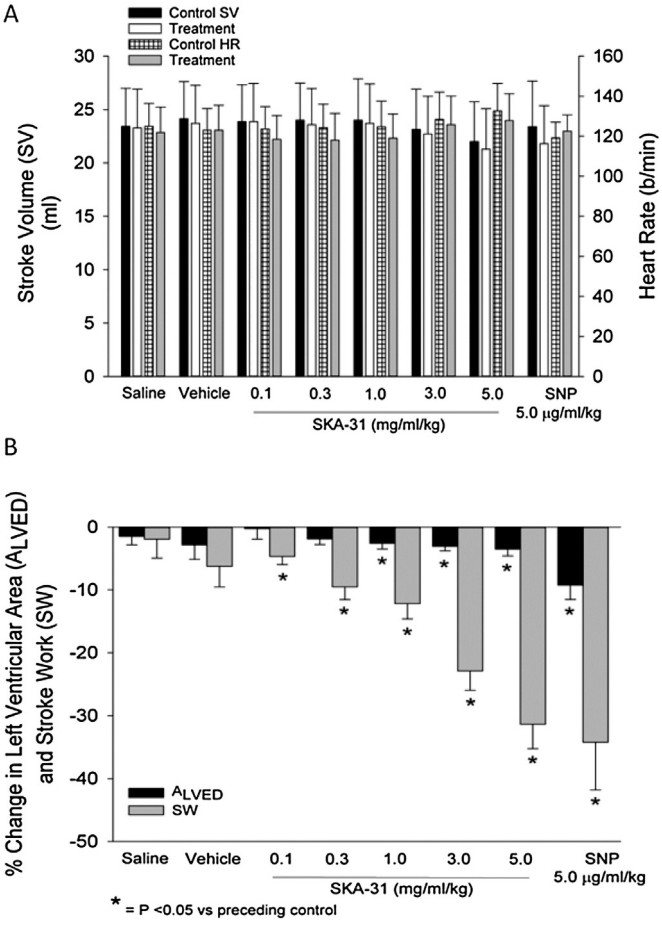

Fig. 7. Quantification of the effects of acute administration of saline, drug vehicle, SKA-31 (0.1–5.0 mg/ml/kg) or SNP (5.0 μg/ml/kg) on left ventricular stroke volume and heart rate (panel A). No significant changes were noted for either stroke volume or heart rate in response to a given infusion compared with the values measured during the preceding control period. The histogram in panel B displays the percentage changes in left ventricular area (A_{LVED}) and stroke work (SW), relative to the baseline values measured during the control period preceding each indicated infusion. For the SKA-31 related data in panels A and B, N = 7 animals; for the SNP-related data, N = 5 animals.

on these results and the fact that drug infusions did not change stroke volume under any condition (see Fig. 7A), we conclude that neither SKA-31 nor SNP directly impaired cardiac contractility.

3.4. Plasma concentrations of SKA-31 following acute infusion

In a separate group of 3 anesthetized and instrumented animals, we analyzed the plasma concentrations of SKA-31 at selected time points following acute intravenous infusion of a 3.0 mg/ml/kg SKA-31 bolus dose. Blood samples were withdrawn from the left jugular vein at ~1 min, 35 min and 75 min following complete infusion of the drug. The average free plasma concentration of SKA-31 measured at each of the above time points (n = 3) was 77.5 ± 27.7 μM, 27.3 ± 3.8 μM and 27.4 ± 4.9 μM, respectively. The volume of distribution for SKA-31 calculated from a semi-logarithmic plot of average SKA-31 plasma concentrations versus sampling times was 0.19 l/kg.

4. Discussion

Using an anesthetized and instrumented porcine model, we have provided the first detailed description of the systemic hemodynamic actions of SKA-31, a small molecule activator of KCa 2.x and 3.1 channels [2], on key cardiovascular parameters in a large animal and have shown how these actions compare with those of SNP, an established nitrovasodilator and blood pressure-lowering agent. As shown in Figs. 1 and 2, intravenous administration of SKA-31 dose-dependently evoked significant decreases in mean aortic pressure, with the highest dose utilized in our study (5.0 mg/ml/kg SKA-31) producing a similar decrease in mP_{AO} as that observed with SNP (−23 ± 3 and −28 ± 4 mm Hg, respectively) (Fig. 2B). In previous studies [2,5,8], acute in vivo administration of SKA-31 was shown to lower blood pressure in both normotensive and hypertensive mice and, more recently, Köhler and colleagues [4] have reported that acute infusion of SKA-31 (0.4 and 2.0 mg/kg) transiently decreases systemic blood pressure in conscious dogs. We also noted that the decrease in mP_{AO} evoked by 5.0 mg/ml/kg SKA-31 was more rapid compared with SNP (Fig. 3), even though both agents lowered mean aortic pressure to a similar extent (Fig. 2). The slower time course of the SNP-mediated drop in mP_{AO} may reflect the fact that SNP requires vascular conversion/decomposition to release nitric oxide and induce subsequent cellular actions in arterial smooth muscle [17], while SKA-31 directly hyperpolarizes the endothelium by activating

KCa channels. Collectively, these observations are in agreement with the reported vasodilatory actions of SKA-31 in the intact coronary [7] and skeletal muscle circulations [5,8] of rodents and the systemic circulation of the dog [4].

SKA-31 had no significant effect on mean inferior vena cava pressure (mP_{IVC}) (Fig. 4). In the case of SNP, we did observe a trend toward lower mP_{IVC}, which would be in agreement with the known clinical effects of SNP to lower central venous pressure, due to its ability to increase venous capacitance [18]. One possible reason for our observation is that mP_{IVC} was already quite low under basal experimental conditions (~8 mm Hg) and a further drug-induced decrease in mP_{IVC} may have been difficult to detect in our anesthetized pigs. Although KCa2.3 and KCa3.1 channel mRNA and whole cell K^+ currents have been reported in venous endothelial cells (e.g. HUVECs) [1,3,19], we are unaware of data describing a direct vasodilatory effect of KCa channel activators on veins or the venous circulation.

SKA-31 dosages of 1.0 to 5.0 mg/ml/kg increased systemic arterial conductance, with the highest dose producing an increase in conductance similar to that induced by SNP (Fig. 5B). We also observed increases in both carotid and coronary arterial conductances at 3.0 and 5.0 mg/ml/kg SKA-31 (Fig. 6), which were similar to those observed with SNP at the highest dosage of SKA-31. Interestingly, renal conductance appeared to be unaffected by either SKA-31 or SNP. In the case of SNP, this is somewhat unexpected, as other investigators have reported that renal arteries are sensitive to nitrovasodilators [20,21]. The renal microcirculation is known to exhibit strong autoregulatory behavior [22,23], which is critical for ensuring adequate blood flow to glomerular units and protecting them from arterial pressure-induced damage. One possible explanation for this apparent insensitivity of the renal conductance to SKA-31 and SNP is that the renal circulation may have already been near-maximally dilated, due to a combination of intrinsic autoregulation and the somewhat lower mP_{AO} present in our anesthetized pigs. Alternatively, it is possible that reduced arterial resistance triggered an increase in peripheral sympathetic tone to counteract reduced blood pressure, which then limited renal arterial dilation. However, this possibility is less likely, as we observed no concomitant increase in heart rate with declines in mP_{AO}, which one would anticipate with the activation of a baroreceptor feedback mechanism acting on the heart.

4.1. Cardiac function

As small-conductance Ca^{2+}-activated K^+ channels have been reported in the atria and pacemaker/conducting cells of murine and human cardiac tissue [24–27] and thus may be activated in response to systemic SKA-31 administration, we recorded various indices of myocardial performance during SKA-31 infusions. Importantly, we observed no significant change in left ventricular stroke volume following administration of either SKA-31 or SNP. In contrast to Köhler and coworkers [4], who reported a pronounced increase in heart rate (HR) following acute SKA-31 infusion in dogs, we did not detect a significant change in HR in response to SKA-31 or SNP infusions in the anesthetized pig (Fig. 7). The difference in HR responses in these two studies could be attributed to the difference in experimental models, as Köhler and colleagues examined conscious dogs (presumably with unsuppressed baroreceptor reflexes providing autonomic nerve input to the heart) versus our anesthetized, instrumented pig model. Importantly, the absence of SKA-31 induced changes in HR observed in our study strongly suggests that plasma levels of SKA-31 sufficient to evoke substantial decreases in blood pressure do not directly impact either pacemaker function or action potential propagation in the heart, as revealed under conditions of minimal baroreceptor reflex activity.

Neither SKA-31 nor SNP significantly reduced central venous pressure (Fig. 4). One possible explanation is that the relative magnitude of the arterial and venous effects of these vasodilatory agents may differ [18,28–30] or an increase in total venous capacitance may have been limited by a modest elevation in arterial capacitance as a result of evoked vasodilation. Hemodynamically, a minor, undetected rise in total venous capacitance could explain the slight decrease we observed in A_{LVED}, our measure of left ventricular end-diastolic volume, in response to SKA-31 and more so to SNP (Fig. 7B). Since left ventricular stroke work (SW) is a function of both ventricular volume and pressure, the decreases observed in SW following administration of SKA-31 and SNP could be explained, in part, by the minor reduction in A_{LVED}. However, further analysis of these data clearly showed that changes in A_{LVED} accounted for less than 25% of the variance in SW, whereas changes in mP_{AO} accounted for more than 80%. Thus, the observed decreases in SW could be largely attributed to the reductions in mP_{AO} associated with drug administration (Fig. 8). Furthermore, the observed reductions in mP_{AO}, indicative of left ventricular afterload, would be expected to offset the slight decreases in A_{LVED} observed with SKA-31 and SNP administrations, and the balance of these two effects would tend to maintain stroke volume near control levels in the presence of either SKA-31 or SNP (Fig. 7A).

Our attempt to explore the pharmacokinetic behavior of SKA-31 revealed that its plasma levels measured 35–75 min following intravenous infusion of a 3.0 mg/ml/kg dose were higher than the reported EC_{50} values of SKA-31 for KCa3.1 channels (~0.3 μM) and KCa2.3 channels (~2 μM) [2], suggesting that sustained activation of these endothelial channels might be anticipated. However, the absence of prolonged hypotension following administration of SKA-31 in our anesthetized pigs suggests that the relationship between the free plasma concentration of SKA-31 (plasma protein binding of SKA-31 in mice and dogs is reported to be 35–40%) [2,4] and its vasodilatory actions may not be a direct one and may be complicated by the availability of additional drug binding sites or a more complex whole body distribution pattern.

Another explanation for the relatively short-lived hemodynamic response following SKA-31 infusion could be a “desensitization” of the pharmacological targets for SKA-31 actions. Both KCa3.1 and KCa2.3 channels are subject to regulation by intracellular second messengers or protein kinases and phosphatases. For example, phosphorylation of channel-associated calmodulin by casein kinase 2 reduces the affinity of KCa2 channels for the membrane phospholipid PIP_2 [31] and likely contributes to the inhibition of KCa2 channel activity by Gq-associated G-protein coupled receptors. In immune cells, KCa3.1 activity is increased by phosphorylation of His358 in the channel's C-terminus through the histidine kinase nucleoside diphosphate kinase B (NDPK-B) [32], while the PI_3P phosphatase myotubalarin related protein 6 (MTMR6) and the histidine phosphatase phosphohistidine phosphatase-1 (PHPT-1) inhibit KCa3.1 function [33,34]. Additionally, KCa3.1 currents can be regulated by cAMP-dependent protein kinase (PKA) via Ser phosphorylation sites in the C-terminus [35–37], which may impair endothelium-dependent vasodilation [38].

Finally, the ability of a KCa channel activator to lower blood pressure more effectively in hypertensive versus normotensive mice [2,5] suggests that this class of compound may be beneficial in the acute or chronic treatment of elevated blood pressure. Our results showing that SKA-31 reduces blood pressure and increases systemic conductance in the pig suggest that translational studies examining the potential blood pressure-lowering actions of a KCa channel activator in a large animal model of hypertension or vascular disease are likely feasible.

4.2. Summary and conclusions

The results of our study demonstrate that the KCa channel activator SKA-31 effectively and reversibly increases systemic conductance in a dose-dependent manner and lowers mean aortic pressure in a large animal model. The observed hemodynamic actions of SKA-31 closely mimic those evoked by SNP. SKA-31 did not directly affect cardiac contractility, nor did it appear to impact heart rate or excitability. The common and overlapping cardiovascular responses to SKA-31 and SNP are consistent with the conclusion that SKA-31 acts primarily on blood vessels to evoke its effects on the systemic vasculature. Based on our

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observations, SKA-31 and related compounds represent a novel pharmacologic approach to induce local and systemic vasodilation in a large mammal, due to the unique cellular targets and pathways activated by these agents. Endothelial KCa channel activators thus do not mimic SNP, nitroglycerin and other classes of clinically prescribed cardiovascular drugs in their mechanism of action. As a consequence, KCa channel activators may be useful as an alternative pharmacologic strategy to evoke acute decreases in systemic resistance in situations, such as left ventricular failure and hypertensive emergencies, where nitrate tolerance can be an issue.

Conflicts of interest

On behalf of all the authors, the corresponding author states that no conflicts of interest exist.

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