Heart and Vessels
https://doi.org/10.1007/s00380-019-01489-9

ORIGINAL ARTICLE

Small and intermediate Ca^{2+}-sensitive K^+ channels do not play a role in vascular conductance during resting blood flow in the anaesthetised pig

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Received: 23 May 2019 / Accepted: 23 August 2019

Abstract
Flow-induced dilation in resistance arteries is mediated by endothelium-dependent hyperpolarisation via small and intermediate conducting Ca^{2+} sensitive K^+ channels. The aim of the current study was to assess the effect of blocking both channels, using the toxins apamin and charybdotoxin, on flow-induced dilation in a conduit artery and vascular conductance. Experiments were carried out on the iliac and its vascular bed in anaesthetised pigs ($n = 4$). Flow-induced dilation and vascular conductance ($\Delta F/\Delta P$) were assessed before and after administration of toxins intra-arterially (i.a.) at 50 μg kg^{-1}. Iliac diameter increased from baseline to 2.39 ± 0.4 mm before and 2.09 ± 0.46 mm after toxin administration, which was not significantly different ($P = 0.63$, Student's paired t test). Control conductance was 1.49 ± 0.27 ml min^{-1} $mmHg^{-1}$ ($P < 0.00001$, ANOVA), and 1.53 ± 0.18 ml min^{-1} $mmHg^{-1}$ ($P < 0.00001$, ANOVA) in the presence of the toxins which was not significantly different ($P = 0.93$ homogeneity of regression analysis). There was a small but significant increase in mean arterial pressure after the toxins were administered, from 74 ± 5 to 80 ± 9 mmHg ($P = 0.03$, Student's paired t test); but all other measured parameters were not significantly affected. Small- and intermediate-conducting Ca^{2+}-sensitive K^+ channels are not involved in flow-mediated dilation in conduit arteries and do not play a role in resistance vessel diameter maintenance at resting blood flow.

Keywords Small-conducting Ca^{2+}-sensitive K^+ channels · Intermediate-conducting Ca^{2+}-sensitive K^+ channels · EDH · Nitric oxide · Conductance

Introduction

There is consensus that the mechanism of flow-induced arterial diameter increase differs depending on the artery type. In large conduit arteries, the effect is mediated by release of nitric oxide from the endothelium in response to the increased shear stress in the vessel lumen [1]. Whereas, in resistance arteries, diameter is increased by endothelium hyperpolarisation via small- and intermediate-conducting Ca^{2+}-sensitive K^+ channels [2]; but not large conductance K^+ channels [3]. In all artery types, the dilatory response requires an intact endothelium [4]. However, it is unclear whether the small- and intermediate-conducting Ca^{2+}-sensitive K^+ channels are active and contribute to the maintenance of resistance vessel diameter under normal, baseline physiological flow conditions. This is an important question since, in the intact animal, resistance vessel diameter is a determinant of blood pressure and hence blood flow.

Therefore, the aim of this study was to assess the effect of antagonism of both channels on vascular conductance in vivo during baseline blood flow. The toxin apamin was used to block the small-conducting Ca^{2+}-sensitive K^+ channels and charybdotoxin was used to block the intermediate-conducting Ca^{2+}-sensitive K^+ channels. Their combined administration has been shown to prevent endothelium-dependent hyperpolarisation in vitro [5].

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Published online: 31 August 2019

Methods

Ethical approval

Procedures on live animals were performed under licence in accordance with Irish and European directive 2010/63/EU following approval by University College Cork animal research ethics committee (project authorisation number: AE19130-P025.

Surgery and instrumentation

4 female landrace pigs (15.86–19.47 kg) were sedated with ketamine (14 mg kg^{-1}) and xylazine (2.7 mg kg^{-1}) i.m; a cannula was inserted into an ear vein and the animal was anesthetized with a bolus, followed by a continuous infusion of sodium pentobarbital (induction 30 mg kg^{-1}; maintenance 6 mg kg^{-1} h^{-1} i.v.). Continuous infusion was maintained via a catheter in the jugular vein, connected to an infusion pump (Harvard). End-tidal carbon dioxide ($ETCO_2$), pulse oximetry and core temperature were monitored using a SurgiVet Advisor Vital Signs Monitor (Smiths Medical, Dublin, OH, USA). Arterial pH, PCO_2 and PO_2 were assessed using a hand-held i-STAT Blood Gas Analyzer (Abbot Point of Care Inc, Princeton, NJ, USA) and maintained within normal ranges. Following tracheotomy, animals were ventilated with 40% O_2 in room air using a Harvard ventilation pump at a rate adjusted to keep end tidal and arterial PCO_2 within a normal range. A cannula attached to a pressure transducer (Grass; Grass Technologies, West Warwick, RI, USA) was inserted into the left carotid artery (Fig. 1) for measurement of arterial blood pressure. The iliac artery was prepared as described previously [6]. Briefly, either the left or right iliac artery was dissected from the aortic bifurcation to the deep femoral branch. A cannula attached to a 3-way tap was inserted into the deep femoral artery for injection of acetylcholine and the two toxins. Ultrasonic piezoelectric crystals were placed on diametrically opposite sides of the iliac artery for continuous measurement of the diameter using a sonomicrometer (Sonometrics Corporation, London, Ontario, Canada). An ultrasonic transit time flow transducer (Transonic Systems Inc, Ithaca, New York, NY, USA) was placed around the artery to measure blood flow. In addition, an adjustable snare was placed just below the iliac–aorta bifurcation to produce controlled restrictions to blood flow (Fig. 1). Pressure within the iliac was measured with a catheter-tipped manometer (Millar). Haemodynamic signals were recorded using Power lab pre-amplifiers, software (AD Instruments Ltd, Oxford, UK) and a Dell computer.

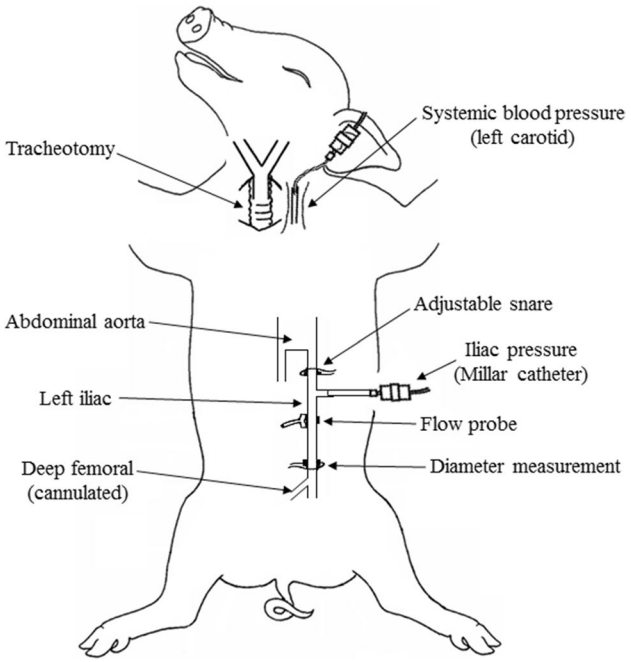

Fig. 1 A schematic showing the experimental setup and instrumentation of the anaesthetised pig

Electronic measurements of hemodynamic variables were taken off-line using Chart 7 software (AD Instruments). Following experimental procedures, animals were killed using a lethal intravenous injection of pentobarbitone and KCL.

Experimental protocol

1. Flow-induced dilation in the iliac
   In each pig control, flow-induced dilation was accomplished by infusing acetylcholine (20–40 µg min^{-1}) via the deep femoral artery, downstream of the diameter measurement site on the iliac; an example of records from this experiment is shown in Fig. 2.
2. Conductance
   Conductance was obtained using the flow restriction protocol previously described [6], and an example of records obtained is shown in Fig. 4. Briefly, the adjustable snare was used to restrict blood flow once every 2–3 min (a total of 4 occlusions were carried out per experimental run).

After the control iliac dilation and conductance were obtained, apamin and charybdotoxin (50 µg kg^{-1} i.a. for both) were injected systemically via the deep femoral artery. This was done to maximise the concentration of the toxins in the arterial vessels of the study area. Subsequently, the acetylcholine infusion to assess flow-induced dilation and the conductance protocol was repeated.

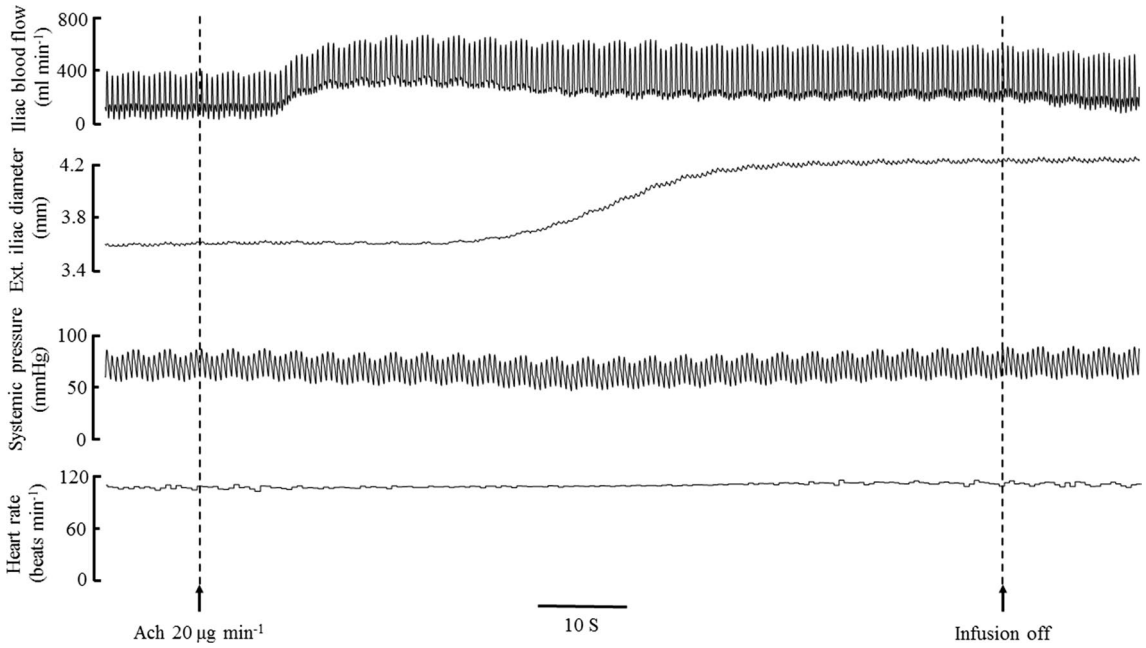

Fig. 2 An example of records from pig 5/17 showing a flow induced increase in iliac artery diameter. Iliac diameter is measured by placing two piezoelectric crystals in the vessel sheath opposite to one another. Diameter returned to baseline within 5–10 min. Arterial blood pressure was unaffected

Drugs used

Apamin and charybdotoxin were both obtained from Smartox Biotechnology (Saint Egrève, France) and acetylcholine was bought from Sigma (Dublin, Ireland).

Statistical analysis

Data are expressed as mean ± SE. Vascular conductance was obtained from the slope of the relationship between flow and pressure ($\Delta F/\Delta P$), slope significance was assessed using ANOVA (SPSS) and the slopes were compared to one another using homogeneity of regressions analysis (SPSS), mean values were compared using Student's paired *t* test. $P < 0.05$ was considered significant.

Results

Baseline parameters at the onset of experimentation are given in Table 1. There was a small but significant increase in mean arterial pressure flowing toxin injection.

Table 1 Cardiovascular parameters before and after arterial administration of apamin and charybdotoxin (50 µg/kg i.a. for both)

Pig	Control				Post-apamin + charybdotoxin			
	Heart rate (beat min^{-1})	MABP (mmHg)	ILBF (ml min^{-1})	Iliac diam (mm)	Heart rate (beat min^{-1})	MABP (mmHg)	ILBF (ml min^{-1})	Iliac diam (mm)
Pig 1/17	101	62	110	2.05	101	72	63	1.21
Pig 5/17	109	80	106	2.49	104	85	104	2.41
Pig 1/18	93	70	170	1.00	94	73	169	0.99
Pig 2 18	137	85	116	2.72	115	90	95	2.79
Mean	110	74	126	2.06	104	80*	108	1.85
SD	19	10	30	0.77	9	9	44	0.88
SEM	10	5	15	0.38	4	4	22	0.44

*$P < 0.05$ Student's paired *t* test

Effect of apamin and charybdotoxin (50 μ kg^{-1} i.a. for both) on flow-induced dilation in the iliac ($n=4$)

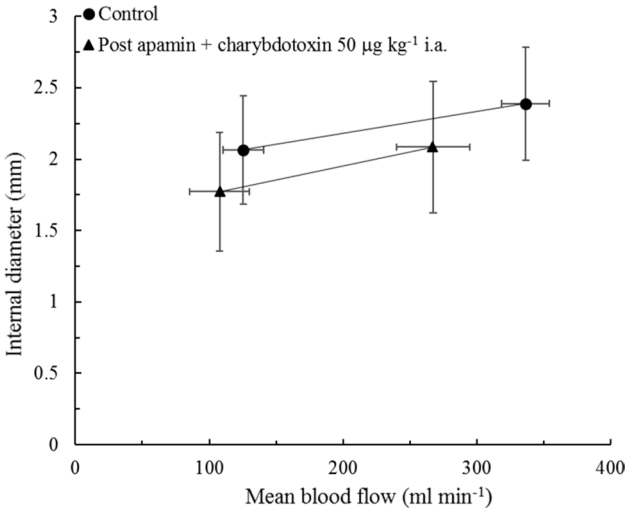

Fig. 3 The effect of apamin and charybdotoxin (50 μg kg^{-1} i.a. for both) on flow-induced dilation of the iliac ($n=4$). Points on the left show the average control diameter and blood flow of the iliac at rest and points they are connected to on the right show the increase in blood flow and diameter following flow increase, for both protocols. The diameter and blood flow were not significantly different either at rest or at the peak of the response (Student's paired *t* test)

Figure 2 shows an example of records obtained from one experiment, the iliac artery dilates in response to an increase in blood flow. The data from this series of experiments are summarised in Fig. 3. The maximum diameter achieved in the control flow-induced dilation experiment averaged to 2.39 ± 0.4 mm which was not significantly different from the maximum diameter increase in the presence of the toxins which was 2.09 ± 0.46 mm ($P=0.63$, Student's paired *t* test). Blood flow reached an average maximum of 337 ± 18 ml min^{-1} for the control protocol and 267 ± 27 ml min^{-1} in the presence of the toxins, which was also not significantly different ($P=0.08$, Student's paired *t* test).

This series of experiments shows that antagonism of small and intermediate Ca^{2+}-sensitive K^+ channels does not affect flow-induced dilation in the iliac, a conduit artery.

Effect of apamin and charybdotoxin (50 μ kg^{-1} i.a. for both) on conductance in the iliac vascular bed ($n=4$)

Figure 4 shows an example of records from a control occlusion study and Fig. 5 summarises the results obtained in this series of experiments. Control conductance before administration of the toxins was 1.49 ± 0.27 ml min^{-1} mmHg^{-1} ($P<0.00001$, ANOVA), and 1.53 ± 0.18 ml min^{-1} mmHg^{-1} ($P<0.00001$, ANOVA) in the presence of the toxins.

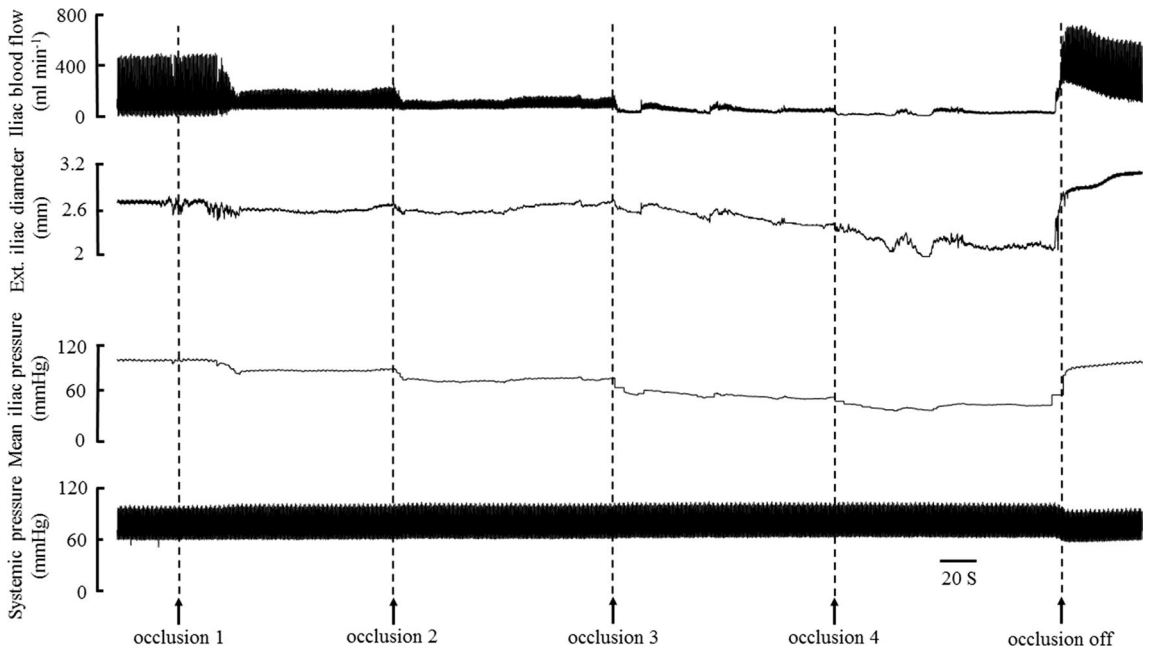

Fig. 4 An example of records from pig 1/18 showing the effect of serial occlusions of the iliac on flow and pressure. As can be seen four occlusions were carried out and there is a step decrease in iliac artery pressure and flow without a change in systemic pressure (the adjustable snare is placed above the iliac pressure and flow measurement site) which is monitored in the left carotid. A reactive hyperaemia occurred when the occlusion was removed at the end of the protocol. The slope of the relationship between flow and pressure ($\Delta F/\Delta P$) gives the vascular conductance

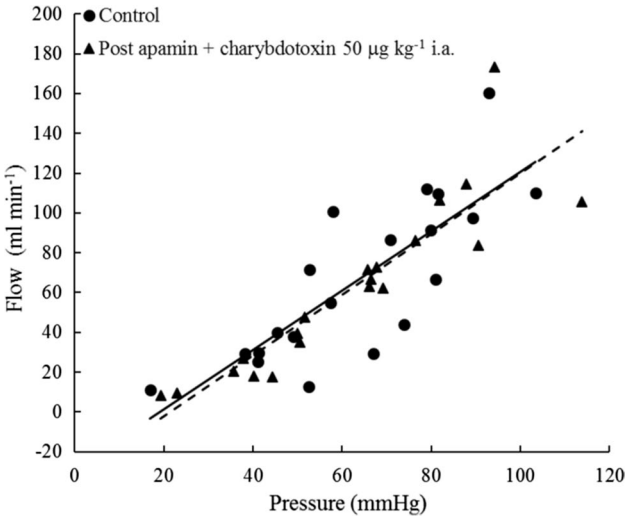

Fig. 5 The effect of apamin and charybdotoxin (50 µg kg^{-1} i.a. for both) on vascular conductance in the iliac vascular bed ($n=4$). The conductance (slope of the line) was not significantly affected by small and intermediate Ca^{2+}-sensitive K^+ channel antagonism (homogeneity of regression analysis). Solid line: control; dashed line: post-toxins

Comparing slope values using homogeneity of regression analysis indicated that they were not significantly different ($P=0.93$).

Therefore, small and intermediate Ca^{2+}-sensitive K^+ channels do not play a role in conductance, and hence maintenance of resistance vessel diameter, during resting blood flow in vivo.

Discussion

The main finding of this study is that antagonism of small- and intermediate-conducting Ca^{2+}-sensitive K^+ channels did not affect vascular conductance under resting blood flow conditions, which suggests that these two channels are not engaged during normal non-active blood flow in the intact animal. A secondary finding that flow-induced dilation of the iliac was unaffected confirms the previous findings in vivo [4]. Both apamin and charybdotoxin were administered intra-arterially directly into the vascular bed supplied by the iliac to maximise their effect, and the dose was previously used in vivo in Wistar rats [7]. Experimentally, there is too much emphasis on vessel diameter measurement in vascular research with respect to conduit arteries, since nitric oxide release is primarily to prevent platelet and cell adhesion [8], and the concomitant increase in diameter is a secondary effect. However, the diameter changes in resistance vessels are important since this is a major determinant of pressure and flow in the arterial system in vivo. One other notable observation was the small but significant increase in mean arterial pressure after the administration of the two Ca^{2+}-sensitive channel blockers. A previous study in dogs showed that pharmacological activation of endothelial Ca^{2+}-activated K^+ channels reduced arterial pressure significantly [9], a result that is in line with the increased mean blood pressure observed in the present study. Whether these findings point to a potential new target for antihypertensives require further investigation; especially since the conclusions are based on experiments conducted in only four pigs, which is a limitation of the present study. One other limitation is that the efficacy of Ca^{2+}-sensitive K^+ channels antagonism was not and could not be assessed in vivo. Ideally this would be accomplished by challenging the effectiveness of apamin and charybdotoxin using pharmacological agonists; however, all available small and intermediate Ca^{2+}-sensitive K^+ channels agonists are only soluble in either dimethyl sulfoxide or ethanol. Injecting either of these solvents intravascularly is not possible in intact animals.

In conclusion, blockade of small- and intermediate-conducting Ca^{2+}-sensitive K^+ channels using the toxins apamin and charybdotoxin indicate that these channels are not involved in flow-mediated dilation in conduit arteries and, more importantly, do not play a role in the vascular conductance during resting blood flow.

Acknowledgements Surgivet Monitor was purchased with a grant from the Strategic Research Fund University College Cork (UCC) 2012. This study was funded by the Dept. Physiology, UCC.

Compliance with ethical standards

Conflict of interest The authors have no conflict to declare

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