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Down-Regulation of Inwardly Rectifying Kir2.1 K^+ Channels by Human Parvovirus B19 Capsid Protein VP1

Musaab Ahmed · Bernat Elvira · Ahmad Almilaji · C.-Thomas Bock · Reinhard Kandolf · Florian Lang

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Abstract Parvovirus B19 (B19V) has previously been shown to cause endothelial dysfunction. B19V capsid protein VP1 harbors a lysophosphatidylcholine producing phospholipase A2 (PLA2). Lysophosphatidylcholine inhibits Na^+/K^+ ATPase, which in turn may impact on the activity of inwardly rectifying K^+ channels. The present study explored whether VP1 modifies the activity of Kir2.1 K^+ channels. cRNA encoding Kir2.1 was injected into *Xenopus* oocytes without or with cRNA encoding VP1 isolated from a patient suffering from fatal B19V-induced inflammatory cardiomyopathy or the VP1 mutant ^{H153A}VP1 lacking a functional PLA2 activity. K^+ channel activity was determined by dual electrode voltage clamp. In addition, Na^+/K^+-ATPase activity was estimated from K^+-induced pump current (I_{pump}) and ouabain-inhibited current ($I_{ouabain}$). Injection of cRNA encoding Kir2.1 into *Xenopus* oocytes was followed by appearance of inwardly rectifying K^+ channel activity (I_K), which was significantly decreased by additional injection of cRNA encoding VP1, but not by additional injection of cRNA encoding ^{H153A}VP1. The effect of VP1 on I_K was mimicked by lysophosphatidylcholine (1 μg/ml) and by inhibition of Na^+/K^+-ATPase with 0.1 mM ouabain. In the presence of lysophosphatidylcholine, I_K was not further decreased by additional treatment with ouabain. The B19V capsid protein VP1 thus inhibits Kir2.1 channels, an effect at least partially due to PLA2-dependent formation of lysophosphatidylcholine with subsequent inhibition of Na^+/K^+-ATPase activity.

Keywords Parvovirus B19 · Phospholipase A2 · Channels · *Xenopus* · ATPase

M. Ahmed · B. Elvira · A. Almilaji · F. Lang (✉)
Department of Physiology, University of Tübingen, Gmelinstr. 5, 72076 Tübingen, Germany
e-mail: florian.lang@uni-tuebingen.de

C.-T. Bock · R. Kandolf
Department of Molecular Pathology, University of Tübingen, Tübingen, Germany

Introduction

Parvovirus B19 (B19V) leads to common infections (Brown et al. 1994b) with variable clinical features (Koch 2001; Lehmann et al. 2003; Pattison 1988) including *erythema infectiosum* (fifth disease), *hydrops fetalis*, and transient aplastic anemia (Anderson et al. 1983; Young and Brown 2004), arthritis (Dingli et al. 2000; Trapani et al. 1999), hepatitis (Drago et al. 1999; Hillingso et al. 1998; Karetnyi et al. 1999; Sokal et al. 1998; Yoto et al. 1996), vasculitic syndromes (Corman and Dolson 1992; Finkel et al. 1994), neurological disorders (Yoto et al. 1996), and myocarditis (Brown et al. 1994a; Kuhl et al. 2003; Malm et al. 1993; Schowengerdt et al. 1997). B19V infection of cardiac endothelial cells may result in isolated left ventricular diastolic dysfunction (Tschope et al. 2005) and is an important pathogenic agent in the etiology of inflammatory cardiomyopathy (iCMP) (Bock et al. 2010). B19V infection may occur during pregnancy leading to maternal and fetal myocarditis, congenital malformations, stillbirth, and abortion (Brown 1989; Crane 2002; Katz et al. 1990; Oyer et al. 2000). The consequences of antenatal infections are particularly severe as B19V preferably enters proliferating cells (Telerman et al. 1993). B19V may enter cells by binding to blood group P-antigen (Brown et al. 1993), α5β1 integrin and Ku80 autoantigen (Munakata et al. 2005; Weigel-Kelley et al. 2003). B19V, thus, preferably invades erythroid progenitor cells but may enter fetal myocytes,

follicular dendritic cells, and endothelial cells (Brown et al. 1993; Ellis et al. 2003; Munakata et al. 2005; Weigel-Kelley et al. 2003). In fatal iCMP, the virus was particularly abundant in endothelial cells (Bultmann et al. 2003; Klingel et al. 2004).

The B19V genome encodes the NS1 protein, which may function as helicase and transcription factor (Cotmore et al. 1986; Doerig et al. 1987; Heegaard and Brown 2002), transactivator of cellular and viral promoters (Mitchell 2002), stimulator of apoptosis (Hsu et al. 2004; Moffatt et al. 1998; Sol et al. 1999), and modulates inflammatory signaling by activation of the STAT3/PIAS3 pathway (Duechting et al. 2008). The B19V genome further encodes the structural capsid proteins VP1 and VP2 (Cotmore et al. 1986), which are decisive for the viral life cycle (Gigler et al. 1999; Young and Brown 2004). The VP1 protein contains a sequence similar to secreted phospholipase A2 (sPLA2) (Canaan et al. 2004; Li et al. 2001; Zadori et al. 2001), which probably generates eicosanoids (Dorsch et al. 2002; Zadori et al. 2001). The vPLA2 enzyme activity is disrupted by replacement of a histidine at position 153 with alanine (^{H153A}VP1) (Dorsch et al. 2002; Zadori et al. 2001).

VP1 influences Ca^{2+} entry (Lupescu et al. 2006), voltage-gated K^+ channels (Ahmed et al. 2014) and Na^+/K^+ ATPase (Almilaji et al. 2013b), effects abrogated by loss of function mutation of the PLA2 sequence, and mimicked by the vPLA2 product lysophosphatidylcholine (Almilaji et al. 2013b; Rabini et al. 1998).

Na^+/K^+ ATPase activity has previously been shown to strongly influence the activity of inwardly rectifying K^+ channels (Lang and Rehwald 1992). The present study explored, whether expression of VP1 influences the activity of the inwardly rectifying Kir2.1 K^+ channels, which have previously been shown to be expressed in endothelial cells (Nilius and Droogmans 2001).

Materials and Methods

Xenopus Oocytes

Xenopus oocytes were explanted from adult *Xenopus laevis* (NASCO), which were anesthetized by a 0.1 % Tricaine solution. After confirmation of anesthesia and disinfection of the skin, a small abdominal incision was made and oocytes were removed, following by closure of the skin by sutures. All animal experiments were conducted in accordance with the Helsinki Declaration of 1975 and according to the German law for the welfare of animals and the surgical procedures on the adult *X. laevis* were reviewed and approved by the respective government authority of the state Baden-Württemberg (Regierungspräsidium) prior to the start of the study (Anzeige für Organentnahme nach §6).

Plasmids

B19V DNA was isolated from deparaffinized myocardial tissue of a patient with fatal B19V-associated iCMP after proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation (accession number: DQ225150). For cloning of the pWHE163-VP1 plasmid, the respective region was amplified by PCR using a high fidelity polymerase system (Roche, Basel, Switzerland). Constructs encoding wild-type VP1 (Almilaji et al. 2013b), PLA2-negative ^{H153A}VP1 mutant (Almilaji et al. 2013b), and wild-type Kir2.1 (Munoz et al. 2014) were used for generation of cRNA as described previously (Hosseinzadeh et al. 2013).

Voltage Clamp in *Xenopus* Oocytes

Xenopus oocytes were prepared as previously described (Bogatikov et al. 2012; Henrion et al. 2012). cRNA encoding VP1 (10 ng) was injected on the same day of preparation of the *Xenopus* oocytes (Hosseinzadeh et al. 2014; Munoz et al. 2013). All experiments were performed at room temperature (about 22 °C) 3 days after the injection (Hosseinzadeh et al. 2012b). In two-electrode voltage clamp experiments, Kir2.1 currents were elicited every 20 s with 1 s pulses from −150 to +30 mV applied from a holding potential of −60 mV. The data were filtered at 1 kHz and recorded with a Digidata 1322A A/D–D/A converter and ClampexV.9.2 software for data acquisition (Axon Instruments) (Shojaiefard et al. 2012; Warsi et al. 2014). The analysis of the data was performed with Clampfit 9.2 (Axon Instruments) software (Almilaji et al. 2013a; Pakladok et al. 2014). The oocytes were maintained at 17 °C in ND96 solution containing: 88.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaC1_2$, 5 mM HEPES, tetracycline (50 mg/l), ciprofloxacin (1.6 mg/l), refobacin (100 mg/l), and theophylline (90 mg/l) as well as sodium pyruvate (5 mM) were added to the ND96, pH was adjusted to 7.5 by addition of NaOH. The control superfusate (ND96) contained 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH was adjusted to 7.4 by addition of NaOH (Alesutan et al. 2012; Hosseinzadeh et al. 2012a). The flow rate of the superfusion was 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s (Mia et al. 2012; Pathare et al. 2012).

Statistical Analysis

Data are provided as mean ± SEM, *n* represents the number of oocytes investigated. All experiments were repeated with at least three batches of oocytes; in all repetitions, qualitatively similar data were obtained. Data were tested for significance using analysis of variance (ANOVA)

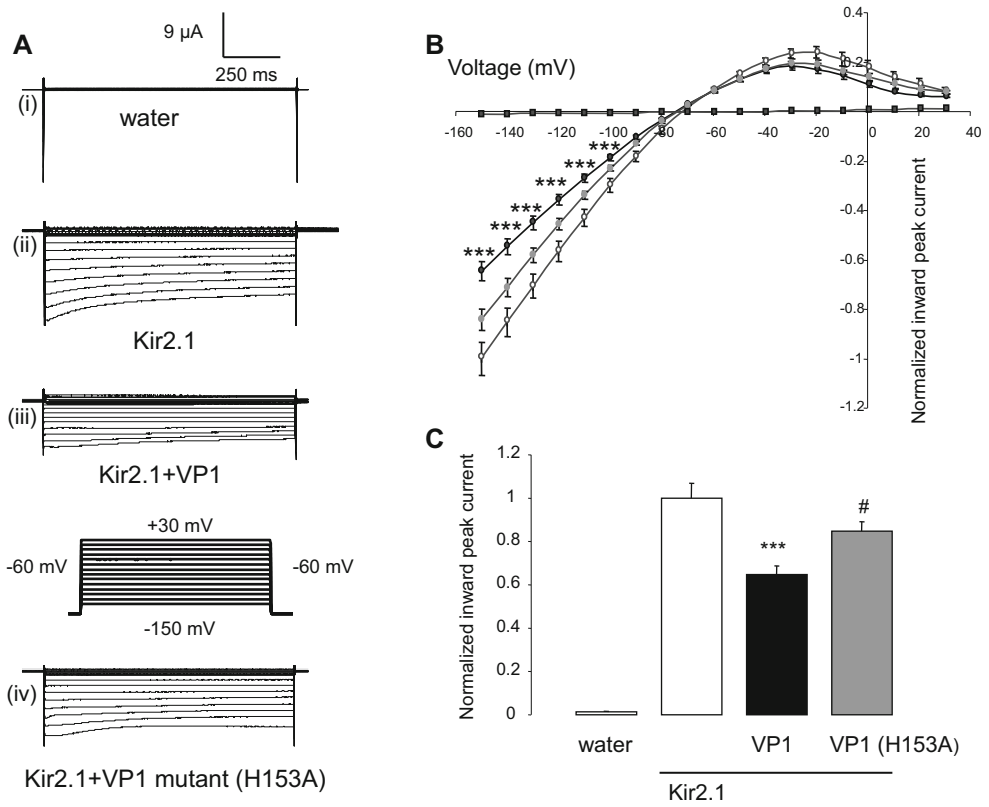

Fig. 1 Inhibition of K^+ currents in Kir2.1 expressing *Xenopus* oocytes by coexpression of VP1 but not of ^{H153A}VP1. **a** Original tracings recorded in *Xenopus* oocytes injected with water (*i*), with cRNA encoding Kir2.1 alone (*ii*), with cRNAs encoding both, Kir2.1 and VP1 (*iii*), and with cRNA encoding both, Kir2.1 and the PLA2-negative ^{H153A}VP1 mutant (*iv*). The currents were elicited every 20 s with 1 s pulses from −150 to +30 mV applied from a holding potential of −60 mV. **b** Arithmetic mean ± SEM (n = 6–22) of the normalized Kir2.1 current as a function of voltage in *Xenopus* oocytes injected with water (*black squares*), or with cRNA encoding Kir2.1 alone (*white circles*), or with cRNA encoding both, Kir2.1 and VP1 (*black circles*), or with cRNA encoding Kir2.1 and PLA2-negative VP1 mutant (*gray circles*). Peak currents were normalized to the mean peak current at −150 mV in *Xenopus* oocytes injected with cRNA encoding Kir2.1. **c** Arithmetic mean ± SEM (n = 6–22) of the normalized Kir2.1 peak current at −150 mV in *Xenopus* oocytes injected with water (*dotted bar*), with cRNA encoding Kir2.1 alone (*white bar*), with cRNA encoding both, Kir2.1 and VP1 (*black bar*), or with cRNA encoding Kir2.1 and ^{H153A}VP1 mutant (*gray bar*). ***$p < 0.001$ indicates statistically significant difference from *Xenopus* oocytes injected with cRNA encoding Kir2.1 alone, $^{\#}p < 0.05$ indicates statistically significant difference from *Xenopus* oocytes injected with cRNA encoding wild-type VP1 (ANOVA-one way)

or t test, as appropriate. Results with $p < 0.05$ were considered statistically significant.

Results

The present study explored, whether coexpression of parvovirus capsid protein VP1 influences the activity of Kir2.1 K^+-channels. To this end, Kir2.1 was expressed in *Xenopus* oocytes with or without additional expression of VP1 and inwardly rectifying K^+-currents taken as a measure of K^+ channel activity.

As shown in Fig. 1, inwardly rectifying currents were low in *Xenopus* oocytes injected with water. Expression of Kir2.1 resulted in a strong inwardly rectifying current (I_K). Coexpression of wild-type VP1 was followed by a marked decline of I_K (Fig. 1). In contrast, coexpression of the ^{H153A}VP1 mutant lacking functional PLA2 activity did not significantly modify Kir2.1 currents (Fig. 1). Accordingly, the K^+ current was significantly higher following coexpression of Kir2.1 with ^{H153A}VP1 than following coexpression of Kir2.1 with VP1 (Fig. 1).

As PLA2 of VP1 is known to generate lysophosphatidylcholine, additional experiments were performed to test whether lysophosphatidylcholine influences K^+ currents in Kir2.1 expressing *Xenopus* oocytes. As illustrated in Fig. 2, the treatment of Kir2.1 expressing *Xenopus* oocytes with lysophosphatidylcholine (1 μg/ml) within 10 min significantly decreased the K^+ currents.

Additional experiments were performed to test whether the effect of VP1 expression or lysophosphatidylcholine treatment could be mimicked by inhibition of the Na^+/K^+ ATPase with ouabain (0.1 mM). As illustrated in Fig. 3, the treatment of Kir2.1 expressing *Xenopus* oocytes with

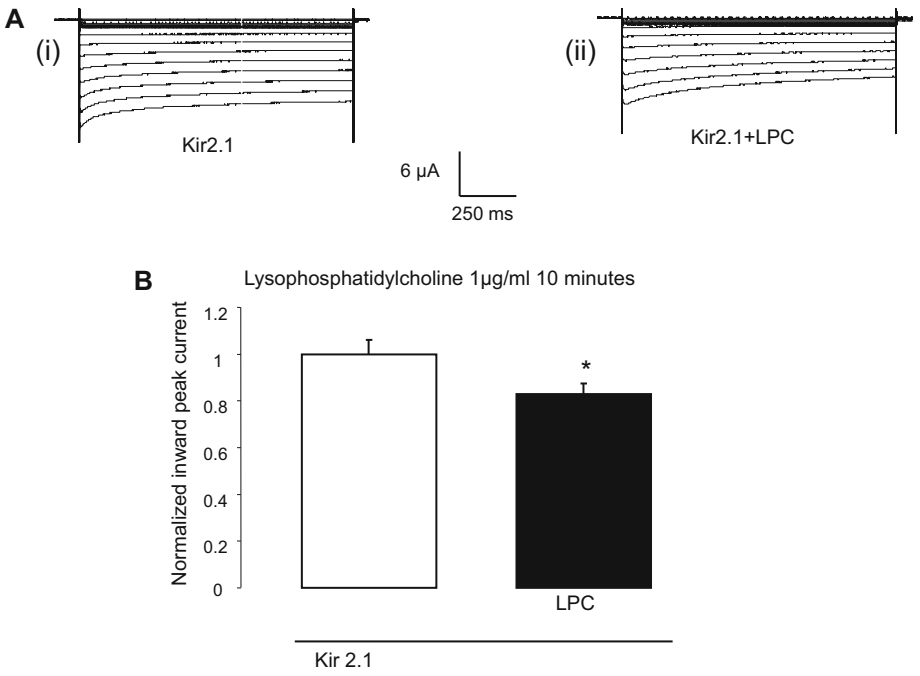

Fig. 2 Inhibition of K^+-channel activity in Kir2.1 expressing *Xenopus* oocytes by lysophosphatidylcholine. **a** Original tracings recorded in oocytes injected with cRNA encoding Kir2.1 alone in the absence (*i*) or presence (*ii*) of lysophosphatidylcholine (1 μg/ml). The currents were elicited every 20 s with 1 s pulses from −150 to +30 mV applied from a holding potential of −60 mV. **b** Arithmetic mean ± SEM ($n = 18$) of the normalized K^+-peak current in oocytes injected with cRNA encoding Kir2.1 alone in the absence (*white bar*) and presence (*black bar*) of lysophosphatidylcholine (1 μg/ml). *Asterisk* indicates statistically significant ($p < 0.05$) difference from absence of lysophosphatidylcholine (unpaired Student's *t* test)

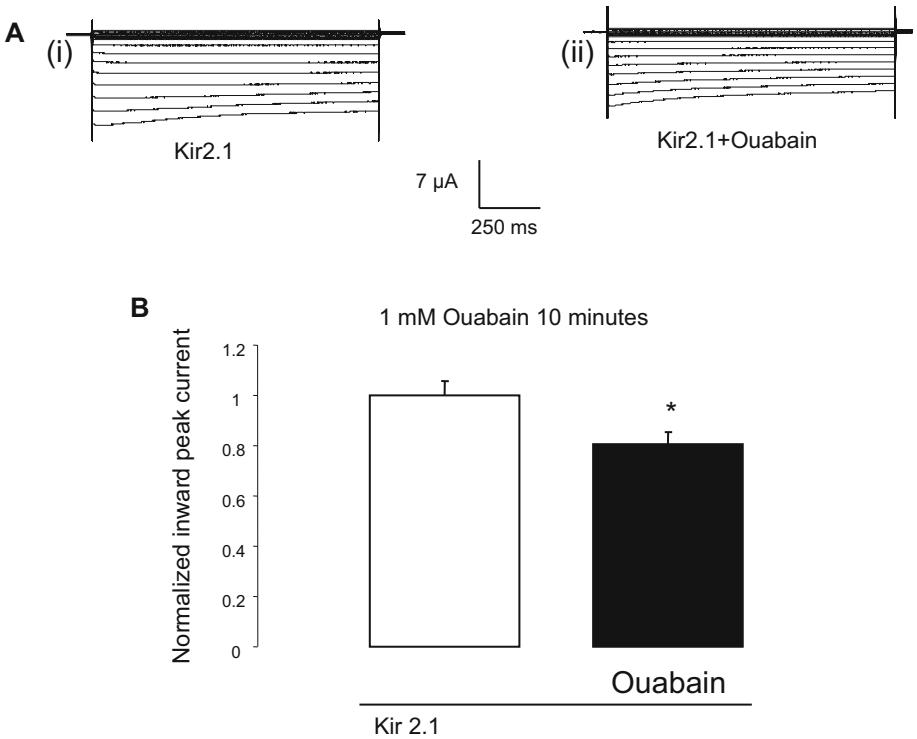

Fig. 3 Inhibition of K^+-channel activity in Kir2.1 expressing *Xenopus* oocytes by ouabain. **a** Original tracings recorded in oocytes injected with cRNA encoding Kir2.1 alone in the absence (*i*) or presence (*ii*) of ouabain (0.1 mM). The currents were elicited every 20 s with 1 s pulses from −150 to +30 mV applied from a holding potential of −60 mV. **b** Arithmetic mean ± SEM ($n = 18$) of the normalized K^+-peak current in oocytes injected with cRNA encoding Kir2.1 alone in the absence (*white bar*) and presence (*black bar*) of ouabain (0.1 mM). *Asterisk* indicates statistically significant ($p < 0.05$) difference from absence of ouabain (unpaired Student's *t* test)

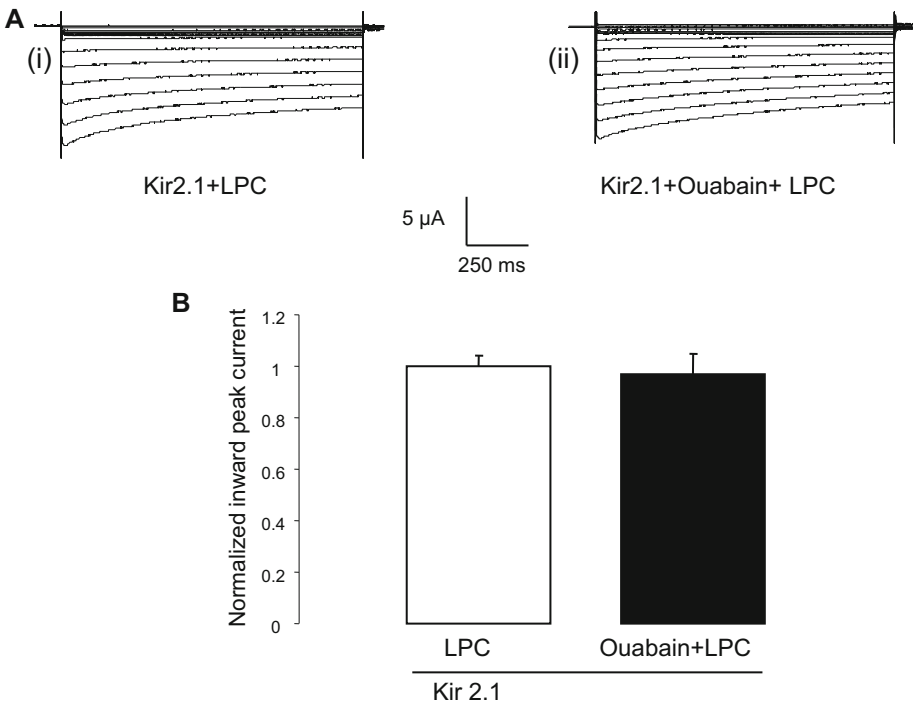

Fig. 4 Nonadditivity of lysophosphatidylcholine and ouabain on Kir2.1 K^+ channel activity. **a** Original tracings recorded in oocytes injected with cRNA encoding Kir2.1 alone and treated for 3 min with lysophosphatidylcholine (1 µg/ml) (*i*) or with both, lysophosphatidylcholine (1 µg/ml) and ouabain (0.1 mM) (*ii*). The currents were elicited every 20 s with 1 s pulses from −150 to +30 mV applied from a holding potential of −60 mV. **b** Arithmetic mean ± SEM ($n = 19$) of the normalized K^+-peak current in oocytes injected with cRNA encoding Kir2.1 alone in the presence of lysophosphatidylcholine (1 µg/ml) (*white bar*), and presence (*black bar*) of both lysophosphatidylcholine (1 µg/ml) and ouabain (0.1 mM)

ouabain within 10 min significantly decreased the K^+ currents.

Further experiments tested, whether the inhibition of Na^+/K^+ ATPase was required for the inhibitory effect of lysophosphatidylcholine on Kir2.1. To this end, the Kir2.1 expressing oocytes were treated either with lysophosphatidylcholine (1 µg/ml) alone or with both lysophosphatidylcholine (1 µg/ml) and ouabain (0.1 mM). As illustrated in Fig. 4, the decline of I_K in Kir2.1 expressing oocytes was similar following combined treatment with lysophosphatidylcholine (1 µg/ml) and ouabain (0.1 mM) and following treatment with lysophosphatidylcholine alone.

Discussion

The present observations disclose a novel effect of the B19V capsid protein VP1, i.e., the down-regulation of the inwardly rectifying K^+ channel Kir2.1. As shown previously for Kv1.3 and Kv1.5 channels (Ahmed et al. 2014), Ca^{2+} entry (Lupescu et al. 2006) and Na^+/K^+ ATPase activity (Almilaji et al. 2013b), the effect of VP1 requires its PLA2-like motif (Canaan et al. 2004; Dorsch et al. 2002). Loss of function mutation of the motif disrupts the effect of VP1 on Kir2.1. Again, similar to what has been observed previously on the regulation of voltage-gated K^+ channels (Ahmed et al. 2014), Ca^{2+} entry (Lupescu et al. 2006) and Na^+/K^+ ATPase activity (Almilaji et al. 2013b), the effect of VP1 expression on Kir2.1 channel activity was mimicked by the vPLA2 product lysophosphatidylcholine.

The effect of B19V on K^+ channels could contribute to the triggering of endothelial dysfunction, as B19V enters myocardial endothelial cells (Bultmann et al. 2003; Klingel et al. 2004). Inhibition of K^+ channels is expected to foster cell swelling (Lang 2007; Lang et al. 1998), as reduced K^+ channel activity leads to impaired K^+ exit, depolarization, Cl^- entry, and thus cellular accumulation of KCl with the respective osmotically obliged water (Lang 2007; Lang et al. 1998). The depolarization is further fostered by inhibition of Na^+/K^+-ATPase activity (Almilaji et al. 2013b) with the resulting dissipation of the ion gradients across the cell membrane.

The inhibition of Kir2.1 channels could at least partially result from the inhibitory effect of lysophosphatidylcholine on the Na^+/K^+-ATPase (Almilaji et al. 2013b), as the channels are similarly down-regulated by the Na^+/K^+-ATPase inhibitor ouabain. Inwardly rectifying K^+ channels have previously been shown to be highly sensitive to Na^+/K^+-ATPase activity and to be rapidly down-regulated following pump inhibition (Lang and Rehwald 1992).

In conclusion, VP1 down-regulates the inwardly rectifying K^+channel Kir2.1, an effect involving PLA2 activity of the parvovirus protein, lysophosphatidylcholine formation, and inhibition of Na^+/K^+-ATPase activity. The inhibition of endothelial K^+ channels may lead to cell swelling, and thus participate in the pathophysiology of endothelial dysfunction during B19V infection.

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Conflict of interest There are no conflicts to disclose.

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