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Original Paper

Down-Regulation of Na^+/K^+ ATPase Activity by Human Parvovirus B19 Capsid Protein VP1

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Key Words
Viral myocarditis • VP1 • Phospholipase A2 • Na^+/K^+ ATPase • Lysophosphatidylcholine • 4-bromophenacylbromide

Abstract
Background/Aims: Human parvovirus B19 (B19V) may cause inflammatory cardiomyopathy (iCMP) which is accompanied by endothelial dysfunction. The B19V capsid protein VP1 contains a lysophosphatidylcholine producing phospholipase A2 (PLA) sequence. Lysophosphatidylcholine has in turn been shown to inhibit Na^+/K^+ ATPase. The present study explored whether VP1 modifies Na^+/K^+ ATPase activity. ***Methods:*** *Xenopus* oocytes were injected with cRNA encoding VP1 isolated from a patient suffering from fatal B19V-iCMP or cRNA encoding PLA2-negative VP1 mutant (H153A) and K^+ induced pump current (I_{pump}) as well as ouabain-inhibited current ($I_{ouabain}$) both reflecting Na^+/K^+-ATPase activity were determined by dual electrode voltage clamp. ***Results:*** Injection of cRNA encoding VP1, but not of VP1(H153A) or water, was followed by a significant decrease of both, I_{pump} and $I_{ouabain}$ in *Xenopus* oocytes. The effect was not modified by inhibition of transcription with actinomycin (10 µM for 36 hours) but was abrogated in the presence of PLA2 specific blocker 4-bromophenacylbromide (50 µM) and was mimicked by lysophosphatidylcholine (0.5 - 1 µg/ml). According to whole cell patch clamp, lysophosphatidylcholine (1 µg /ml) similarly decreased I_{pump} in human microvascular endothelial cells (HMEC). ***Conclusion:*** The B19V capsid protein VP1 is a powerful inhibitor of host cell Na^+/K^+ ATPase, an effect at least partially due to phospholipase A2 (PLA2) dependent formation of lysophosphatidylcholine.

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Introduction

Infection with human parvovirus B19 (B19V), a member of the *Erythroviruses* within the family of *Parvoviridae* [1], is common, as reflected by the high prevalence of specific immunoglobulin G (IgG) antibodies in young children (5% to15%), adults (60%) and seniors older than 69 years (85%) [2]. B19V infection may result in *erythema infectiosum* (fifth disease), *hydrops fetalis* and transient aplastic anaemia [3, 4]. B19V infection is associated with further diseases [5-7], such as arthritis [8, 9], hepatitis [10-14], vasculitic syndromes [15, 16], and neurological disorders [14, 17]. Most importantly, B19V infections are associated with acute and chronic myocarditis [18-26]. Endothelial B19V-infection may result in isolated left ventricular diastolic dysfunction [27]. Infection of pregnant women may result in maternal and fetal myocarditis, congenital abnormalities, stillbirth and abortion [28-31]. The severe course of the antenatal disease has been related to the preference of B19V for proliferating tissues [32].

Cellular entry of B19V requires blood group P-antigen [33] together with $\alpha5\beta1$ integrin and Ku80 autoantigen [34, 35]. B19V thus infects mainly erythroid progenitor cells expressing high levels of P antigen as well as the coreceptors $\alpha5\beta1$ integrin and Ku80 autoantigen. Moreover, non-erythroid cell lineages, such as fetal myocytes, follicular dendritic cells and endothelial cells may be infected by B19V [33-36]. In patients with fatal inflammatory cardiomyopathy (iCMP) B19V genomes could be detected in endothelial cells (ECs) of myocardial tissue predominantly of small intramyocardial arteries and venoles, but not in cardiac myocytes or epicardial coronaries [19, 26]. The presence of B19V genomes is paralleled by expression of the adhesion molecule E-selectin, margination, adherence, penetration, and perivascular infiltration of the heart by T-lymphocytes and macrophages [19, 26].

The DNA genome of B19V contains three open reading frames (ORFs). The first ORF encodes the NS1 protein with transcriptional and helicase activities [1, 37, 38] and functions as transactivator on cellular and viral promoters [39]. NS1 presumably largely accounts for host cell apoptosis [40-42]. The other two ORF located in the 3´-half of the B19V genome encode the major VP1 and minor VP2 structural capsid proteins [37], which are important for the viral life cycle [4, 43]. A highly conserved domain of VP1 shares homologies to the catalytic site and Ca^{2+}-binding loop of secreted phospholipase A2 (sPLA2) [44-46]. A PLA2 like motif was found in VP1 of a wide variety of parvoviruses [45-47]. Viral PLA2 is presumably required for viral entry and nuclear targeting of the viral genome, production of eicosanoids [46, 47] and virus infectivity [46]. A mutation at position 153 with a histidine to aspartic acid (H153A) exchange abolishes enzyme activity of vPLA2 [46, 47] and results in loss of infectiosity [46].

In a previous study, VP1 has been shown to upregulate Ca^{2+} entry [48]. The effect was abolished by loss of function mutation of the PLA2 like motif and was mimicked by the phospholipase A2 product lysophosphatidylcholine (1 μg/ml) [48]. Lysophosphatidylcholine is further known to down-regulate Na^+/K^+ ATPase activity [49]. The present study thus explored, whether expression of VP1 influences Na^+/K^+ ATPase activity and whether this effect is sensitive to inhibition of PLA2 and is mimicked by lysophosphatidylcholine.

Materials and Methods

Plasmids

B19V DNA was isolated from deparaffinized myocardial tissue of a patient with fatal B19V-associated inflammatory cardiomyopathy 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).

Voltage clamp in Xenopus oocytes

Wild-type VP1 and PLA2-negative VP1(H153A) mutant were subcloned from pCMV vector into pGHJ, a *Xenopus* oocyte expression vector using AflII - XbaI restriction sites. Constructs encoding wild-type VP1 and PLA2-negative VP1(H153A) mutant were used for the generation of cRNA as described previously [50, 51]. *Xenopus* oocytes were prepared as previously described [52, 53]. cRNA encoding VP1 and VP1(H153A) (10 ng) was injected on the first day after preparation of the *Xenopus* oocytes [54]. All experiments were performed at room temperature (about 22° C) 3 days after the injection. Two-electrode voltage-clamp recordings were performed at a holding potential of -30 mV for determination of the endogeneous Na^+/K^+-ATPase activity. The data were filtered at 10 Hz and recorded with a GeneClamp 500 amplifier, a DigiData 1300 A/D-D/A converter and the pClamp 9.2 software packages for data acquisition and analysis (Axon Instruments, Foster City, CA, USA) [55]. The oocytes were maintained at 17°C in ND96 solution containing 88.5 mM NaCl, 2 mM KCl, 1 mM $MgC1_2$, 1.8 mM $CaC1_2$, 5 mM HEPES, tretracycline (Sigma, 0.11 mM), ciprofloxacin (Sigma, 4 µM), gentamycin (Refobacin© 0.2 mM), theophylline (Euphylong©, 0.5 mM) and sodium pyruvate (Sigma, 5 mM), pH was adjusted to 7.5 by addition of NaOH. The flow rate of the superfusion was 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s. To determine electrogenic transport by Na^+/K^+-ATPase, the oocytes were incubated for 6 hours in a potassium-free solution containing 96 mM NaCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES and 25 mM sucrose titrated to the pH 7.5 using NaOH [56]. Subsequently, the oocytes were exposed to the same solution containing, in addition, 5 mM $BaCl_2$ (replacing 10 mM sucrose) for inhibition of K^+ channels. Then, 5 mM KCl (replacing 10 mM sucrose) was added in the continuous presence of $BaCl_2$. Where indicated, ouabain (1 mM) was added to inhibit the K^+-induced outward current.

Endothelial Cell Culture

Human microvascular endothelial cells (HMEC) were maintained in Dulbecco´s modified Eagle´s Medium supplemented with 10 % (v/v) heat-inactivated foetal calf serum, 1 % non-essential amino acids, 1 % L-glutamine, 5 mM glucose, 100 units/ml penicillin, 100 µg/ml streptomycin, 1 µg/ml hydrocortisone, 10 ng/ml endothelial cell growth supplement, 300 µU/ml hygromycin and 300 µg/ml G418 (Calbiochem, Bad Soden, Germany) in an atmosphere containing 5 % CO_2 at 37 °C. All cell culture reagents were purchased from Invitrogen (Karlsruhe, Germany). Cells were allowed to recover for 16 hours prior to experiments. Thereafter cells were harvested and analyzed for whole cell patch clamp.

Patch clamp

Cells were cultured for 24 hours at appropriate cell densities ($2x10^7$ cells/ml) in mini dishes and ouabain-sensitive currents ($I_{ouabain}$) reflecting Na^+/K^+-ATPase activity was determined by whole cell patch clamp recording. Patch clamp experiments were performed at room temperature in voltage-clamp, fast whole cell mode [57]. Cells were continuously superfused through a flow system inserted into the dish. The bath was grounded via a bridge filled with Ringer solution. Borosilicate glass pipettes (2- to 4-MΩ resistance; Harvard Apparatus, UK), heat polished manufactured by a microprocessor-driven DMZ puller (Zeitz, Augsburg, Germany), were applied in combination with a MS314 electrical micromanipulator (MW, Märzhäuser, Wetzlar, Germany). The currents were recorded on an EPC-9 amplifier (Heka, Lambrecht, Germany) and analyzed with Pulse software (Heka) and an ITC-16 Interface (Instrutech, Port Washington, NY). Currents were recorded at an acquisition frequency of 10 kHz and 3 kHz low-pass filtered.

To measure Na^+/K^+ ATPase activity ouabain (1 mM) sensitive outward currents were recorded. The pipette solution used contained (in mM): 30 NaCl, 20 KCl, 70 CsCl, 5 $MgCl_2$, 5 HEPES, 5 Na_2ATP and 5 ethylene glycol tetraacetic acid (EGTA) (pH 7.2, CsOH). The external solution contained (in mM): 60 NaCl, 80 TEA-Cl, 1 $MgCl_2$, 2.5 $CaCl_2$, 5 $NiCl_2$, 5 $BaCl_2$, 5 glucose, 10 HEPES (pH 7.4, CsOH), and 0.5 EGTA. Na^+/K^+ ATPase currents were elicited by switching to a bath solution that contained (in mM): 60 NaCl, 80 TEA-Cl, 5 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 5 $NiCl_2$, 5 $BaCl_2$, 5 glucose, 10 HEPES (pH 7.4, CsOH). The currents were measured at -40 mV.

Statistical analysis

Data are provided as means ± SEM, n represents the number of oocytes/cells investigated. All experiments were repeated with at least 3 batches of oocytes; in all repetitions qualitatively similar data were obtained. As the expression of proteins may vary between different batches of oocytes, statistical comparisons were always made between oocytes from the same batch. No statistical comparisons were

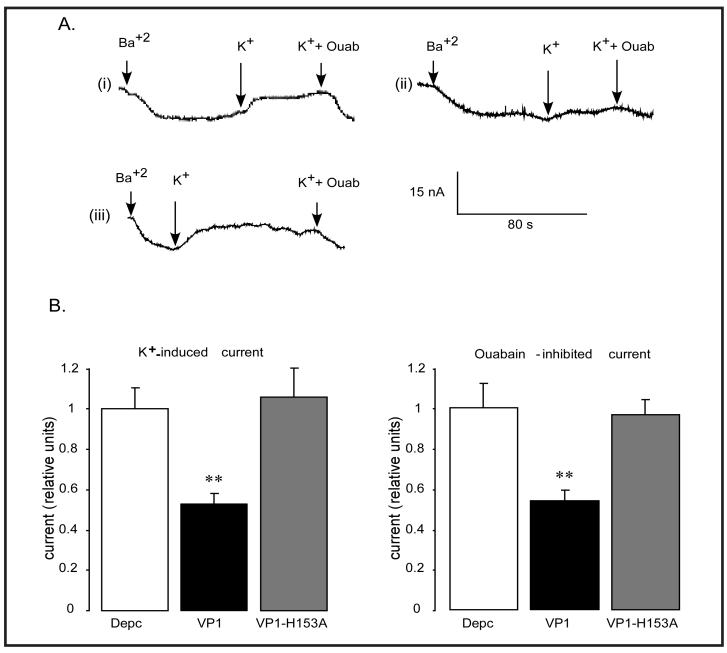

Fig. 1. Decrease of Na^+/K^+-ATPase activity in *Xenopus* oocytes by VP1 expression. A. Original tracings recorded in oocytes injected with water (i), with cRNA encoding VP1 (ii) or with cRNA encoding mutated VP1(H153A) (iii). The arrows indicate the addition of the respective solutions. B. Arithmetic means ± SEM (n = 9-22) of the K^+-induced current (left bars) and ouabain-inhibited current (right bars) measured in *Xenopus* oocytes injected with water (white bars), with cRNA encoding VP1 (black bars) or with cRNA encoding mutated VP1(H153A) (grey bars). ** indicates statistically significant ($p<0.01$) difference from water-injected *Xenopus* oocytes (ANOVA).

made between different series of experiments done in different batches of oocytes. Data were tested for significance using analysis of variance (ANOVA) or Student's unpaired two-tailed **t**-test, as appropriate. Results with $p < 0.05$ were considered statistically significant.

Results

To elucidate the effect of VP1 on Na^+/K^+-ATPase activity, VP1 was expressed in *Xenopus* oocytes and K^+-induced pump currents (I_{pump}) as well as ouabain inhibited currents ($I_{ouabain}$) taken as a measure of Na^+/K^+-ATPase activity. To achieve K^+ depletion, *Xenopus* oocytes were preincubated for 6 hours in K^+-free solution, superfused for a few minutes with K^+-free bath solution and subsequently with a K^+ free solution containing the K^+-channel blocker Ba^{2+} (5 mM) to prevent K^+ fluxes through K^+ channels. The readdition of K^+ in the continued presence of Ba^{2+} was followed by an outwardly directed pump current (I_{pump}) due to electrogenic extrusion of 3 Na^+ in exchange for 2 K^+ (Fig. 1). I_{pump} was suppressed in the presence of the Na^+/K^+-ATPase inhibitor ouabain (1 mM). Accordingly, inhibition of the I_{pump} by application of ouabain (1 mM) was followed by an inward current ($I_{ouabain}$). Both I_{pump} and $I_{ouabain}$ were significantly smaller in *Xenopus* oocytes injected with cRNA encoding VP1 than in oocytes injected with PLA2-negative VP1(H153A) or water (Fig. 1). Thus, VP1 inhibited the endogenous Na^+/K^+-ATPase in *Xenopus* oocytes.

Further experiments addressed whether the effect of VP1 on *Xenopus* oocyte Na^+/K^+ ATPase current was due to its phospholipase A2 (PLA2) activity. Treatment of *Xenopus* oocytes for 3 and 6 hours with PLA2 specific inhibitor 4-bromophenacylbromide (50 μM) prior to the experiment abrogated the effect of VP1 expression on both, I_{pump} and $I_{ouabain}$ (Fig. 2). Fitting the normalized I_{pump} and $I_{ouabain}$ currents of the VP1 expressing *Xenopus* oocytes to Boltzmann function yielded 4BPB half times of inhibition amounting to 154 and 170 minutes, respectively.

Since the PLA2 like motif of VP1 is known to generate lysophosphatidylcholine, additional experiments were performed to explore whether Na^+/K^+ ATPase activity of *Xenopus* oocytes is sensitive to lysophosphatidylcholine. As a result, incubation of *Xenopus* oocytes with 0.5 and 1 μg/ml lysophosphatidylcholine for 5 minutes prior to the measurements decreased significantly both, I_{pump} and $I_{ouabain}$ (Fig. 3A, B). The effect of lysophosphatidylcholine on Na^+/K^+ ATPase activity was dose-dependent, fitting the normalized values of K^+-induced current I_{pump} to an exponential decay function yielded a half maximal inhibitory concentration of lysophosphatidylcholine IC_{50} amounting to 59 ng (Fig. 3C).

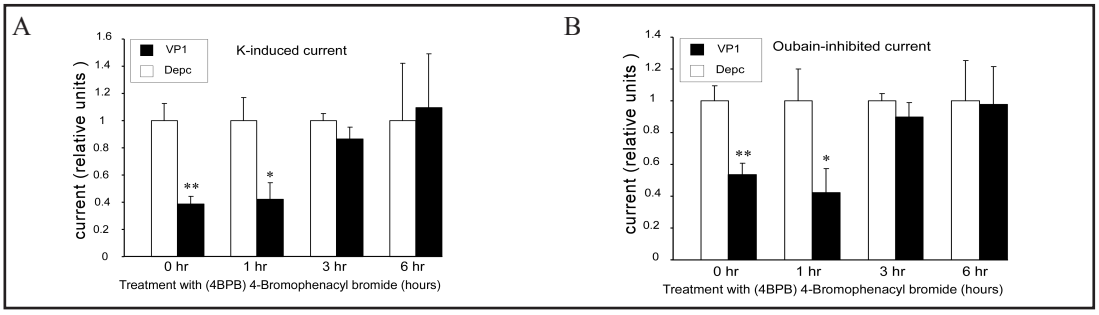

Fig. 2. Effect of VP1 expression on Na^+/K^+-ATPase activity in *Xenopus* oocytes in the presence of PLA2 inhibitor 4-bromophenacylbromide (4BPB). Arithmetic means ± SEM (n = 5-11) of the K^+-induced current (A) and ouabain-induced current (B) measured in *Xenopus* oocytes pretreated for 0, 1, 3 and 6 hours with specific phospholipase A2 inhibitor 4BPB (4 bromophenacyl bromide, 50 μM) and injected with water (white bars) or with cRNA encoding wild type VP1 (black bars).

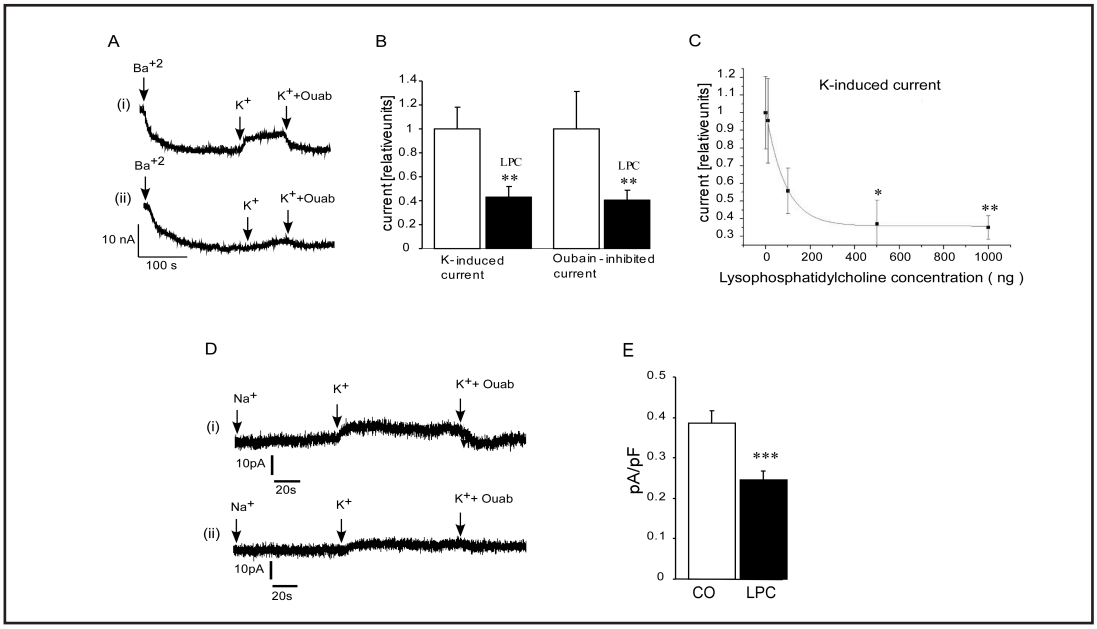

Fig. 3. Inhibition of Na^+/K^+-ATPase activity by lysophosphatidylcholine. A. Original tracings recorded in *Xenopus* oocytes without (i) or with (ii) prior lysophosphatidylcholine (1 μg/ml) treatment for 5 minutes. The arrows indicate the addition of the respective solutions. B. Arithmetic means ± SEM (n = 14) of the K^+-induced current (left bars) and ouabain-inhibited current (right bars) measured in *Xenopus* oocytes whithout (white bars) or with (black bars) lysophosphatidylcholine treatment (1 μg/ml) for 5 minutes. ** indicates statistically significant ($p<0.01$) difference from untreated *Xenopus* oocytes (unpaired t- test). C. Lysophosphatidylcholine dose-response curve. Arithmetic means ± SEM (n = 10-14) of the K^+-induced current measured in *Xenopus* oocytes pretreated for 5 minutes with 0, 10, 100, 500 and 1000 ng/ml lysophosphatidylcholine. *,** indicates statistically significant ($p<0.05$, 0.01) difference from untreated *Xenopus* oocytes (unpaired t- test). The data points were fitted with the exponential decay function with IC_{50} of 59 ng. D. Original tracings of K^+-induced currents (K^+) with subsequent inhibition by ouabain (Ouab) in whole cell patch clamp on human microvascular endothelial cells (HMEC) non-treated h (i) or treated with (ii) lysophosphatidylcholine (1 μg/ml) for 5 minutes. The arrows indicate the addition of the respective solutions. E. Arithmetic means ± SEM (n = 10-18) of the K^+-induced current measured in HMEC whithout (white bar) or with (black bar) lysophosphatidylcholine treatment (1 μg/ml) for 5 minutes.*** indicates statistically significant ($p<0.001$) difference from untreated cells (unpaired t- test).

Additional series of experiments were performed in human microvascular endothelial cells (HMEC). In those cells the Na^+/K^+ ATPase activity was recorded utilizing whole cell patch

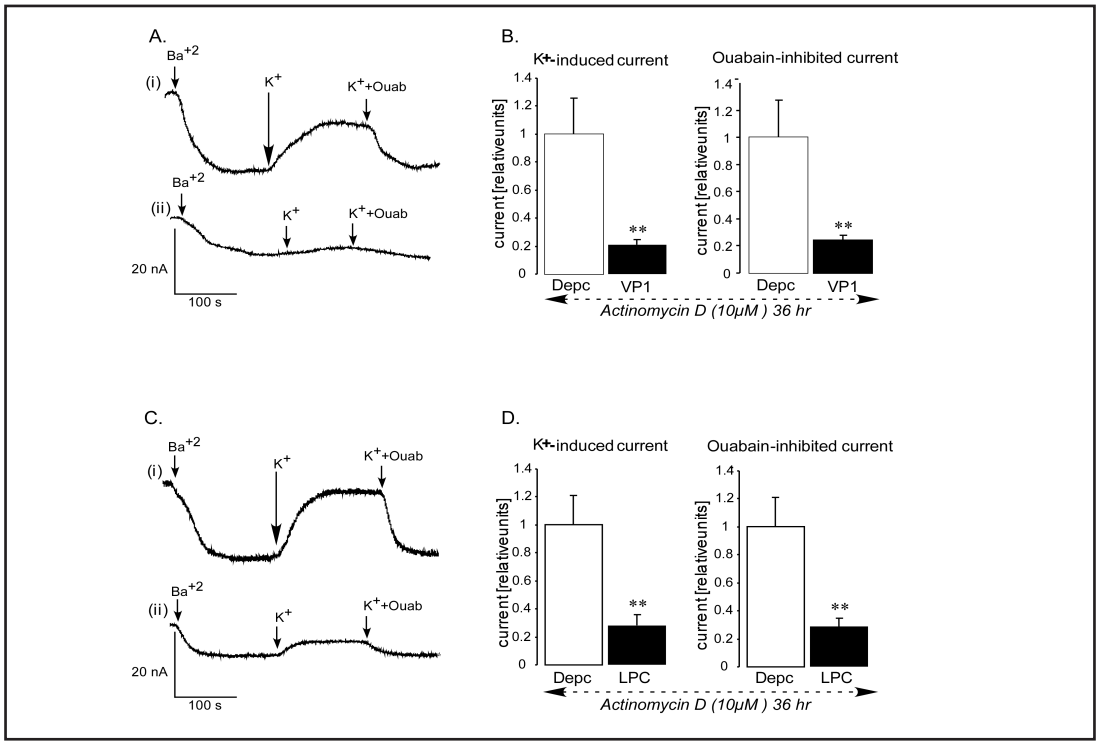

Fig. 4. Decrease of Na^+/K^+-ATPase activity in *Xenopus* oocytes by VP1 expression in presence of actinomycin. A. Original tracings recorded in oocytes pretreated for 36 hours with 10 μM actinomycin and injected with water (i) or with cRNA encoding VP1 (ii). The arrows indicate the addition of the respective solutions. B. Arithmetic means ± SEM (n =9-14) of the K^+-induced current (left bars) and ouabain-inhibited current (right bars) measured in *Xenopus* oocytes pretreated for 36 hours with 10 μM actinomycin and injected with water (white bars) or with cRNA encoding VP1 (black bars). ** indicates statistically significant (p<0.01) difference from water-injected *Xenopus* oocytes (unpaired t- test). C. Original tracings recorded in oocytes injected with water pretreated for 36 hours with 10 μM actinomycin and then incubated without (i) or with (ii) lysophosphatidylcholine (1 μg/ml) for 5 minutes prior to experiments. The arrows indicate the addition of the respective solutions. D. Arithmetic means ± SEM (n =11-14) of the K^+-induced current (left bars) and ouabain-inhibited current (right bars) measured in *Xenopus* oocytes injected with water and pretreated for 36 hours with 10 μM actinomycin and then incubated without (white bars) or with (black bars) lysophosphatidylcholine (1 μg/ml) for 5 minutes prior to experiments. ** indicates statistically significant (p<0.01) difference from water-injected *Xenopus* oocytes (unpaired t- test).

clamp. According to those experiments, I_{pump} was significantly (p<0.001) down-regulated by treatment with lysophosphatidylcholine (1 μg/ml) (Fig. 3D, E). Thus, similar to its effect on *Xenopus* oocytes, lysophosphatidylcholine decreased Na^+/K^+ ATPase activity in endothelial cells.

In order to test whether the effect of VP1 on Na^+/K^+ ATPase activity of *Xenopus* oocytes required transcription, experiments were performed in the presence of actinomycin (10 μM, added 36 hours prior to the experiment). In the presence of actinomycin, expression of VP1 significantly decreased both I_{pump} (by 79 ± 19%, n=9-14, Fig. 4A, B) and $I_{ouabain}$ (by 76 ± 23%, n=9-14, Fig. 4A, B). Since the effect of VP1 was seemingly stronger in the presence of actinomycin than in its absence (Fig. 1), we tested the effect of lysophosphatidylcholine (1 μg/ml) in actinomycin-treated oocytes (Fig. 4C, D). Pretreatment of *Xenopus* oocytes with lysophosphatidylcholine (1 μg/ml) for 5 minutes in the presence of actinomycin inhibited I_{pump} (by 72 ± 13%, n=11-14 Fig. 4C, D) and $I_{ouabain}$ (72 ± 22%, n=9-14, Fig. 4C, D) in *Xenopus* oocytes to a similar extent as VP1.

Discussion

The present experiments disclose a novel action of the B19V capsid protein VP1, i.e. inhibition of the Na^+/K^+ ATPase. The present observations further provide some insight into the mechanism involved in the inhibition of Na^+/K^+ ATPase activity. The VP1 protein contains a phospholipase A2-like motif [44, 47]. As shown earlier [48], the effect of VP1 on Ca^{2+} entry depended on phospholipase A2 activity of VP1, as it was abolished by mutation of the PLA2 motif, where histidine was replaced by alanine in the putative catalytic site (^{H153A}VP1). As shown previously [48], the mutation completely abolished the effect of VP1 overexpression on Ca^{2+} entry. Moreover, the effect of VP1 overexpression was mimicked by addition of lysophosphatidylcholine, a product of phospholipase A2 [48]. The present study provides both pharmacological and genetic evidence that intact PLA2 is similarly required for the VP1 induced down-regulation of Na^+/K^+ ATPase activity. Moreover, the present study reveals that the effect of VP1 on Na^+/K^+ ATPase activity is mimicked by lysophosphatidylcholine.

Myocardial endothelial cells have previously been shown to be targets for parvovirus B19 [19, 26]. Accordingly, parvovirus induced acute myocarditis may result in a clinical course mimicking myocardial infarction which results from dysregulation of endothelial and vascular smooth muscle cell function [19, 26].

The presently observed inhibition of the Na^+/K^+-ATPase may well contribute to endothelial dysfunction. Inhibition of the pump is expected to increase cytosolic Na^+ concentration and decrease cytosolic K^+ concentration [58, 59]. As endothelial cells express Na^+/Ca^{2+} exchangers [60], an increase of Na^+ is expected to increase cytosolic Ca^{2+} activity. As a matter of fact, ouabain [61], lysophosphatidylcholine [48], and VP1 expression [48] have been shown to increase cytosolic Ca^{2+} activity, an effect, however, in part attributed to stimulation of Ca^{2+} entry through cation channels [48, 61]. Excessive Ca^{2+} entry is in turn known to trigger suicidal cell death [62-65].

As endothelial cells express Ca^{2+} sensitive K^+ channels [66], an increase of cytosolic Ca^{2+} activity could activate those channels leading to (transient) hyperpolarization, Cl^- exit and thus cell shrinkage due to cellular loss of KCl with osmotically obliged water [58, 59]. The dissipation of Na^+ and K^+ gradients following inhibition of Na^+/K^+-ATPase should, however, eventually lead to cell swelling [58, 59]. The decline of intracellular K^+ activity following sustained impairment of Na^+/K^+-ATPase decreases the K^+ equilibrium potential resulting in depolarization of the cell membrane [58, 59]. The outside positive cell membrane potential difference is required to drive Cl^- exit and to maintain the low cytosolic Cl^- concentration, which outweighs the high concentration of osmotically active organic substances. The depolarization following inhibition of the Na^+/K^+-ATPase leads to cellular accumulation of Cl^- and thus to cell swelling [58, 59]. Cell swelling during compromized Na^+/K^+-ATPase activity depends on the rate of Na^+ entry [58, 59]. At least in theory, if the cell is completely Na^+ impermeable, cytosolic K^+ and Cl^- concentrations approach an equilibrium, which does not require maintenance by continued activity of Na^+/K^+-ATPase. Following stimulation of Na^+ entry, however, Na^+/K^+-ATPase activity is required for prevention of increasing cytosolic Na^+ concentration and subsequent cell swelling [67].

The increase of cytosolic Na^+ activity following impaired Na^+/K^+-ATPase activity further dissipates the chemical gradients for Na^+ coupled transport processes [68]. Impairment of the Na^+/K^+-ATPase activity by pharmacological inhibition [69], by hypothermia [70] or by cellular energy depletion [71], further decreases the K^+ conductance, an effect accelerating the depolarization and loss of driving force for Na^+ coupled transport processes.

Inhibition of endothelial cell Na^+/K^+ ATPase by ouabain affects the expression of a wide variety of genes. Among those, the expression of several transcription factors (Fos, Jun, Hes1, Nfkbia), interleukin-6, protein phosphatase 1 regulatory subunit, dual specificity phosphatase (Dusp8), prostaglandin-endoperoxide synthase 2, and cyclin L1 were regulated by inhibition inhibition of Na^+/K^+ ATPase even if increases of cytosolic Ca^{2+} activity were prevented by Ca^{2+} chelation. The influence of Na^+/K^+ ATPase inhibition on expression of metallopeptidase Adamts1, adrenomedulin, Dups1, Dusp10 and Dusp16, however, required

cytosolic Ca^{2+} [72]. Moreover, cardiotonic steroids such as ouabain may trigger endothelial cell death by mechanisms other than inhibition of Na^+/K^+ ATPase mediated ion fluxes and respective alteration of the cytosolic Na^+ and K^+ concentrations [73]. Inhibition of Na^+/K^+ ATPase may activate p38 kinase [74], which is in turn well known to trigger suicidal death of a variety of cells[75-82].

In conclusion, the present observations demonstrate that the phospholipase A2 activity of the parvoviral B19 protein VP1 leads to down-regulation of Na^+/K^+ ATPase activity, an effect likely participating in the pathophysiology of parvovirus B19 infection.

Acknowledgements

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References

1 Heegaard ED, Brown KE: Human parvovirus B19. Clin Microbiol Rev 2002;15:485-505.
2 Brown KE, Young NS, Liu JM: Molecular, cellular and clinical aspects of parvovirus B19 infection. Crit Rev Oncol Hematol 1994;16:1-31.
3 Anderson MJ, Jones SE, Fisher-Hoch SP, Lewis E, Hall SM, Bartlett CL, Cohen BJ, Mortimer PP, Pereira MS: Human parvovirus, the cause of erythema infectiosum (fifth disease)? Lancet 1983;1:1378.
4 Young NS, Brown KE: Parvovirus B19. N Engl J Med 2004;350:586-597.
5 Koch WC: Fifth (human parvovirus) and sixth (herpesvirus 6) diseases. Curr Opin Infect Dis 2001;14:343-356.
6 Lehmann HW, Knoll A, Kuster RM, Modrow S: Frequent infection with a viral pathogen, parvovirus B19, in rheumatic diseases of childhood. Arthritis Rheum 2003;48:1631-1638.
7 Pattison JR: Diseases caused by the human parvovirus B19. Arch Dis Child 1988;63:1426-1427.
8 Dingli D, Pfizenmaier DH, Arromdee E, Wennberg P, Spittell PC, Chang-Miller A, Clarke BL: Severe digital arterial occlusive disease and acute parvovirus B19 infection. Lancet 2000;356:312-314.
9 Trapani S, Ermini M, Falcini F: Human parvovirus B19 infection: its relationship with systemic lupus erythematosus. Semin Arthritis Rheum 1999;28:319-325.
10 Drago F, Semino M, Rampini P, Rebora A: Parvovirus B19 infection associated with acute hepatitis and a purpuric exanthem. Br J Dermatol 1999;141:160-161.
11 Hillingso JG, Jensen IP, Tom-Petersen L: Parvovirus B19 and acute hepatitis in adults. Lancet 1998;351:955-956.
12 Karetnyi YV, Beck PR, Markin RS, Langnas AN, Naides SJ: Human parvovirus B19 infection in acute fulminant liver failure. Arch Virol 1999;144:1713-1724.
13 Sokal EM, Melchior M, Cornu C, Vandenbroucke AT, Buts JP, Cohen BJ, Burtonboy G: Acute parvovirus B19 infection associated with fulminant hepatitis of favourable prognosis in young children. Lancet 1998;352:1739-1741.
14 Yoto Y, Kudoh T, Haseyama K, Suzuki N, Chiba S: Human parvovirus B19 infection associated with acute hepatitis. Lancet 1996;347:868-869.
15 Corman LC, Dolson DJ: Polyarteritis nodosa and parvovirus B19 infection. Lancet 1992;339:491.
16 Finkel TH, Torok TJ, Ferguson PJ, Durigon EL, Zaki SR, Leung DY, Harbeck RJ, Gelfand EW, Saulsbury FT, Hollister JR, et al.: Chronic parvovirus B19 infection and systemic necrotising vasculitis: opportunistic infection or aetiological agent? Lancet 1994;343:1255-1258.
17 Barah F, Vallely PJ, Chiswick ML, Cleator GM, Kerr JR: Association of human parvovirus B19 infection with acute meningoencephalitis. Lancet 2001;358:729-730.
18 Brown KE, Hibbs JR, Gallinella G, Anderson SM, Lehman ED, McCarthy P, Young NS: Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte P antigen). N Engl J Med 1994;330:1192-1196.

19 Bultmann BD, Klingel K, Sotlar K, Bock CT, Baba HA, Sauter M, Kandolf R: Fatal parvovirus B19-associated myocarditis clinically mimicking ischemic heart disease: an endothelial cell-mediated disease. Hum Pathol 2003;34:92-95.
20 Kuhl U, Pauschinger M, Bock T, Klingel K, Schwimmbeck CP, Seeberg B, Krautwurm L, Poller W, Schultheiss HP, Kandolf R: Parvovirus B19 infection mimicking acute myocardial infarction. Circulation 2003;108:945-950.
21 Malm C, Fridell E, Jansson K: Heart failure after parvovirus B19 infection. Lancet 1993;341:1408-1409.
22 Murry CE, Jerome KR, Reichenbach DD: Fatal parvovirus myocarditis in a 5-year-old girl. Hum Pathol 2001;32:342-345.
23 Nigro G, Bastianon V, Colloridi V, Ventriglia F, Gallo P, D'Amati G, Koch WC, Adler SP: Human parvovirus B19 infection in infancy associated with acute and chronic lymphocytic myocarditis and high cytokine levels: report of 3 cases and review. Clin Infect Dis 2000;31:65-69.
24 Orth T, Herr W, Spahn T, Voigtlander T, Michel D, Mertens T, Mayet WJ, Dippold W, Meyer zum Buschenfelde KH: Human parvovirus B19 infection associated with severe acute perimyocarditis in a 34-year-old man. Eur Heart J 1997;18:524-525.
25 Schowengerdt KO, Ni J, Denfield SW, Gajarski RJ, Bowles NE, Rosenthal G, Kearney DL, Price JK, Rogers BB, Schauer GM, Chinnock RE, Towbin JA: Association of parvovirus B19 genome in children with myocarditis and cardiac allograft rejection: diagnosis using the polymerase chain reaction. Circulation 1997;96:3549-3554.
26 Klingel K, Sauter M, Bock CT, Szalay G, Schnorr JJ, Kandolf R: Molecular pathology of inflammatory cardiomyopathy. Med Microbiol Immunol (Berl) 2004;193:101-107.
27 Tschope C, Bock CT, Kasner M, Noutsias M, Westermann D, Schwimmbeck PL, Pauschinger M, Poller WC, Kuhl U, Kandolf R, Schultheiss HP: High prevalence of cardiac parvovirus B19 infection in patients with isolated left ventricular diastolic dysfunction. Circulation 2005;111:879-886.
28 Brown KE: What threat is human parvovirus B19 to the fetus? A review. Br J Obstet Gynaecol 1989;96:764-767.
29 Crane J: Parvovirus B19 infection in pregnancy. J Obstet Gynaecol Can 2002;24:727-743.
30 Katz VL, Chescheir NC, Bethea M: Hydrops fetalis from B19 parvovirus infection. J Perinatol 1990;10:366-368.
31 Oyer CE, Ongcapin EH, Ni J, Bowles NE, Towbin JA: Fatal intrauterine adenoviral endomyocarditis with aortic and pulmonary valve stenosis: diagnosis by polymerase chain reaction. Hum Pathol 2000;31:1433-1435.
32 Telerman A, Tuynder M, Dupressoir T, Robaye B, Sigaux F, Shaulian E, Oren M, Rommelaere J, Amson R: A model for tumor suppression using H-1 parvovirus. Proc Natl Acad Sci U S A 1993;90:8702-8706.
33 Brown KE, Anderson SM, Young NS: Erythrocyte P antigen: cellular receptor for B19 parvovirus. Science 1993;262:114-117.
34 Weigel-Kelley KA, Yoder MC, Srivastava A: Alpha5beta1 integrin as a cellular coreceptor for human parvovirus B19: requirement of functional activation of beta1 integrin for viral entry. Blood 2003;102:3927-3933.
35 Munakata Y, Saito-Ito T, Kumura-Ishii K, Huang J, Kodera T, Ishii T, Hirabayashi Y, Koyanagi Y, Sasaki T: Ku80 autoantigen as a cellular coreceptor for human parvovirus B19 infection. Blood 2005; 106:3449-3456.
36 Ellis PD, Metcalfe JC, Hyvonen M, Kemp PR: Adhesion of endothelial cells to NOV is mediated by the integrins alphavbeta3 and alpha5beta1. J Vasc Res 2003;40:234-243.
37 Cotmore SF, McKie VC, Anderson LJ, Astell CR, Tattersall P: Identification of the major structural and nonstructural proteins encoded by human parvovirus B19 and mapping of their genes by procaryotic expression of isolated genomic fragments. J Virol 1986;60:548-557.
38 Doerig C, Beard P, Hirt B: A transcriptional promoter of the human parvovirus B19 active in vitro and in vivo. Virology 1987;157:539-542.
39 Mitchell LA: Parvovirus B19 nonstructural (NS1) protein as a transactivator of interleukin-6 synthesis: common pathway in inflammatory sequelae of human parvovirus infections? J Med Virol 2002;67:267-274.
40 Hsu TC, Wu WJ, Chen MC, Tsay GJ: Human parvovirus B19 non-structural protein (NS1) induces apoptosis through mitochondria cell death pathway in COS-7 cells. Scand J Infect Dis 2004;36:570-577.
41 Moffatt S, Yaegashi N, Tada K, Tanaka N, Sugamura K: Human parvovirus B19 nonstructural (NS1) protein induces apoptosis in erythroid lineage cells. J Virol 1998;72:3018-3028.
42 Sol N, Le Junter J, Vassias I, Freyssinier JM, Thomas A, Prigent AF, Rudkin BB, Fichelson S, Morinet F: Possible interactions between the NS-1 protein and tumor necrosis factor alpha pathways in erythroid cell apoptosis induced by human parvovirus B19. J Virol 1999;73:8762-8770.
43 Gigler A, Dorsch S, Hemauer A, Williams C, Kim S, Young NS, Zolla-Pazner S, Wolf H, Gorny MK, Modrow S: Generation of neutralizing human monoclonal antibodies against parvovirus B19 proteins. J Virol 1999;73:1974-1979.

44 Canaan S, Zadori Z, Ghomashchi F, Bollinger J, Sadilek M, Moreau ME, Tijssen P, Gelb MH: Interfacial enzymology of parvovirus phospholipases A2. J Biol Chem 2004;279:14502-14508.
45 Li Y, Zadori Z, Bando H, Dubuc R, Fediere G, Szelei J, Tijssen P: Genome organization of the densovirus from Bombyx mori (BmDNV-1) and enzyme activity of its capsid. J Gen Virol 2001;82:2821-2825.
46 Zadori Z, Szelei J, Lacoste MC, Li Y, Gariepy S, Raymond P, Allaire M, Nabi IR, Tijssen P: A viral phospholipase A2 is required for parvovirus infectivity. Dev Cell 2001;1:291-302.
47 Dorsch S, Liebisch G, Kaufmann B, von Landenberg P, Hoffmann JH, Drobnik W, Modrow S: The VP1 unique region of parvovirus B19 and its constituent phospholipase A2-like activity. J Virol 2002;76:2014-2018.
48 Lupescu A, Bock CT, Lang PA, Aberle S, Kaiser H, Kandolf R, Lang F: Phospholipase A2 activity-dependent stimulation of Ca^{2+} entry by human parvovirus B19 capsid protein VP1. J Virol 2006;80:11370-11380.
49 Rabini RA, Fumelli P, Zolese G, Amler E, Salvolini E, Staffolani R, Cester N, Mazzanti L: Modifications induced by plasma from insulin-dependent diabetic patients and by lysophosphatidylcholine on human Na^+,K^+-adenosine triphosphatase. J Clin Endocrinol Metab 1998;83:2405-2410.
50 Mohamed MR, Alesutan I, Foller M, Sopjani M, Bress A, Baur M, Salama RH, Bakr MS, Mohamed MA, Blin N, Lang F, Pfister M: Functional analysis of a novel I71N mutation in the GJB2 gene among Southern Egyptians causing autosomal recessive hearing loss. Cell Physiol Biochem 2010;26:959-966.
51 Strutz-Seebohm N, Pusch M, Wolf S, Stoll R, Tapken D, Gerwert K, Attali B, Seebohm G: Structural basis of slow activation gating in the cardiac I Ks channel complex. Cell Physiol Biochem 2011;27:443-452.
52 Bohmer C, Sopjani M, Klaus F, Lindner R, Laufer J, Jeyaraj S, Lang F, Palmada M: The serum and glucocorticoid inducible kinases SGK1-3 stimulate the neutral amino acid transporter SLC6A19. Cell Physiol Biochem 2010;25:723-732.
53 Rexhepaj R, Dermaku-Sopjani M, Gehring EM, Sopjani M, Kempe DS, Foller M, Lang F: Stimulation of electrogenic glucose transport by glycogen synthase kinase 3. Cell Physiol Biochem 2010;26:641-646.
54 Alesutan IS, Ureche ON, Laufer J, Klaus F, Zurn A, Lindner R, Strutz-Seebohm N, Tavare JM, Boehmer C, Palmada M, Lang UE, Seebohm G, Lang F: Regulation of the glutamate transporter EAAT4 by PIKfyve. Cell Physiol Biochem 2010;25:187-194.
55 Hosseinzadeh Z, Bhavsar SK, Sopjani M, Alesutan I, Saxena A, Dermaku-Sopjani M, Lang F: Regulation of the glutamate transporters by JAK2. Cell Physiol Biochem 2011;28:693-702.
56 Dermaku-Sopjani M, Sopjani M, Saxena A, Shojaiefard M, Bogatikov E, Alesutan I, Eichenmuller M, Lang F: Downregulation of NaPi-IIa and NaPi-IIb Na-coupled phosphate transporters by coexpression of Klotho. Cell Physiol Biochem 2011;28:251-258.
57 Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 1981;391:85-100.
58 Lang F, Busch GL, Ritter M, Volkl H, Waldegger S, Gulbins E, Haussinger D: Functional significance of cell volume regulatory mechanisms. Physiol Rev 1998;78:247-306.
59 Lang F: Mechanisms and significance of cell volume regulation. J Am Coll Nutr 2007;26:613S-623S.
60 Kaye DM, Kelly RA: Expression and regulation of the sodium-calcium exchanger in cardiac microvascular endothelial cells. Clin Exp Pharmacol Physiol 1999;26:651-655.
61 Pittner J, Rhinehart K, Pallone TL: Ouabain modulation of endothelial calcium signaling in descending vasa recta. Am J Physiol Renal Physiol 2006;291:F761-F769.
62 Berridge MJ, Lipp P, Bootman MD: The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 2000;1:11-21.
63 Fang KM, Chang WL, Wang SM, Su MJ, Wu ML: Arachidonic acid induces both Na^+ and Ca^{2+} entry resulting in apoptosis. J Neurochem 2008;104:1177-1189.
64 Green DR, Reed JC: Mitochondria and apoptosis. Science 1998;281:1309-1312.
65 Orrenius S, Zhivotovsky B, Nicotera P: Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 2003;4:552-565.
66 Kerr PM, Tam R, Narang D, Potts K, McMillan D, McMillan K, Plane F: Endothelial calcium-activated potassium channels as therapeutic targets to enhance availability of nitric oxide. Can J Physiol Pharmacol 2012;90:739-752.
67 Bers DM, Despa S: Na/K-ATPase--an integral player in the adrenergic fight-or-flight response. Trends Cardiovasc Med 2009;19:111-118.
68 Lang F, Rehwald W: Potassium channels in renal epithelial transport regulation. Physiol Rev 1992;72:1-32.

69 Messner G, Wang W, Paulmichl M, Oberleithner H, Lang F: Ouabain decreases apparent potassium-conductance in proximal tubules of the amphibian kidney. Pflugers Arch 1985;404:131-137.
70 Volkl H, Geibel J, Greger R, Lang F: Effects of ouabain and temperature on cell membrane potentials in isolated perfused straight proximal tubules of the mouse kidney. Pflugers Arch 1986;407:252-257.
71 Rehwald W, Lang F: The effect of cyanide on apparent potassium conductance across the peritubular cell membrane of frog proximal tubules. Pflugers Arch 1986;407:607-610.
72 Koltsova SV, Trushina Y, Haloui M, Akimova OA, Tremblay J, Hamet P, Orlov SN: Ubiquitous [Na+]i/[K+] i-sensitive transcriptome in mammalian cells: evidence for Ca(2+)i-independent excitation-transcription coupling. PLoS One 2012;7:e38032.
73 Orlov SN, Hamet P: The death of cardiotonic steroid-treated cells: evidence of Na+i,K+i-independent H+i-sensitive signalling. Acta Physiol (Oxf) 2006;187:231-240.
74 Akimova OA, Lopina OD, Rubtsov AM, Gekle M, Tremblay J, Hamet P, Orlov SN: Death of ouabain-treated renal epithelial cells: evidence for p38 MAPK-mediated Na (i) (+) /K (i) (+) -independent signaling. Apoptosis 2009;14:1266-1273.
75 Gatidis S, Zelenak C, Fajol A, Lang E, Jilani K, Michael D, Qadri SM, Lang F: p38 MAPK activation and function following osmotic shock of erythrocytes. Cell Physiol Biochem 2011;28:1279-1286.
76 Shaik N, Lupescu A, Lang F: Sunitinib-sensitive suicidal erythrocyte death. Cell Physiol Biochem 2012;30:512-522.
77 Allan LA, Clarke PR: Apoptosis and autophagy: Regulation of caspase-9 by phosphorylation. FEBS J 2009;276:6063-6073.
78 Hagemann C, Blank JL: The ups and downs of MEK kinase interactions. Cell Signal 2001;13:863-875.
79 Kostenko S, Dumitriu G, Laegreid KJ, Moens U: Physiological roles of mitogen-activated-protein-kinase-activated p38-regulated/activated protein kinase. World J Biol Chem 2011;2:73-89.
80 Myers CR, Myers JM, Kufahl TD, Forbes R, Szadkowski A: The effects of acrolein on the thioredoxin system: implications for redox-sensitive signaling. Mol Nutr Food Res 2011;55:1361-1374.
81 Sari FR, Widyantoro B, Thandavarayan RA, Harima M, Lakshmanan AP, Zhang S, Muslin AJ, Suzuki K, Kodama M, Watanabe K: Attenuation of CHOP-mediated myocardial apoptosis in pressure-overloaded dominant negative p38alpha mitogen-activated protein kinase mice. Cell Physiol Biochem 2011;27:487-496.
82 Shiryaev A, Moens U: Mitogen-activated protein kinase p38 and MK2, MK3 and MK5: menage a trois or menage a quatre? Cell Signal 2010;22:1185-1192.