ACTA PHYSIOLOGICA

Acta Physiol 2016

Biosensor cell assay for measuring real-time aldosterone-induced release of histamine from mesenteric arteries

E. G. Dalgaard, K. Andersen, P. Svenningsen* and P. B. L. Hansen*

Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense C, Denmark

Received 15 February 2016, revision requested 14 March 2016, accepted 14 March 2016

Correspondence: Pernille B. L. Hansen, Ph.D., Department of Cardiovascular and Renal Research, University of Southern Denmark, Winsloewparken 21,3, 5000 Odense C, Denmark.
E-mail: pbhansen@health.sdu.dk

*These authors contributed equally to this work.

Abstract

Aims: The aims were to develop a method for real-time detection of histamine release and to test whether incubation with aldosterone induces histamine release from isolated, perfused mice mesenteric arteries.

Methods: Fura-2-loaded HEK-293 cells transfected with the histamine H1 receptor was used as a sensitive biosensor assay for histamine release from isolated mouse mesenteric arteries. Activation of the H1 receptor by histamine was measured as an increased number of intracellular Ca^{2+} transient peaks using fluorescence imaging.

Results: The developed biosensor was sensitive to histamine in physiological relevant concentrations and responded to substances released by the artery preparation. Aldosterone treatment of mesenteric arteries from wild-type mice for 50 min resulted in an increased number of intracellular Ca^{2+} transient peaks in the biosensor cells, which was significantly inhibited by the histamine H1 blocker pyrilamine. Mesenteric arteries from mast cell-deficient SASH mice induced similar pyrilamine-sensitive Ca^{2+} transient response in the biosensor cells. Mesenteric arteries from wild-type and SASH mice expressed histamine decarboxylase mRNA, indicating that mast cells are not the only source of histamine release.

Conclusion: The developed biosensor assay can measure release of substances from vascular preparations. Histamine is released from the vessel preparation in response to aldosterone treatment independently of mast cells. The assay enables us to study a new signaling mechanism for vascular responses induced by aldosterone.

Keywords aldosterone, biosensor, histamine detection, mast cells, perfused mesenteric artery.

The hormone aldosterone is now known to have vascular effects, and clinical observations underline substantial adverse effects of aldosterone on cardiovascular function (Pitt *et al.* 1999, 2014). Aldosterone antagonists lower blood pressure in essential hypertension (Levy *et al.* 2004) and reduce mortality and morbidity following acute myocardial infarction and stroke more than what can be ascribed to the blood pressure lowering effects alone (Pitt *et al.* 1999). These findings encouraged investigation of extra-renal effects of aldosterone and direct vascular effects have now been described (McCurley & Jaffe 2012, Nguyen Dinh Cat & Jaisser 2012, Tarjus *et al.* 2015a,b). Aldosterone affects the vasculature by activation of the genomic mineralocorticoid receptor (MR)-mediated pathway, but it also exerts rapid non-genomic effects in both VSMCs and endothelial cells (Golestaneh *et al.* 2001, Kornel 1994) and MR-independent effects (Christ *et al.* 1995). The response to aldosterone can include both vasodilator responses

© 2016 Scandinavian Physiological Society. Published by John Wiley & Sons Ltd, doi: 10.1111/apha.12680

through nitric oxide (NO) formation and vasoconstriction through a changed calcium handling and reactive oxygen species (ROS) (Arima *et al.* 2003, Uhrenholt *et al.* 2003, Wehling *et al.* 1995). Furthermore, aldosterone leads to different vascular effects depending on whether the exposure is acute (<1 h) or chronic (Uhrenholt *et al.* 2003). The acute response can lead to a NO-mediated dilatation, whereas the chronic response to aldosterone is contractile. The mechanisms through which aldosterone induce constriction involves increased ROS formation, calcium channel activation and/or activation of histamine release from mast cells (Maron *et al.* 2012, Schjerning *et al.* 2013, Tarjus *et al.* 2015a,b). The involvement of histamine in the aldosterone-mediated vasoconstriction was first shown in perfused mice mesenteric arteries (Schjerning *et al.* 2013). Depolarization induces a rapid constriction followed by a secondary NO-dependent vasodilatation. This secondary dilatation is attenuated by 50-min exposure to physiological relevant aldosterone levels (10^{-9} M) (Schjerning *et al.* 2013). Interestingly, the aldosterone effect on secondary dilatation was abolished by histamine H_2 receptor blockade using cimetidine. Furthermore, peri-vascular mast cells (PVMCs) are known to be present in the tunica adventitia of mesenteric vessels, cheek pouch arterioles and penetrating brain arterioles (Doyle *et al.* 1994, Schjerning *et al.* 2013, Strbian *et al.* 2009). In response to aldosterone, an involvement of histamine in vasoconstriction has been observed in previous studies in mesenteric arteries, cheek pouch arterioles and arterioles of skeletal muscle (Doyle *et al.* 1994, Payne *et al.* 2003, 2004, Schjerning *et al.* 2013). PVMCs may therefore play an important role in the regulation of vascular resistance, and aldosterone may be a key regulator of the histamine release from these cells.

The study by Schjerning *et al.* (2013) led to the hypothesis that aldosterone induces histamine release from PVMCs, and a method for measuring histamine release from resistance vessels was therefore needed. To detect release of substances upon exposure to a candidate stimulatory drug, cells transfected with the appropriate receptor can be used as biosensors. Transfected HEK cells have previously been used as biosensors for real-time detection of acetylcholine and ATP (Peti-Peterdi *et al.* 2003, Rodriguez-Diaz *et al.* 2012). The histamine H1 receptor is a Gq-coupled receptor that upon binding of histamine induces an increase in intracellular Ca^{2+} (Carrillo *et al.* 2003). This makes it ideal for use in histamine-detecting biosensor cells.

The aim of this study was to develop a new method using a biosensor cell approach to directly measure real-time histamine release from mast cells located on mesenteric arteries. This will enable us to test the hypothesis that aldosterone mediates histamine release from peri-vascular mast cells.

Methods and materials

Animals

Animal care was followed the guidelines of National Institutes of Health and was approved by the Danish Animal Experiments Inspectorate. Studies were conducted in male and female C57BL/6J (WT) mice (Taconic Farms, Ry, Denmark) and STOCK *Kit*$^{W\text{-}sh}$/HNihrJaeBsmJ (SASH, Jackson laboratory, USA), aged 8–15 weeks. The SASH mice were backcrossed into a C57BL/6J every third generation. Animals were kept at 21 ± 3 °C air temperature, 55 ± 15% air humidity, 12 : 12 h light/dark cycle and with free access to water and rodent chow (Altromin, Brogaarden, Denmark).

Development of histamine biosensor

For the initial experiments, HEK293 cells (ATCC, USA) were transiently transfected with plasmid encoding mouse H1 receptor cDNA (Source Bioscience Product ID: OCACo5052B095D) using Lipofectamine 2000 (Invitrogen, USA) on coverslips in Dulbecco's modified Eagle medium: Nutrient Mixture F-12 (Gibco, USA) supplemented with 10% foetal calf serum (Gibco, USA) and 1% penicillin–streptomycin (Sigma–Aldrich, Germany). A stable cell line expressing H1 receptor was made by transfecting HEK293 cells with H1 receptor cDNA and subsequently selection of transfected cells by addition of blasticidin (1 μg mL^{-1}, Invitrogen, USA) to the cell culture medium. Single clones were isolated and tested for their intracellular Ca^{2+} response to 10^{-8} M histamine. The calcium response was tested using Fluo4 (Invitrogen, USA) on an Enspire plate reader (Perkin Elmer, USA). A single positive clone was identified and cultured for use in further experiments.

Cells were grown on 10 mm glass coverslips (Thermo Scientifics, USA). To improve cell adhesion, five different coatings were tested: (i) no-coat; (ii) acid treatment, 6 h in 1N HCl at 50 °C; (iii) Matrigel (BD Biosciences, Denmark) 15 mg mL^{-1}, 30 min, 37 °C; (iv) poly-l-lysine (PLL, Innoprot, Spain) 0.1 mg mL^{-1}, 60 min, 37 °C; (V) Extracellular MatrixGel (ECM, Sigma–Aldrich, Germany), diluted 1 : 70 in culture medium, 30 min, 37 °C. Cells were plated at the same density and tested for histamine responsiveness as described below.

For biosensor experiments, the following cells were used: (i) HEK-293 cells; (ii) HEK-293 cells stably transfected with the histamine H1 receptor (hereafter

 © 2016 Scandinavian Physiological Society. Published by John Wiley & Sons Ltd, doi: 10.1111/apha.12680

named HEK-H1). All cells were cultured at 37 °C in ambient air with 5% CO_2 and maintained by splitting 1 : 4 every third day. For culturing of HEK-H1 cells, blasticidin (5 μg mL^{-1}) was added to the medium. The cells were used for imaging experiments at 60–90% confluence 48 h after plating on ECM-coated coverslips.

Intracellular calcium measurements with Fura-2

Cells were loaded with 5 μM Fura-2 (Invitrogen) for 30–45 min and rinsed twice in physiological saline solution (PSS) before transferring to the experimental chamber mounted on an inverted microscope (Olympus IX71, Hamburg, Germany). Cells were excited alternately at 340 nm and 387 nm (Olympus Imaging Station cell^R MT20E), and the emitted light was collected through a 510-nm long-pass filter with binding of 2 × 2 pixels, 1 picture per 2 s (Olympus XM10 monochrome camera) using an Olympus UPlanFLN 20X objective (0.3 numerical aperture) with 1.6× extra magnification applied. The measured calcium response of Fura-2-loaded cells is reported as ratio of emitted light from 340 of 387 nm excitation (340/387 ratio). Response of HEK-293 and HEK-H1 cells upon addition of PSS, aldosterone and increasing amounts of histamine (10^{-12}–10^{-5} M) was tested by adding 20 μL solution to the chamber in a cumulative manner equal to 1% of the total volume. The physiological relevant concentration of aldosterone 10^{-9} M (Sigma–Aldrich, Germany) was tested. The mast cell secretagogue compound 48 of 80 (C48/80, 20 μg mL^{-1}, Sigma–Aldrich, Germany) and incubation with a slow (8–16 mL h^{-1}) flow of PSS and stimulation with 70 mM high potassium solution (KPSS) were tested.

Perfusion of mesenteric arteries

The perfusion method was modified from Schjerning *et al.* (2013). A second-order mesenteric artery cut close to branching points was dissected and transferred to a thermostated (TC-324B temperature controller, Warner Instruments, USA) perfusion chamber (custom made, stainless steel with glass bottom, 2 mL capacity). The blood vessel was mounted on glass pipettes and secured with sutures (11-0, ETHILON, ETHICON, USA). The chamber was mounted on an inverted microscope (Olympus IX71). Perfusion pressure was set to 60 mmHg at the inlet and 55 mmHg at the outlet, resulting in a flow rate of 1–2 μL min^{-1}. All solutions were applied abluminally. The temperature was raised to 37 °C followed by a 30-min equilibration period of constant superfusion with physiological saline solution (PSS, 16.5 mL h^{-1}) and flow of ambient, heated gas (5% CO_2 in atmospheric air, 37 °C). The viability of the artery was tested by addition of 70 mM K^+ (KPSS).

Combining the perfused vessel with the HEK-H1 cells

Fura-2-loaded HEK-H1 cells were placed right below (approx. 50–70 μm) the perfused vessel leaving the vessel and cells in close vicinity (<50 μm). The following protocols were hereafter performed, and all experiments were ended by performing a concentration–response test for histamine (10^{-10}–10^{-4} M).

(1) 50-min incubation with 10^{-9} M of aldosterone (aldo) (+ vessel, + aldo);
(2) 50-min incubation with vehicle (+ vessel, − aldo);
(3) 50-min incubation with aldosterone 10^{-9} M, without a vessel (− vessel, + aldo).

Aldosterone or vehicle was added 1 min after start of recording. The experiments were conducted in the presence of the purinergic P2 receptor blocker suramin (10^{-4} M) and repeated with suramin plus the H1 receptor antagonist pyrilamine (10^{-5} M).

Analysis of sensor cell experiments

Analysis of biosensor cell response was carried out by marking individual cells as regions of interest (ROIs). Cells responding to histamine 10^{-4} M were included, resulting in a total of 20–51 cells marked per experiment. Background light was subtracted from all ROIs, and the 340/387 ratio was calculated using Olympus Xcellence RT software. Ratio measurements for each cell were analysed individually by counting of peaks. All peaks with a ratio >Δ0.5 above the baseline were included.

Toluidine blue staining

Toluidine blue (TB) staining was performed on freshly dissected secondary mesenteric arteries from C57BL/6J and mast cell-deficient SASH mice. TB is a metachromatic dye, which stains acidic tissue having high sensitivity and specificity for mast cells (Leclere *et al.* 2006). TB working solution was made by diluting a stock solution of 1% TB (Sigma–Aldrich) (mass/volume in 70% alcohol) into 1% NaCl to a final TB concentration of 0.1%. pH was adjusted to ~2.2 with HCl. The preparation was placed in TB working solution for 2–3 min followed by 2 × 10 times wash in water.

Saline solutions

The physiological saline solution (PSS) used, contained in mM: NaCl 115, $NaHCO_3$ 25, K_2HPO_4 2.5, $MgSO_4$

© 2016 Scandinavian Physiological Society. Published by John Wiley & Sons Ltd, doi: 10.1111/apha.12680

1.2, glucose 5.5, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) 10 and $CaCl_2$ 1.3. The 70 mM K+ in PSS (70 mM KPSS) contained in mM: KCl 65, NaCl 50, $NaHCO_3$ 25, K_2HPO_4 2.5, $MgSO_4$ 1.2, glucose 5.5, HEPES 10 and $CaCl_2$ 1.3. All saline solutions were equilibrated with 5% CO_2 in air for 20 min before use. Bovine serum albumin (BSA) was added in concentrations of 1% to the perfusate, and 0.1% to the superfusate before pH was adjusted to 7.39.

Reverse transcription PCR

RNA isolation from mesenteric arteries and cDNA synthesis was carried out as previously described (Schjerning *et al.* 2013). RT-PCR consisted of 36 cycles and each cycle included incubation at 95 °C for 20 s, 60 °C for 20 s and 72 °C for 20 s. To test for the presence of mast cells, specific primers were used for mast cell protease 2 (mmCPT2) and to test for possible histamine production, the expression of histidine decarboxylase was tested (mHDC). mmCPT2: (sense 5′-GCA CTT CTT TTG CCT TCT GG-3′ and antisense 5′-TGT GCA GCA GTC ATC ACA AA-3′, 170 bp), mHDC (sense: 5′-GCT TTC GCT CCA TTA AGC TG-3′ and antisense 5′ CCA AAC CAA GGT GCC TCT TA-3′, 159 bp) and mTBP (sense: 5′-CAG CCT TCC ACC TTA TGC TC-3′ and antisense: 5′-TTG CTG CTG CTG TCT TTG TT-3′, 169 bp). Negative controls included RNA where no reverse transcriptase was added to the reaction (-RT) and water (H_2O).

Statistics

Significance was calculated by analysis of variance (ANOVA) followed by Bonferronis post-test for comparing replicate means and Student's *t*-test for comparison of two groups. Data are presented as mean ± SEM, and $P < 0.05$ was considered significant. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, USA).

Results

Development of the histamine biosensor

Histamine H1 receptor cDNA-transfected HEK293 cells responded to histamine concentrations below 10^{-8} M, and one clone was chosen for further analysis. HEK293 cells stable transfected with histamine H1 receptor adhered poorly to uncoated coverslips and acid-treated coverslips (data not shown), and cell adhesion of transfected cells was optimized by coating of coverslips. ECM coating improved morphology and cell growth compared to matrigel and PLL (data not shown). Histamine increased the intracellular calcium concentration and the response measured as increase in 340/387 ratio was significant higher in cells grown on ECM compared to uncoated coverslips ($P < 0.05$) (Fig. 1a); thus, ECM coating of the coverslips was chosen for all further experiments with the biosensor cells.

Non-transfected HEK cells responded consistently to histamine 10^{-5} M (Fig. 1b), while transfected HEK-H1 cells responded consistently to histamine 10^{-9} M (Fig. 1c). The 340/387 ratio increased significantly in HEK-H1 cells from 0.68 ± 0.03 at rest to 2.60 ± 0.54 at maximum response to histamine 10^{-9} M ($P < 0.01$, Fig. 1d). Aldosterone stimulation of non-transfected HEK or HEK-H1 cells did not produce Ca^{2+} transients (Fig. 1b and c). However, HEK-H1 cells responded to the histamine releasing agent C48/80 with an increase in 340/387 ratio from 0.69 ± 0.012 to 2.12 ± 0.23 ($P < 0.001$) (Figure S1a).

Aldosterone induces histamine release from mesenteric arteries independent of mast cells

Toluidine blue staining of dissected mesenteric vessels showed mast cells in the adventitia (Fig. 2a). The number of PVMC did, however, vary among the 50 stainings. The transfected HEK-H1 cells were placed below the perfused vessel as shown in Fig. 2b and Figure S1b, and were in focus during the experiment. The biosensor cells responded to the presence of blood vessels treated with (Fig. 2c) or without aldosterone (not shown) with significant changes in intracellular concentration (340/387 ratio) compared to biosensor cells with no vessels present. The increase in intracellular calcium significantly diminished in the presence of the purine receptor antagonist suramin (Fig. 2d).

In the presence of an artery and suramin, the biosensor cells responded to aldosterone treatment by an increased number of intracellular calcium transients, which was inhibited by the histamine receptor H1 antagonist pyrilamine (Fig. 2e). The number of Ca^{2+} peaks was not significantly affected by pyrilamine in mesenteric arteries incubated without aldosterone (Fig. 2f).

To test whether mast cells are required for the aldosterone-induced histamine release, mesenteric arteries from SASH mice were used. Aldosterone induced Ca^{2+} transient that was inhibited by pyrilamine in mesenteric arteries from the SASH mice (Fig. 3a and b). mRNA for the mouse mast cell marker mMCPT2 (mast cell protease 2) was not detected in mesenteric arteries from SASH mice (Fig. 3c). Histidine synthesis is dependent on the enzyme histidine

 © 2016 Scandinavian Physiological Society. Published by John Wiley & Sons Ltd, doi: 10.1111/apha.12680

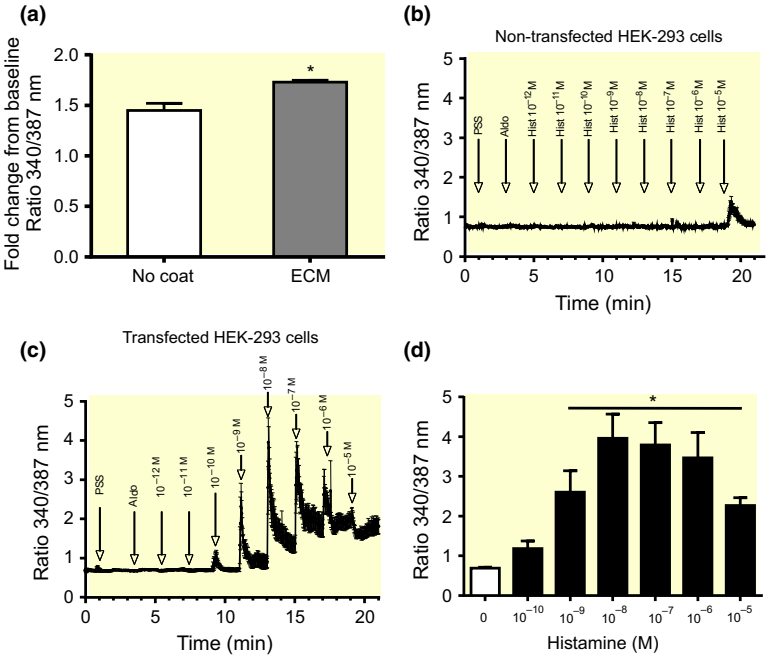

Figure 1 Development and testing of biosensor cells. (a) Increase in calcium response (340/387 ratio) to histamine 10^{-8} M in HEK-H1 cells (transfected HEK-293 cells) grown on uncoated vs. ECM-coated coverslips (n = 6 and 10 respectively). (b) Intracellular calcium concentration in non-transfected HEK-293 cells in response to administration of physiological saline solution (PSS), aldosterone (aldo, 10^{-9} M) and increasing amounts of histamine (Hist, 10^{-12}–10^{-5} M) (single experiment, 26 regions of interests (ROIs) from different cells. (c) As B but performed in HEK-H1 cells (n = 6 experiments, 29–39 ROIs per experiment from different cells). (d) Peak responses to increased histamine concentrations. Maximum responses from C. Data are mean values ± SEM. * $P < 0.05$ compared to resting value.

decarboxylase (HDC), and HDC mRNA was detected in mesenteric arteries from WT and SASH mice (Fig. 3c), indicating that PVMC are not the only source for histamine.

Discussion

A new experimental assay using biosensor cells to detect local histamine releases in physiologically relevant concentrations has been established. Through the use of a histamine biosensor cell, the hypothesis that aldosterone can led to histamine release from PVMCs was tested. Aldosterone stimulation in the combined perfusion and biosensor assay led to release of histamine as the biosensor cells responded to aldosterone by an increased number of Ca^{2+} transients. This response was inhibited by the H1 antagonist pyrilamine. The response was, however, also present in the mast cell-deficient SASH mice, indicating another source for histamine. In support of this notion, the histamine synthesizing enzyme HDC was found to be expressed in mesenteric arteries from both WT and SASH mice at the mRNA level. The present data reveal an aldosterone-induced histamine release from mesenteric blood vessels independent on peri-vascular mast cells. Furthermore, ATP was found to be released from mesenteric arteries as the Ca^{2+} transient peaks were attenuated in the presence of the non-selective purine receptor antagonist suramin. This occurred independently of the investigated aldosterone pathway and show that the present assay can be used as a general approach to measure in real-time release of relevant substances from blood vessel preparations.

A prerequisite for the development of the biosensor cell in the current set-up is the coupling between extracellular histamine and intracellular Ca^{2+} concentration transients. The histamine biosensor cells were sensitive to nanomolar concentrations of histamine. Previously, this concentration of histamine has been shown to attenuate secondary dilatation in mesenteric vessels in a similar manner as aldosterone (Schjerning *et al.* 2013) and indicate that the sensitivity of the HEK-H1 cells is in the relevant concentration range to detect the vascular responses. Although our current set-up allowed us to measure real-time release of histamine, there might be options to increase the sensitivity of the assay. The biosensor cells were plated on coverslips, and diffusion and dilution of histamine in the chamber could potentially affect the sensitivity of the assay. To reduce this problem in the current set-up, the HEK-H1 cells were placed in close vicinity to the vessels. Nevertheless, it seems plausible that the sensitivity of the assay could be improved using a single biosensor cell attached to a holding pipette (Peti-Peterdi *et al.* 2003). An added benefit of using the single biosensor cell approach is that analysis of the Ca^{2+} transient is easier. In the current setup several approaches for analysis were tested: several automatic cell-tracking programs were tested which could have allowed for analysis of all cells in the recorded field. However, the programs gave different cell counts for each picture, which made tracking of individual cells impossible. Analysis of the entire picture as one ROI resulted in very low histamine sensitivity and was prone to artefacts. Instead, a manual analysis method was used measuring all cells (ROI) responding to

© 2016 Scandinavian Physiological Society. Published by John Wiley & Sons Ltd, doi: 10.1111/apha.12680

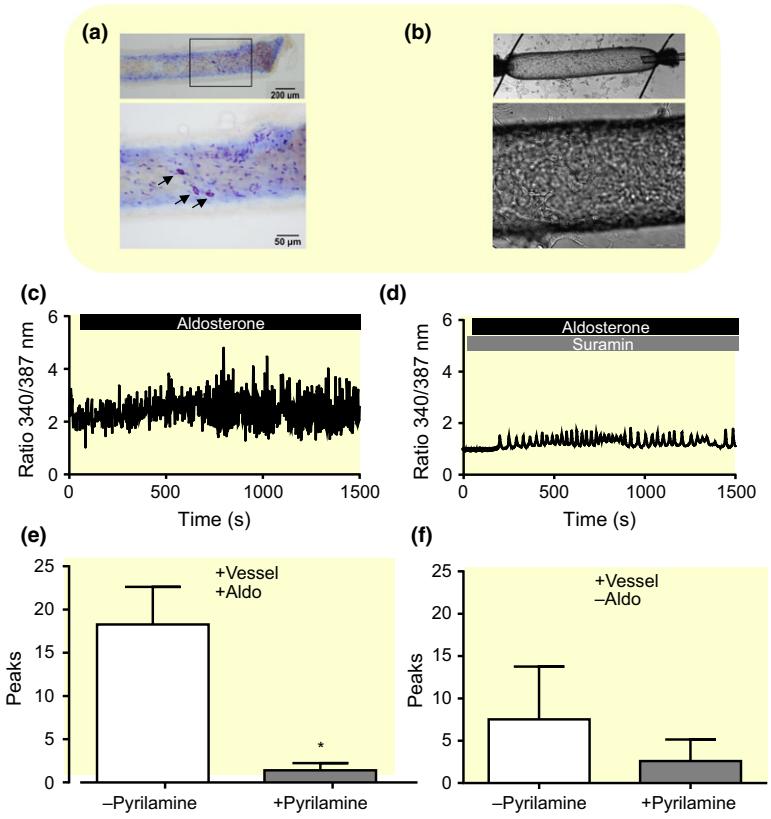

Figure 2 Aldosterone induces histamine release from mesenteric arteries. (a) Mesenteric artery with mast cells (C57BL6 mouse) stained with toluidine blue (TB) showing three mast cells (arrows). (b) (top) Perfused vessel above HEK-H1 cells, with (bottom) showing the imaging field during the experiment. Changes in intracellular Ca^{2+} concentration (340/387 ratio) in biosensor cells upon exposure to aldosterone (aldo, 10^{-9} M) without (c) and with (d) the presence of the purine receptor blocker suramin (suramin, 10^{-4} M). (e) Number of calcium peaks in response to aldosterone (aldo, 10^{-9} M) in presence (n = 8 experiments, 13–40 ROIs per experiment) or absence (n = 5 experiments, 15–31 ROIs per experiment) of the histamine receptor H1 antagonist pyrilamine (10^{-5} M). (f) Number of calcium peaks in the absence of aldosterone (aldo) in presence (n = 5 experiments, 7–34 ROIs per experiment) or absence (n = 4 experiments, 4–11 ROIs per experiment) of the histamine receptor H1 antagonist pyrilamine. All experiments were made in the presence of suramin. Data are mean values ± SEM. * $P < 0.05$.

control administration of histamine in the end of the experiment. By conducting and analysing control experiments in the same way, the effect of selection bias and potential risk of type 1 error was minimized. Further development of custom-written algorithms would aid in the analysis task.

The HEK-H1 cells did not respond to aldosterone, whereas the mast cell activator compound 48/80 gave rise to a calcium increase. C48/80 was intended as a control stimulator of mast cell degranulation, but as the HEK-H1 cells responded to C48/80, it was discarded. Moreover, the mesenteric arteries appeared to release ATP independently of aldosterone stimulation. The Ca^{2+} induced by ATP was effectively blocked by suramin, and it was concluded that the biosensor cell could be used for measuring aldosterone-mediated histamine release from a vessel preparation in the presence of suramin.

It was found that aldosterone-induced histamine release from the mesenteric arteries in wild-type and mast cell-deficient mice, which does not support the hypothesis that mast cells are the only source for histamine although it support the idea that histamine can be released from isolated blood vessels In agreement, mesenteric arteries from WT and SASH mice expressed histamine decarboxylase (HDC) and suggest a non-PVMC synthesis of histamine in the vasculature. HDC has previously been shown to be expressed in vascular smooth muscle cells (VSMCs) from rats and in human endothelial and VSMCs indicating that these cells types have the ability to synthesis histamine (Tippens *et al.* 2004, Tippens & Gruetter 2004).

The harmful vascular effect of aldosterone during cardiovascular diseases is well known (Lyngsø *et al.* 2015); however, the role for the aldosterone–histamine pathway is surprising as activation of

 © 2016 Scandinavian Physiological Society. Published by John Wiley & Sons Ltd, doi: 10.1111/apha.12680

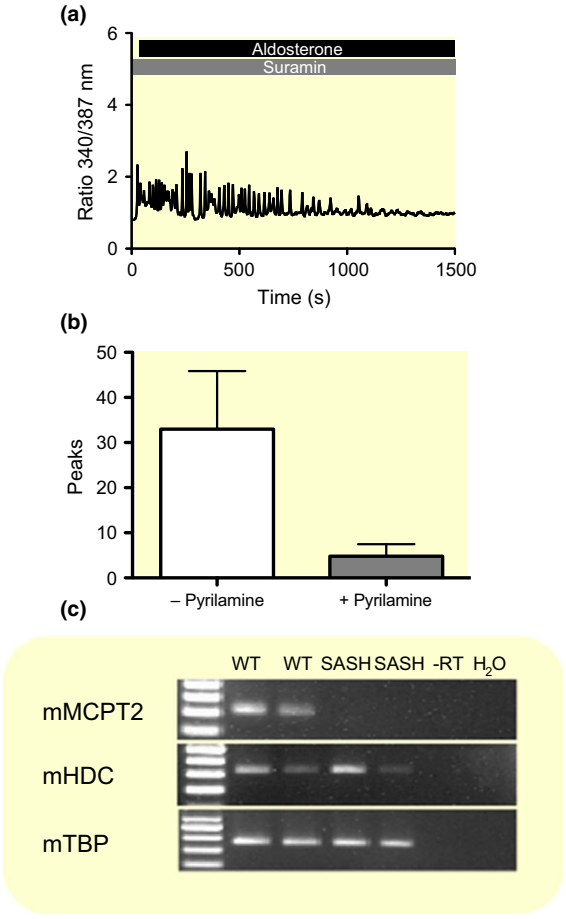

Figure 3 Aldosterone induces histamine release in mast cell deficient SASH mice. (a) Trace showing the intracellular Ca^{2+} concentration (340/387 ratio) in biosensor cells upon exposure of a mesenteric artery from SASH mice to aldosterone (aldo, 10^{-9} M). (b) Number of calcium peaks in the presence of aldosterone (aldo) in presence (n = 4 experiments) or absence (n = 7 experiments) of the histamine receptor H1 antagonist pyrilamine. The experiments were carried out in the presence of suramin. (c) PCR of mesenteric arteries from wild-type (WT) and SASH mice shows that although the mouse mast cell marker MCPT2 is not expressed in SASH mice, both WT and SASH mice express mouse histamine decarboxylase (mHDC). mTBP is TATA-box binding protein used as loading control.

histamine H_2 receptors usually leads to vasodilatation and decrease in blood pressure. Aldosterone can attenuate NO-dependent dilatation by degranulation of adventitial mast cells, which results in activation of H_2 receptors and a decreased NO sensitivity of the smooth muscle cell (Schjerning *et al.* 2013). Histamine can inhibit NO-dependent vasodilatation (Payne *et al.* 2003, 2004, Schjerning *et al.* 2013); however, the mechanism underlying aldosterone-induced histamine release is unknown. The present study suggests that not only peri-vascular mast cells but also vascular cells are involved in the aldosterone–histamine pathway. The aldosterone–histamine coupling is an entirely new pathway for aldosterone action with the potential for new drug targets and treatment of patients with a wide range of cardiovascular diseases associated with hyperaldosteronism and/or MR activation (Milliez *et al.* 2005, Pitt *et al.* 1999).

An increased interest in communication between the vasculature and the surrounding tissue has led to many new discoveries. Many different substances are released from peri-vascular adipocytes and other peri-vascular cell types. The present method can directly be used to measuring histamine release, but it could potentially also be used for investigations of many other paracrine pathways that affect the vascular tone.

Conclusion

Aldosterone is involved in a wide range of cardiovascular diseases, and new treatment options are therefore highly demanded. The present histamine-biosensor assay demonstrated that aldosterone induces histamine release from mesenteric arteries. We detected histamine release in mast cell-deficient SASH mice, indicating that other vascular cells are involved in the aldosterone-induced histamine release. This study shows a new pathway of aldosterone signalling acting through histamine release. Furthermore, the developed method can be used for measuring real-time release of substances relevant for vascular function.

Conflict of interest

No conflict of interests to disclose.

The author would like to thank Kristoffer Rosenstand for helpful and valuable technical guidance and assistance. This work was supported by grants from the Danish Medical Research Council (11-107552), The Danish Heart Foundation (11-04-R84-A3492-22663). Furthermore, we would like to thank the EU COST action 'ADMIRE' for support and the Stiftelsen Nordisk Fysiologi, SNF for generous support for the AP Symposium on 'Endothelium-dependent hyperpolarizations 2015'.

References

Arima, S., Kohagura, K., Xu, H.L., Sugawara, A., Abe, T., Satoh, F., Takeuchi, K. & Ito, S. 2003. Nongenomic vascular action of aldosterone in the glomerular microcirculation. *J Am Soc Nephrol* **14**, 2255–2263.

Carrillo, J.J., Pediani, J. & Milligan, G. 2003. Dimers of class A G protein-coupled receptors function via agonist-mediated trans-activation of associated G proteins. *J Biol Chem* **278**, 42578–42587.

Christ, M., Meyer, C., Sippel, K. & Wehling, M. 1995. Rapid aldosterone signaling in vascular smooth muscle

cells: involvement of phospholipase C, diacylglycerol and protein kinase C alpha. *Biochem Biophys Res Commun* **213**, 123–129.

Doyle, M.P., Linden, J. & Duling, B.R. 1994. Nucleoside-induced arteriolar constriction: a mast cell-dependent response. *Am J Physiol* **266**, H2042–H2050.

Golestaneh, N., Klein, C., Valamanesh, F., Suarez, G., Agarwal, M.K. & Mirshahi, M. 2001. Mineralocorticoid receptor-mediated signaling regulates the ion gated sodium channel in vascular endothelial cells and requires an intact cytoskeleton. *Biochem Biophys Res Commun* **280**, 1300–1306.

Kornel, L. 1994. Colocalization of 11 beta-hydroxysteroid dehydrogenase and mineralocorticoid receptors in cultured vascular smooth muscle cells. *Am J Hypertens* **7**, 100–103.

Leclere, M., Desnoyers, M., Beauchamp, G. & Lavoie, J.P. 2006. Comparison of four staining methods for detection of mast cells in equine bronchoalveolar lavage fluid. *J Vet Intern Med* **20**, 377–381.

Levy, D.G., Rocha, R. & Funder, J.W. 2004. Distinguishing the antihypertensive and electrolyte effects of eplerenone. *J Clin Endocrinol Metab* **89**, 2736–2740.

Lyngsø, K.S., Assersen, K., Dalgaard, E.G., Skott, O., Jensen, B.L. & Hansen, P.B. 2015. Does aldosterone play a significant role for regulation of vascular tone? *J Cardiovasc Pharmacol* PMID: 26657712. [Epub ahead of print]

Maron, B.A., Zhang, Y.Y., White, K., Chan, S.Y., Handy, D.E., Mahoney, C.E., Loscalzo, J. & Leopold, J.A. 2012. Aldosterone inactivates the endothelin-B receptor via a cysteinyl thiol redox switch to decrease pulmonary endothelial nitric oxide levels and modulate pulmonary arterial hypertension. *Circulation* **126**, 963–974.

McCurley, A. & Jaffe, I.Z. 2012. Mineralocorticoid receptors in vascular function and disease. *Mol Cell Endocrinol* **350**, 256–265.

Milliez, P., Girerd, X., Plouin, P.F., Blacher, J., Safar, M.E. & Mourad, J.J. 2005. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol* **45**, 1243–1248.

Nguyen Dinh Cat, A. & Jaisser, F. 2012. Extrarenal effects of aldosterone. *Curr Opin Nephrol Hypertens* **21**, 147–156.

Payne, G.W., Madri, J.A., Sessa, W.C. & Segal, S.S. 2003. Abolition of arteriolar dilation but not constriction to histamine in cremaster muscle of eNOS$^{-/-}$ mice. *Am J Physiol Heart Circ Physiol* **285**, H493–H498.

Payne, G.W., Madri, J.A., Sessa, W.C. & Segal, S.S. 2004. Histamine inhibits conducted vasodilation through endothelium-derived NO production in arterioles of mouse skeletal muscle. *FASEB J* **18**, 280–286.

Peti-Peterdi, J., Komlosi, P., Fuson, A.L., Guan, Y., Schneider, A., Qi, Z., Redha, R., Rosivall, L., Breyer, M.D. & Bell, P.D. 2003. Luminal NaCl delivery regulates basolateral PGE2 release from macula densa cells. *J Clin Invest* **112**, 76–82.

Pitt, B., Zannad, F., Remme, W.J., Cody, R., Castaigne, A., Perez, A., Palensky, J. & Wittes, J. 1999. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* **341**, 709–717.

Pitt, B., Pfeffer, M.A., Assmann, S.F., Boineau, R., Anand, I.S., Claggett, B., Clausell, N., Desai, A.S., Diaz, R., Fleg, J.L., *et al.* 2014. Spironolactone for heart failure with preserved ejection fraction. *N Engl J Med* **370**, 1383–1392.

Rodriguez-Diaz, R., Dando, R., Huang, Y.A., Berggren, P.O., Roper, S.D. & Caicedo, A. 2012. Real-time detection of acetylcholine release from the human endocrine pancreas. *Nat Protoc* **7**, 1015–1023.

Schjerning, J., Uhrenholt, T.R., Svenningsen, P., Vanhoutte, P.M., Skøtt, O., Jensen, B.L. & Hansen, P.B. 2013. Histamine-dependent prolongation by aldosterone of vasoconstriction in isolated small mesenteric arteries of the mouse. *Am J Physiol Heart Circ Physiol* **304**, H1094–H1102.

Strbian, D., Kovanen, P.T., Karjalainen-Lindsberg, M.L., Tatlisumak, T. & Lindsberg, P.J. 2009. An emerging role of mast cells in cerebral ischemia and hemorrhage. *Ann Med* **41**, 438–450.

Tarjus, A., Amador, C., Michea, L. & Jaisser, F. 2015a. Vascular mineralocorticoid receptor and blood pressure regulation. *Curr Opin Pharmacol* **21**, 138–144.

Tarjus, A., Belozertseva, E., Louis, H., El Moghrabi, S., Labat, C., Lacolley, P., Jaisser, F. & Galmiche, G. 2015b. Role of smooth muscle cell mineralocorticoid receptor in vascular tone. *Pflugers Arch* **467**, 1643–1650.

Tippens, A.S. & Gruetter, C.A. 2004. Detection of histidine decarboxylase mRNA in human vascular smooth muscle and endothelial cells. *Inflamm Res* **53**, 215–216.

Tippens, A.S., Davis, S.V., Hayes, J.R., Bryda, E.C., Green, T.L. & Gruetter, C.A. 2004. Detection of histidine decarboxylase in rat aorta and cultured rat aortic smooth muscle cells. *Inflamm Res* **53**, 390–395.

Uhrenholt, T.R., Schjerning, J., Hansen, P.B., Nørregaard, R., Jensen, B.L., Sorensen, G.L. & Skott, O. 2003. Rapid inhibition of vasoconstriction in renal afferent arterioles by aldosterone. *Circ Res* **93**, 1258–1266.

Wehling, M., Neylon, C.B., Fullerton, M., Bobik, A. & Funder, J.W. 1995. Nongenomic effects of aldosterone on intracellular Ca^{2+} in vascular smooth muscle cells. *Circ Res* **76**, 973–979.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. a: HEK-H1 cells were stimulated with PSS and compound 48/80 (20 μg mL^{-1}) to HEK-H1 cells (single experiment, $n = 40$ ROIs). b: Schematic drawing of the perfusion set-up with perfused vessel placed above coverslips with HEK-H1 biosensor cells (not drawn to scale).

 © 2016 Scandinavian Physiological Society. Published by John Wiley & Sons Ltd, doi: 10.1111/apha.12680