Mild heating and reduction of bladder spontaneous contractions

Journal:	*BJU International*
Manuscript ID	BJU-2017-0060.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	24-Apr-2017

Complete List of Authors:	Kitney, Darryl; University of Bristol, Department of Physiology, Pharmacology and Neuroscience Jabr, Rita; University of Surrey, Deparment of Biochemical Sciences; University of Bristol, Department of Physiology, Pharmacology and Neuroscience Vahabi, Bahareh; University of the West of England, Department of Biological, Biomedical and Analytical Sciences; University of Bristol, Department of Physiology, Pharmacology and Neuroscience Fry, Christopher; University of Bristol, Department of Physiology, Pharmacology and Neuroscience

Keywords:	Spontaneous contractions, Heating, Overactive bladder

Abstract:	Objectives: To measure the effect of external heating on bladder wall contractile function, histological structure and expression of proteins related to tissue protection and apoptosis. Material and methods: In vitro preparations of bladder wall and ex vivo perfused pig bladders were heated from 37°C to 42, 46 and 50°C for 15 minutes. Isolated preparations were heated by radiant energy and perfused bladders by altering perfusate temperature. Spontaneous contractions or pressure variations were recorded, as well as responses to the muscarinic agonist carbachol or motor nerve excitation in vitro during heating. Tissue histology in control and after heating was analysed using H&E staining and DAPI nuclear labelling. The effects of heating on protein expression levels of i) heat shock proteins HSP27-pSer82 and inducible-HSP70 and ii) caspase-3 and its downstream DNA-repair substrate, PARP were measured. Results: Heating to 42°C reduced spontaneous contractions or pressure variations by about 70%, effects were fully reversible. There were no effects on carbachol or nerve-mediated responses. Tissue histology was unaffected by heating and expression of heat-shock proteins as well as caspase-3 and PARP were also unaltered. A TRPV1 antagonist had no effect on the reduction of spontaneous activity. Heating to 46°C had a similar effect on spontaneous activity and also reduced the carbachol contracture. Urothelial structure was damaged, caspase-3 levels were increased and inducible-HSP70 levels declined. At 50°C evoked contractions were abolished, the urothelium was absent and heat-shock proteins and PARP expression was reduced with raised caspase-3

	expression. Conclusions: Heating to 42°C caused a profound, reversible and reproducible attenuation of spontaneous activity with no tissue damage and no initiation of apoptosis pathways. Higher temperatures caused tissue damage and activation of apoptotic mechanisms. Mild heating offers a novel approach to reduce bladder spontaneous activity.

Mild heating and reduction of bladder spontaneous contractions

Darryl G Kitney[a,b], Ph.D., Rita I Jabr[a,b], Ph.D., Bahareh Vahabi[b,c], Ph.D., Christopher H Fry[b], Ph.D, DSc, FRCS.

Footnotes

[a] University of Surrey, Faculty of Health and Medical Sciences, Guildford, GU2 7XH.

[b] University of Bristol, School of Physiology, Pharmacology & Neuroscience, University Walk, Bristol, BS8 1TD.

[c] University of the West of England, Department of Biological, Biomedical and Analytical Sciences, Bristol, BS16 1QY.

Corresponding author.

Prof C H Fry, School of Physiology, Pharmacology & Neuroscience

University of Bristol

University Walk, Bristol BS8 1TD, UK

email: chris.fry@bristol.ac.uk

Keywords: Heating; overactive bladder; spontaneous contractions.

Abstract:

Objectives: To measure the effect of external heating on bladder wall contractile function, histological structure and expression of proteins related to tissue protection and apoptosis.

Material and methods: In vitro preparations of bladder wall and ex vivo perfused pig bladders were heated from 37°C to 42, 46 and 50°C for 15 minutes. Isolated preparations were heated by radiant energy and perfused bladders by altering perfusate temperature. Spontaneous contractions or pressure variations were recorded, as well as responses to the muscarinic agonist carbachol or motor nerve excitation in vitro during heating. Tissue histology in control and after heating was analysed using H&E staining and DAPI nuclear labelling. The effects of heating on protein expression levels of i) heat shock proteins HSP27-pSer82 and inducible-HSP70 and ii) caspase-3 and its downstream DNA-repair substrate, PARP were measured.

Results: Heating to 42°C reduced spontaneous contractions or pressure variations by about 70%, effects were fully reversible. There were no effects on carbachol or nerve-mediated responses. Tissue histology was unaffected by heating and expression of heat-shock proteins as well as caspase-3 and PARP were also unaltered. A TRPV1 antagonist had no effect on the reduction of spontaneous activity. Heating to 46°C had a similar effect on spontaneous activity and also reduced the carbachol contracture. Urothelial structure was damaged, caspase-3 levels were increased and inducible-HSP70 levels declined. At 50°C evoked contractions were abolished, the urothelium was absent and heat-shock proteins and PARP expression was reduced with raised caspase-3 expression.

Conclusions: Heating to 42°C caused a profound, reversible and reproducible attenuation of spontaneous activity with no tissue damage and no initiation of apoptosis pathways. Higher temperatures caused tissue damage and activation of apoptotic mechanisms. Mild heating offers a novel approach to reduce bladder spontaneous activity.

Introduction

The urinary bladder generates low level spontaneous contractile activity which may maintain bladder wall tone, but enable it to remain compliant during filling [1]. However, with detrusor overactivity spontaneous contractions increase [2], a phenomenon also present in isolated detrusor preparations [3,4]. It is therefore of interest to develop paradigms that reduce spontaneous activity, but without causing structural and functional damage to the bladder wall, nor disrupt the physiological pathways that generate bladder wall tension to initiate voiding. Heating is one such possible paradigm and has been used, for example, as a therapeutic treatment in the heart to protect against the consequences of myocardial ischaemia by inducing expression of heat shock proteins (HSPs) [5]. There is little work with heating the bladder, one study [6] showed that localised microwave heating *in vivo* up to 43°C caused no changes to bladder capacity, whilst higher temperatures caused progressive decline of bladder capacity. However, there is no systematic characterisation of how heating affects contractile function, although cooling from 37°C increases isolated detrusor contractions [7,8].

Heat stress quantification may be estimated by calculating a thermal dose time [9] given by:

$$CEM_{43}\ (\text{minutes}) = \Delta t.R^{(43-T)}$$

with Δt the duration of exposure to temperature T; R=0.25 for T<43°C and 0.5 for T>43°C; i.e. CEM_{43}=3.75 min at T=42°C for 15 min. From [6], data indicate that CEM_{43} < 11 min is below the level for thermal damage to the bladder.

Some transient receptor potential (TRP) channel subtypes are activated by noxious stimuli, including heating. Located on sensory nerves in the sub-urothelium and on urothelial cells $TRPV_1$ channels in particular respond to temperatures between 41-50°C [10,11]. Activation

of $TRPV_1$ channels permits Ca^{2+} influx into cells that may modulate sensory responses to bladder filling and hence spontaneous activity.

Hyperthermia affects protein structure and function and even small increases of temperature can lead to their unfolding and loss of activity [12]. However, this may be offset by upregulation of protective mechanisms such as induction of chaperones including inducible HSP70 and phosphorylated HSP27 [13]. Moreover, there is evidence that pro-apoptotic pathways mediated by activation of caspase-3 and DNA-repair pathways mediated by poly-[ADP-ribose] polymerases (PARP) are also temperature-dependent [14-16].

We hypothesised that heating isolated detrusor preparations below the CEM_{43} threshold of about 11 min would decrease reversibly spontaneous activity, but without causing structural damage, whilst at higher temperatures irreversible functional and structural changes would occur. We tested also the possible role of $TRPV_1$ receptors in mediating any changes to spontaneous activity.

Materials and methods

In vitro preparations and solutions. Male and female pig bladders (*Sus scrofa domestica*, ~6 months) from a local abattoir were transported in under one hour to the laboratory in gassed Tyrode's solution at 4°C. The bladder was opened sagitally through its ventral face and exposed as a sheet, mucosa uppermost, for dissection. Preparations with an intact mucosa (10x20 mm, 2 mm thick) were attached to an isometric force transducer and superfused with Tyrode's solution (12 $ml.min^{-1}$). Stock solutions of carbachol (10 mM in water), the $TRPV_1$ channel antagonist AMG9810 (10 mM in DMSO) and the capsaicin antagonist, capsazepine (10 mM in methanol) were diluted to final concentrations in Tyrode's solution (mM): NaCl, 118; KCl, 4.0; $MgCl_2$, 1.0 $NaHCO_3$, 24, NaH_2PO_4, 0.4; $CaCl_2$, 1.8; glucose, 6.1; Na pyruvate, 5.0; 95% O_2/5% CO_2. pH was 7.49±0.04 at 37°C and increased after heating to 42°C (7.65±0.04), 46°C (7.73±0.0.03) and 50°C (7.75±0.03).

Preparation of heating coil and temperature recording. A heating coil of nichrome wire (7 coils, 0.4 mm diameter; 10.5 $\Omega.m^{-1}$ resistance) was placed immediately above the tissue preparation to radiate heat onto the urothelial surface. Temperature was measured with thermistor probes (33 gauge, 0.2 mm diameter, hypodermic chromega-alomega probe, Omega) placed in the superfusate by the urothelium immediately below the coil and also at the mucosa/detrusor boundary. A temperature gradient of about 1°C between superfusate and mucosa/detrusor boundary was measured, the latter is referred to as the test temperature.

Contraction measurements. Spontaneous contractions were initiated by exposure to carbachol (1 μM) for 10 minutes and stabilised for one-hour before interventions. Preparations were then heated for 15 minutes and allowed to recover for 45 minutes (unless otherwise stated) before the next intervention. The force integral (area-under-the-curve, AUC) of spontaneous activity was measured for the final 10 minutes of each intervention and control period.

Agonist-induced contractions were elicited by carbachol for 10 minutes, with 45 minutes between successive contractures. Continuous electrically stimulation was via electrical current through platinum wires either side of the tissue (0.1 ms pulse-width for 3 s stimulation every 90 s at 8 Hz, 45 V).

Ex vivo whole perfused pig bladders. Whole female pig bladders were excised immediately *post-mortem* at the abattoir, with their associated vasculature, and stored in ice-cold gassed Kreb's solution, as previously described [17]. At the laboratory, the bladder was perfused with Kreb's solution through its arterial supply at constant flow (10 $ml.min^{-1}$), and the lumen filled with 150 ml Kreb's by a urethral catheter. Intravesical pressure was recorded *via* a fluid-filled double-lumen catheter attached to a pressure transducer. The bladder wall was heated by altering the temperature of the perfusate and luminal fluid. Kreb's solution was similar to Tyrode's except (mM): KCl, 4.7, KH_2PO_4, 1.2; glucose, 11.7.

Histology. Intact preparations after exposure to radiant heating, or paired controls (n=6), were immediately stored in 10% neutral-buffered formalin. Samples were wax-embedded, sectioned (superfrost$^+$ slide, 5 μm) and deparaffinised, then either stained with haematoxylin and eosin (H&E) or the nuclear stain, 4′-6-diamidino-2-phenylindole (DAPI, 1:10,000 dilution, Thermofisher Scientific, UK). H&E sections were used for visualisation and DAPI-stained sections for analysis. Images (63x objective) were taken with a wide-field microscope (Leica, DM LB2) attached to a CCD camera (Leica DFC450C: 1280x960 pixels) for H&E samples. For DAPI-stained images, a z-stack was used to obtain the optimum section using different focal distances. The minimum and maximum depths of each section was obtained and the intermediate region analysed. Images of 300-500 μm^2 area were taken by a CCD camera and analysed for nuclear diameter.

Western blots. Bladders were incubated with control Tyrode's solution for 15 minutes at 37, 42, 46, or 50°C, and rapidly snap-frozen in liquid N_2. Whole tissue protein lysate (30 μg) from each sample was prepared using RIPA buffer, resolved by 12% polyacrylamide SDS-PAGE and transferred to polyvinylidene difluoride membranes (PVDF, Invitrogen, UK). Membranes were blocked with Odyssey blocking buffer (LI-COR Biosciences, Ltd, UK) and probed with primary antibodies (Abcam, UK, rabbit polyclonal) to caspase-3 (1:500 dilution), PARP (1:2000 dilution), phosphorylated HSP27 (HSP27-pSer82, 1:2,000 dilution) or inducible HSP70 (iHSP70, 1:500 dilution). Membranes were then washed and incubated with secondary antibodies appropriate to the source of primary antibodies (LI-COR Biosciences; 1:10,000 dilution). Resolved protein bands were imaged using an Odyssey infra-red imaging system and then quantified with Image-J software in arbitrary units. The quantified band densities were normalised to corresponding GAPDH band densities (Santa Cruz, mouse monoclonal, 1:1,000.dilution).

Data analysis and statistics. Contractile function data were normalised to the average of pre- and post-control values at 37°C unless otherwise stated. Data are medians [25,75% interquartiles], except as means±SD where stated. Differences between data sets were compared using ANOVA, with non-parametric or parametric *post hoc* comparisons using GraphPad Prism 5. The association between two variables was tested using a Spearman's rank correlation; the null hypothesis was rejected at $p<0.05$.

Results

Effect of heating to 42°C on contractile responses, tissue structure and protein expression. Radiant heating to 42°C for 15 mins (CEM_{43}=3.75) significantly and reversibly reduced the tension integral (AUC) of spontaneous activity to about 30% of the control value at 37°C (fig 1A). By contrast, contractions generated by carbachol or electrical field stimulation were unaffected by heating to 42°C (fig 1B,C). The calculated CEM_{43} value (3.75 min; see Introduction) for the above protocol is below the threshold for thermal damage (11.0 min) as estimated previously for the bladder. Histology of the tissue confirmed no gross damage to the urothelium or the detrusor layer on heating to 42°C (fig 2A, representative of *n*=6). In addition, urothelial and detrusor muscle nuclear diameters (indicators of cell death (18)) were unchanged compared to those at 37°C, nor were there changes to the protein expression of heat shock proteins iHSP70 and HSP27 (fig 2B), nor indicators of apoptosis, caspase-3 and PARP (fig 2C).

Control experiments. Reproducibility of spontaneous activity reduction was tested by exposing preparations to two 15 min periods at 42°C with a 45 min interval. First and second exposures reduced AUC to 30.5 [24.5, 37.9] *vs* 32.1 [26.4, 43.7]% control (*n*=6; p>0.05). Heating Tyrode's especially to ≥46°C increased superfusate pH from 7.4 to as much as 7.8, which itself might affect AUC values. However, increasing pH from 7.4 to 7.8 at 37°C had no significant effect on AUC: 100.0 [90.6, 114.0] *vs* 109 [13.9,21.6] % control (*n*=6; p>0.05).

Effect of heating on the SA of the isolated, perfused whole pig bladder. A similar heating regime on isolated perfused pig bladders was also done. These preparations also developed spontaneous intravesical pressure transients that, like those in isolated preparations, were also significantly reduced when perfusate temperature was raised to 42°C (fig 3, *n*=5). The

magnitude of reduction was similar to that from *in vitro* isolated preparations and also this reduction recovered to control levels on return to 37°C.

Effect of $TRPV_1$ modulators. The $TRPV_1$ antagonist AMG9810 (0.3 μM) had no significant effect on AUC at 37°C (105.9 [10.5, 4.8] *vs* 101.0 [2.0, 3.0], drug *vs* no drug, p>0.05, *n*=8), nor did it alter its reduction on heating to 42°C (34.9 [31.3, 39.1] *vs* 33.4 [25.4, 56.1], p>0.05, *n*=8). Capsazepine, which blocks activation of $TRPV_1$ by chemicals also had no effect on AUC at 37°C (91.8 [80.8, 107.8] *vs* 100.1 [2.1, 3.1], drug *vs* no drug, p>0.05, *n*=7), nor its attenuation by heating to 42°C (50.0 [35.0, 60.5] *vs* 43.3 [34.2, 53.8], p>0.05, *n*=8).

The effect of different heating regimes on isolated preparations. Heating to 42°C for 15 min has significant, reversible effects on spontaneous contractions, but none on other contractile modes nor tissue architecture. Prolonged heating or to higher temperatures might have more deleterious effects, as indicted by greater calculated CEM_{43} periods. The following conditions were used: i) 42°C for 30 min (CEM_{43}=7.5); ii) 42°C for 60 min (CEM_{43}=15.0); iii) 46°C for 15 min (CEM_{43}=120); iv) 50°C for 15 min (CEM_{43}=1920). In separate experiments heating to 42°C for 30 and 60 min reduced AUC by similar amounts compared to 15 min, all reductions were fully reversible: 15 min, 35.3% [15.0, 69.7]; 30 min, 37.0% [23.4, 66.4]; 60 min, 37.6% [30.6, 67.4]; *n*=9. Histological observations in six preparations again showed no change to the gross appearance of the mucosa.

Heating to 46°C for 15 min also reversibly reduced AUC, similar to that for 42°C. However, carbachol contractures were also diminished, although EFS contractions were unaffected (fig 4A). Moreover, histology showed evidence of urothelial damage, with regions of ablation in all six preparations tested (fig 4B) and urothelial cell nuclear diameter was significantly reduced to 80.7 [66.3, 88.3]% control, *n*=6, there were no changes to detrusor muscle nuclear

diameter. Finally, iHSP70, but not HSP27, expression was decreased, as well as there being an increase of caspase-3 expression and a decrease of PARP expression (fig 4C).

Heating to 50°C produced profound and often irreversible changes. AUC of spontaneous contractions was reduced to 23.5 [14.3, 30.2]% control, *n*=10, with poor recovery in most cases. Moreover, carbachol and EFS contractile responses were almost completely abolished, and in the case of EFS responses did not recover on return to 37°C. Histology showed that the urothelium was almost completely absent and, where possible, nuclear diameter measurement was only 71.2 [47.4,92.6]% control, *n*=6. Moreover, caspase-3 was significantly increased and PARP reduced, to levels not significantly different from those at 46°C. iHSP70 expression was reduced to levels not different from that at 46°C as was HSP27 expression.

Discussion

Heating isolated pig bladder wall preparations from 37°C to between 42 and 50°C for 15 minutes reduced bladder spontaneous contractile activity, and by a similar extent at any temperature. However, at temperatures above 42°C recovery was not always complete on return to 37°C and there was other evidence of contractile failure, histological damage and initiation of apoptosis. The effect was reproducible, such that a second heat exposure generated an identical reversible response. Moreover, prolonging the heating period for up to 60 minutes did not decrease further spontaneous activity and allowed complete recovery to occur after the intervention. A similar reduction of spontaneous pressure changes was observed in the isolated, perfused pig bladder using a similar heating protocol to 42°C. A previous study in young adult rats investigated the temperature-dependence of intravesical pressure transients between 19 and 38°C with findings that may be extrapolated to above data: increasing temperature, decreased the amplitude of spontaneous transients [19]. Of interest also was that spontaneous contractile activity was more temperature–dependent than agonist- or nerve-evoked direct activation of detrusor smooth muscle. Spontaneous activity was reduced to a maximum extent at 42°C, whilst carbachol-mediated and nerve-evoked contractions were unaffected. This is consistent with the hypothesis that spontaneous activity originates away from the detrusor, in the mucosa layer [20].

It was important to determine if heat exposure to 42, 46 or 50°C induced structural changes to the tissue, affected cytoprotective heat-shock protein (HSP) levels and/or pro-apoptotic pathways. Temperature-dependent effects on tissue structure were mainly observed at the urothelium, with a loss of structural integrity and shrinkage of nuclei at 46 and 50°C. However, the detrusor muscle layer was more resilient, with no gross changes or alterations to nuclear diameter at any temperature. The lability of the urothelium to temperature-dependent damage was not due solely to the temperature gradient (about 1°C) between this

and deeper layers as no damage was evident at 50°C in detrusor, whereas changes to the urothelium were evident at 46°C. Of importance, there was no evidence of structural damage at 42°C when spontaneous contractile activity was already reduced to a maximum extent.

Expression of proteins associated with cell protection and cell death also showed no variation at 42°C, whereas changes at 46 and 50°C were evident. Thus, phosphorylated inducible HSP70 and HSP27-pSer82 protein expression levels were unaffected at 42°C, but showed reduction between 46°C and 50°C. In addition, the pro-apoptotic protein caspase-3 was also significantly similar at 37 and 42°C but upregulated at 46 and 50°C. Such an increase in caspase-3 protein expression levels was associated with its enhanced apoptotic activity. This is evident from the significant cleavage and reduction in PARP protein expression levels at 46 and 50°C, which in turn will impact on PARP DNA-repair capacity at these temperatures only, as well as a shrinkage of nuclear diameter consistent with increased apoptosis.

The concept of quantifying the detrimental effects of heat stress through the CEM_{43} variable [9] indicates that in our study a value of about 4.0 (42°C for 15 min) that provides effective reduction of spontaneous activity does so with little evidence of tissue damage. However, a value of 120 (46°C for 15 min), whilst achieving a similar reduction of spontaneous activity is associated with heat-stress damage. Other tissues such as muscle, prostate, oesophagus, and small intestine [21-24] show threshold CEM_{43} values from <20 to >50 min.

A mechanism for heat-induced reduction of SA was hypothesised to be related to activation of $TRPV_1$ channels, as the range of temperatures used here is similar to their activation range. However, no effect of agents that block the channel or hinder the ability of other chemicals to activate the channel had any influence on the heat-induced reduction of spontaneous activity. An alternative route may be through altering the release of neuromodulators such as ATP or

acetylcholine from the mucosa as this influences spontaneous activity [25]. However, cooling from 37 to 4°C has no effect on ATP release [26], although the effect of raising temperature on modulator release has not been measured.

In conclusion, heating the bladder from 37°C to any temperature in the range 42-50°C reversibly reduces spontaneous contractile activity. However, heating to 42°C offers many advantages: the effect has a rapid onset; it is reproducible and reversible; effective for up to one hour; physiological pathways to initiate detrusor contraction are unaffected; no tissue damage is induced; and proteins associated with apoptotic or DNA repair pathways are unaffected although HSPs are still induced. The application of these observations to reduce spontaneous contractions *in vivo* remains to be elucidated.

Limitations of the study. The effect of heating on bladder function was carried out using pig organs and it is unclear to what extent this may be extended to human tissue. Heating consisted of single exposures so any cumulative and long-term effects are unknown, although in some experiments dual exposures were made. Finally heating was due to external radiant energy and it is also unknown if similar effects would be seen with other methods of delivery such as with microwaves.

Acknowledgements: We thank Boston Scientific for financial support

Conflicts of Interest: None

References

1 Drake MJ, Kanai A, Bijos DA et al. The potential role of unregulated autonomous bladder micromotions in urinary storage and voiding dysfunction; overactive bladder and detrusor underactivity. BJU Int. 2017; 119: 22-29.

2 Oger S, Behr-Roussel D, Gorny D et al. Effects of potassium channel modulators on myogenic spontaneous phasic contractile activity in human detrusor from neurogenic patients. BJU Int. 2011; 108: 604-611

3 Kinder RB, Mundy AR. Pathophysiology of idiopathic detrusor instability and detrusor hyper-reflexia. an in vitro study of human detrusor muscle. Br J Urol 1987; 60: 509–515.

4 Sui G, Fry CH, Malone-Lee J, Wu C. Aberrant Ca^{2+} oscillations in smooth muscle cells from overactive human bladders. Cell Calcium 2009; 45: 456-464.

5 Yellon DM, Pasini E, Cargnoni A, Marber MS, Latchman DS, Ferrari R. The protective role of heat stress in the ischaemic and reperfused rabbit myocardium. J Mol Cell Cardiol 1992; 24: 895-907.

6 Haveman J, Smals OA, Rodermond HM. Effects of hyperthermia on the rat bladder: a pre-clinical study on thermometry and functional damage after treatment. Int J Hyperthermia 2003; 19: 45-57.

7 Mustafa SM, Thulesius O. Cooling-induced bladder contraction: studies on isolated detrusor muscle preparations in the rat. Urology 1999; 53: 653-657.

8 Ziganshin AU, Rychkov AV, Ziganshina LE, Burnstock G. Temperature dependency of P2 receptor-mediated responses. Eur J Pharmacol 2002; 456: 107-114.

9 Yarmolenko PS, Moon EJ, Landon C et al. Thresholds for thermal damage to normal tissues: an update. Int J Hyperthermia 2011; 27: 320-343.

10 Avelino A, Cruz C, Nagy I, Cruz F. Vanilloid receptor 1 expression in the rat urinary tract. Neuroscience. 2002; 109: 787-798.

11 Nilius B, Owsianik G, Voets T, Peters JA. Transient receptor potential cation channels in disease. Physiol Rev 2007; 87:165- 217.

12 Singh K, Shandilya M, Kundu S, Kayastha AM. Heat, acid and chemically induced unfolding pathways, conformational stability and structure-function relationship in wheat α-amylase. PLoS One 2015 8; 10: e0129203.

13 Kostenko S, Moens U. Heat shock protein 27 phosphorylation: kinases, phosphatases, functions and pathology. Cell Mol Life Sci 2009; 66: 3289-3307.

14 Wang Y, Knowlton AA, Christensen TG, Shih T, Borkan SC. Prior heat stress inhibits apoptosis in adenosine triphosphate-depleted renal tubular cells. Kidney Int 1999; 55: 2224–2235.

15 Iwashita Y, Kuwabara T, Hayata M et al. Mild systemic thermal therapy ameliorates renal dysfunction in a rodent model of chronic kidney disease. Am J Physiol Renal Physiol 2016; 310: F1206-F1215

16 Tramontano F, Malanga M, Farina B, Jones R, Quesada P. Heat stress reduces poly(ADPR)polymerase expression in rat testis. Mol Hum Reprod 2000; 6: 575-581.

17 Parsons BA, Drake MJ, Gammie A, Fry CH, Vahabi B. The validation of a functional, isolated pig bladder model for physiological experimentation. Front Pharm 2012; 3: 52.

18 Cummings BS, Wills LP, Schnellmann RG. Measurement of cell death in mammalian cells. Curr Protoc Pharmacol 2004 Sep 1; 0 12: 10.1002/0471141755.ph1208s25.

19 Sugaya K, de Groat WC. Influence of temperature on activity of the isolated whole bladder preparation of neonatal and adult rats. Am J Physiol 2000; 278: R238-246.

20 Fry CH, Vahabi B. The role of the mucosa in normal and abnormal bladder function. Basic Clin Pharmacol Toxicol 2016;119 Suppl 3: 57-62.

21 Ichinoseki-Sekine N, Naito H, Saga N et al. Changes in muscle temperature induced by 434 MHz microwave hyperthermia. Br J Sports Med 2007; 41: 425–429.

22 Nau WH, Diederich CJ, Ross Abet al. MRI-guided interstitial ultrasound thermal therapy of the prostate: A feasibility study in the canine model. Med Phys 2005; 32: 733–743.

23 Melodelima D, Salomir R, Chapelon JY, Theillere Y, Moonen C, Cathignol D. Intraluminal high intensity ultrasound treatment in the esophagus under fast MR temperature mapping: In vivo studies. Magn Reson Med. 2005; 54: 975–982.

24 Lambert GP, Gisolfi CV, Berg DJ, Moseley PL, Oberley LW, Kregel KC. Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. J Appl Physiol. 2002; 92: 1750–1761.

25 Kushida N, Fry CH. On the origin of spontaneous activity in the bladder. BJU Int 2016; 117: 982-992.

26 Yu W. Polarized ATP distribution in urothelial mucosal and serosal space is differentially regulated by stretch and ectonucleotidases. Am J Physiol Renal Physiol 2015; 309: F864-872.

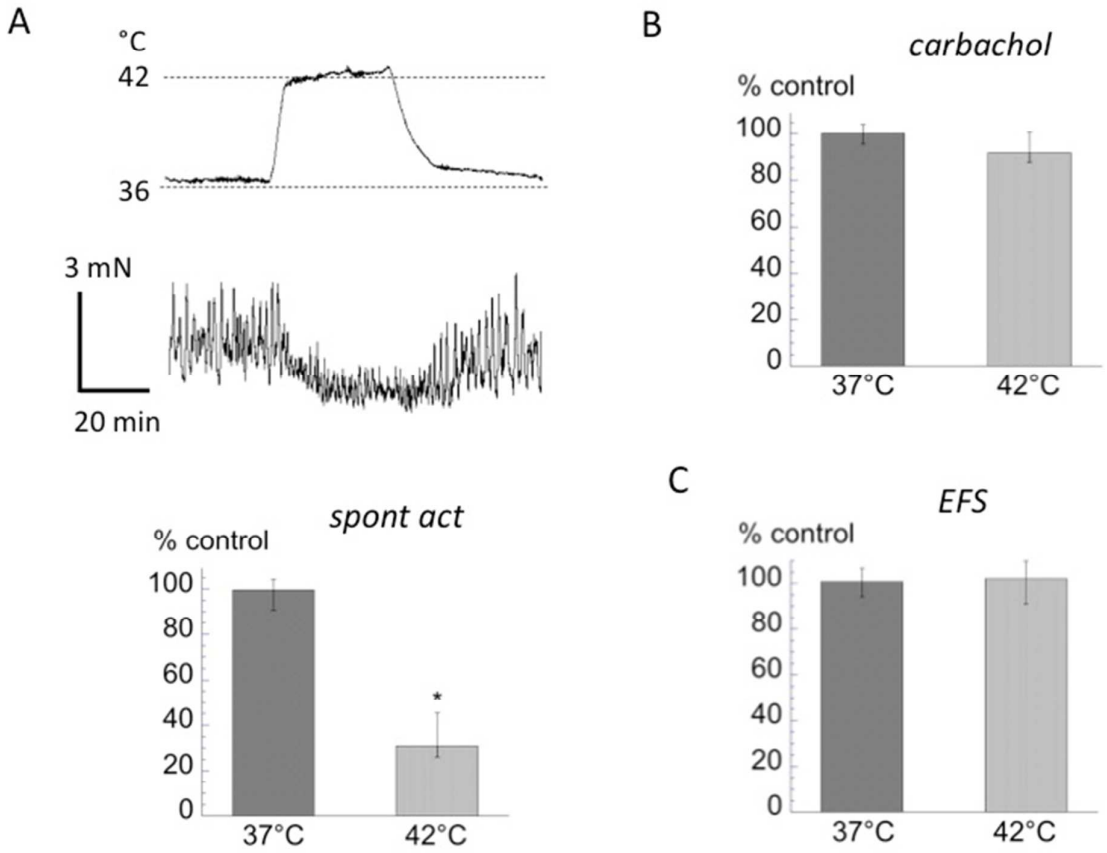

Figure 1. Effect of heating to 42°C on bladder wall contractions. A: lower trace, spontaneous contractions at 37 and 42°C; upper trace, output from a thermistor probe at the mucosa/detrusor boundary. Bar chart: tension integral (AUC) at 42°C as a proportion of that at 37°C. B: magnitude of the carbachol (1 μM) contracture at 37 and 42°C. C: magnitude of the nerve-evoked response (8 Hz stimulation) at 37 and 42°C. All data at 42°C are normalised to the pre-control average (=100%). Median values (25, 75% interquartiles). * $p<0.01$ *vs* control, $n=10$.

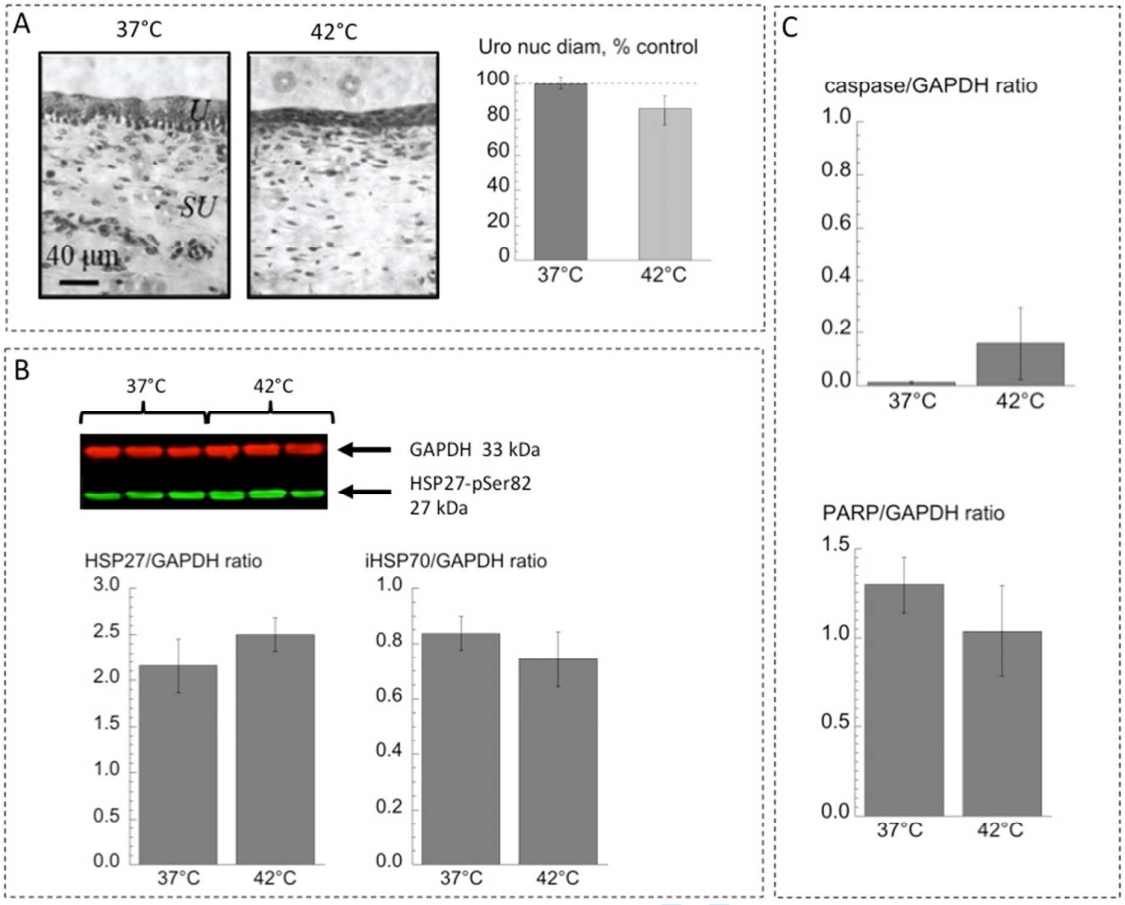

Figure 2. Heating to 42°C on bladder wall histology and protein expression. A: Haematoxylin and eosin stained section of the bladder wall at 37 and 42°C. Bar-chart shows urothelium nuclear diameter at 37 and 42°C. U = urothelium; SU = suburothelium. B: Expression levels of heat-shock proteins HSP27-pSer82 and iHSP70 at 37 and 42°C. The inset shows sample protein blots for the phosphorylated HSP27 and the housekeeping protein GAPDH. C: Expression levels of caspase and PARP, normalised to GAPDH expression. Mean values ± SD.

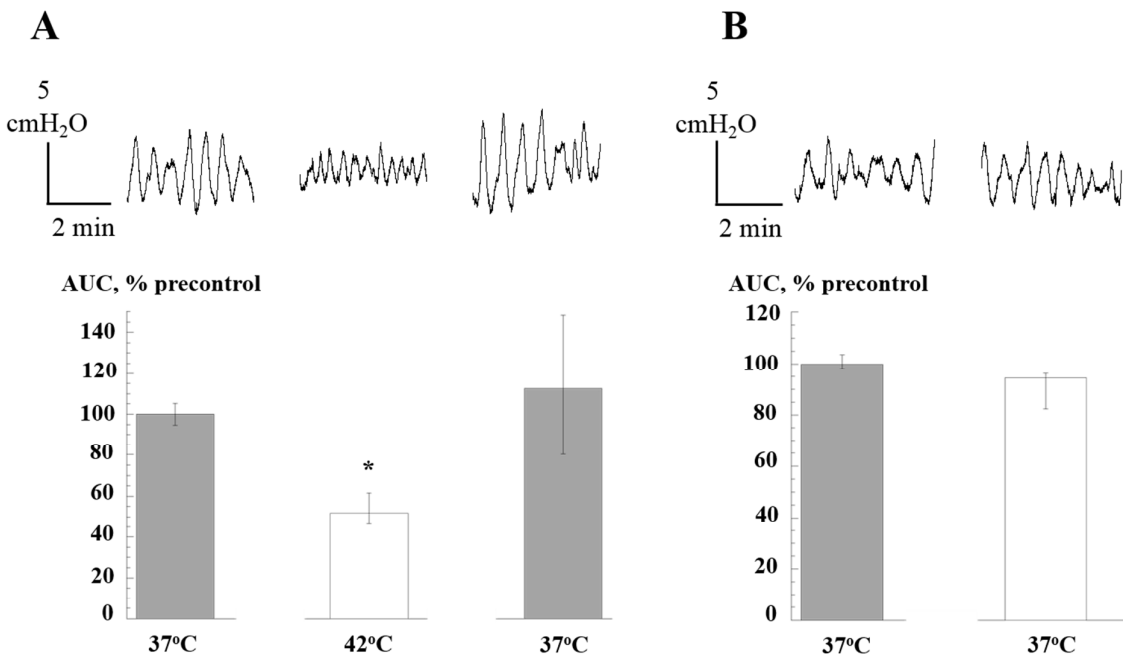

Figure 3. Effect of heating to 42°C on intravesical spontaneous pressure transients in perfused pig bladder. A: Records: examples at 37 and 42°C. Bar charts: AUC values, *n*=6. B: time control of changes to pressure transients over the time course of the experiment in A. AUC values normalised to the pre-control values. Median values (25, 75% interquartiles), *n*=6. * $p<0.05$ *vs* control.

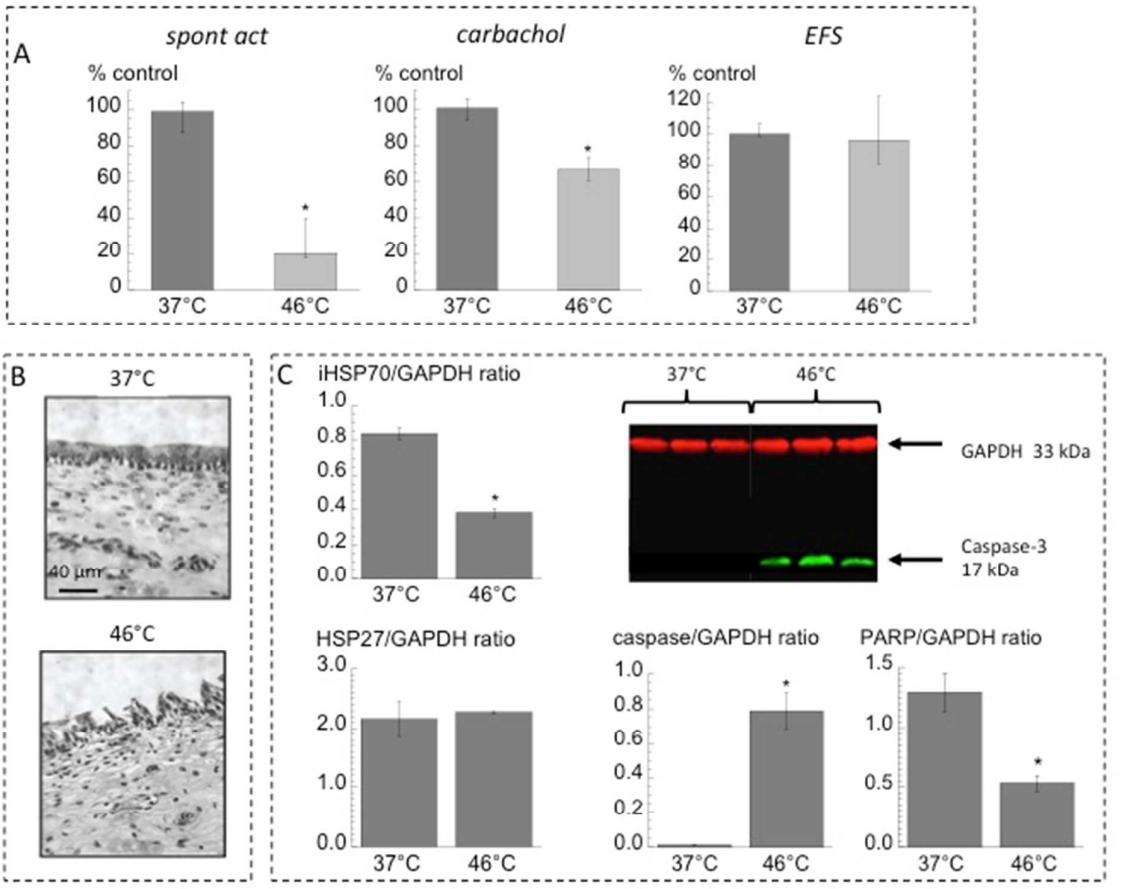

Figure 4. The effect of heating to 46°C on contractile function, tissue structure and protein expression. A: Spontaneous activity (AUC, left), carbachol contractions (middle) and nerve-evoked responses (right) at 37 and 46°C. Data at 46°C are a percentage of the average pre-and post-control values. B: Haematoxylin and eosin stained sections of the bladder wall at 37 and 46°C. C: Expression of the heat-shock proteins iHSP70 and HSP27, normalised to GAPDH expression, at 37 and 42°C (left) and of caspase-3 and PARP (right). The inset shows sample protein blots for caspase-3 and the housekeeping protein GAPDH. Mean values ± SD, *n*=6. * $p<0.05$ *vs* control.