

THE EFFECT OF HEATING (37-41°C) ON DETRUSOR CONTRACTILE FUNCTION IN RABBIT MUCOSA-INTACT AND DENUDED PREPARATIONS.

Hypothesis / aims of study

The bladder may be exposed to small heat changes from either internal pathological conditions or external devices that deliver microwave therapies for cancer. This study addressed the effects of mild heating on bladder contractility. The aims of this study were to determine if: 1) heating from 37 °C to 41-43 °C for prolonged periods (15 minutes) or repeated short exposures (1 minute cycles) altered spontaneous activity of the mucosa-intact bladder 2) heating had a direct effect on detrusor muscle contractility or activation of motor nerves and 3) heating affects the morphology of the bladder wall.

Study design, materials and methods

Two male and ten female rabbits (4.5±0.6 kg; Novartis Pharmaceuticals UK Limited) were sacrificed with an overdose of Na-pentobarbitone. The urinary bladder was placed in gassed (95% O₂ and 5% CO₂) and chilled (4°C) Tyrode's solution (composition, mM: NaCl 114, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, glucose 11.7) and the ventral wall opened longitudinally. Strips of rabbit mucosa-removed and mucosa-intact preparations were approximately 10 x 2 mm², and cut along the craniocaudal axis.

Strips were mounted on a horizontal superfusion trough and tied to an isometric tension transducer (FT03D; Grass instruments, USA) connected to bridge amplifier (TBM-4M; WPI, UK). A thermocouple (0.2 mm diameter; OMEGA Engineering Inc, UK) was inserted into the sub-urothelial layer, and the preparation stretched to 20 mN. Preparations were continuously superfused with gassed Tyrode's solution at approximately 37°C. For mucosa-intact strips a heating device was positioned <5 mm above the mucosa, perpendicular to the preparation and calibrated prior to the main experiment. Mucosa-removed preparations were exposed to electrical field stimulation (EFS: 0.1 ms pulses, 8Hz) or 0.1 µM carbachol (CCH) whilst the Tyrode's solution was heated from 37 °C to 41 °C and back down to 37 °C. The EFS voltage was that required to elicit 70 % maximal contraction. Spontaneous contraction amplitude and frequency were measured during prolonged (15 min) heat exposures; the integral of contractile activity (area under curve, AUC) was recorded during short heat exposures. Data are medians [25, 75% quartiles]; differences between data sets were tested with Mann-Whitney U-tests, the null hypothesis was rejected when p<0.05.

To determine if local heating by the coil caused alteration to the structure of the underlying tissue, tissues were fixed after the physiological experiments in 10% neutral buffered formalin. Fixed tissue underwent a sequence of dehydration processes and infiltrated with paraffin wax and embedded. Sections (5 µm) were cut and transferred onto slides. The slides were stained with haematoxylin and eosin (H&E) and photographed. The image was analysed by ImageJ to quantify any differences in the histology of the sections between the two conditions.

Results

1) During 15 minutes of exposure to 41.3±1.0°C the rabbit mucosa-intact preparations exhibited significantly reduced spontaneous contraction amplitude when compared to control preparations maintained at 36.6±0.7°C (p<0.05, one-tailed unpaired t-test). Post-heating, the amplitude of spontaneous contractions returned to control within 30 minutes (Table 1). During this recovery period there was a reduction in the frequency of contractions in the heated preparations in comparison to the control preparations.

Table 1. Percentage medians (75%, 25% quartiles) of spontaneous contraction amplitude and frequency before (baseline) and during heating to 41°C for 15 minutes and after 30 mins recovery. The control experiment was similar except that the heating coil was not activated. *p<0.05 vs baseline. **p<0.01 vs baseline.

	Heat (41 °C)				Control (37°C)			
	Contraction Amplitude				Contraction Amplitude			
	Median (%)	25%	75%	n	Median (%)	25%	75%	n
Baseline	100.0	100.0	100.0	9	100.0	100.0	100.0	8
Heat	82.5 *	48.0	94.1	9	93.8	83.2	118.5	8
Recovery	94.8	89.7	125.6	9	99.3	83.7	107.9	8
	Frequency				Frequency			
	Median (%)	25%	75%	n	Median (%)	25%	75%	n
	Baseline	100.0	100.0	100.0	9	100.0	100.0	100.0
Heat	98.4	90.2	116.9	9	106.1	97.0	114.8	8
Recovery	90.6 **	81.9	93.6	9	108.5	98.8	114.3	8

Mucosa-intact preparations (n=7) were exposed to eight heating cycles (43.1±1.0°C; one-minute on, one-minute off). Six of the preparations exhibited a decline in AUC of contractions during the heating cycles. In four other preparations capsazepine (TRPV₁ antagonist, 3 µM), was added during the heating cycles. In three preparations the effect of heating on AUC was attenuated by capsazepine.

2) The contraction amplitude of mucosa-removed preparations during EFS at 8 Hz was 2.5 (1.4, 4.8) mN at 37°C (n=5). When Tyrode's solution was heated to 41°C EFS contraction amplitude was not significantly altered (2.1 [1.0, 5.3] mN) and upon return to 37°C contraction amplitude remained similar to baseline (2.9 [1.5, 5.6] mN).

Carbachol (CCH; 0.1 μ M) generated a rise in contraction tone of mucosa-removed preparations at 37°C and 41°C (n=5). The magnitude of the CCH-induced contractions was greater at 37.1 \pm 0.3°C (1.1 [0.3, 3.1] mN) compared to 41.4 \pm 0.5°C (0.7 [0.1, 1.3] mN). Upon return to control temperature (37.4 \pm 0.2°C), the CCH-induced contracture recovered to 113 % of the original value (1.3 [0.4, 2.9] mN).

3) There was no significant difference in the urothelium width, sub-urothelium cellular density or the muscle content between the heated (41°C) and control (37°C) tissues.

Interpretation of results

Local heating suppressed spontaneous activity of mucosa-intact tissue and global heating suppressed CCH (0.1 μ M)-induced contraction of mucosa-removed tissue. The EFS contraction amplitude did not significantly alter during and post-heating, indicating there was no evidence of nerve block. An effect of heat on CCH-induced contracture implies a detrusor specific action of heating.

The data indicate that the suppression of spontaneous contractions on heating may be mediated by TRPV1 receptors. There was no effect of heat on the integrity of the tissue.

Concluding message

Mild heating of mucosa-intact and denuded preparations reduced the amplitude of spontaneous and agonists-evoked contractions. These data suggest an effect directly on detrusor smooth muscle.

Disclosures

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