

## THE INFLUENCE OF HEATING (37-50 °C) ON SPONTANEOUS CONTRACTILE ACTIVITY OF ISOLATED BLADDER

### Hypothesis / aims of study

To quantify the effect of graded heating between 37 and 50 °C on spontaneous contractile function in isolated pig bladder tissue. We tested the hypothesis that heating reduces detrusor contractile function.

### Study design, materials and methods

Pig bladders were obtained from the local abattoir, stored in ice-cold Ca-free Tyrode's solution and transported immediately to the laboratory for use within one hour. Segments of bladder wall ( $\approx 4 \text{ cm}^2$  square), with an intact mucosa, were dissected and superfused with Tyrode's solution at 36°C. Opposite sides were clamped to an anchor or to an isometric force transducer to record spontaneous contractions. Tyrode's solution contained (mM): NaCl, 118; KCl, 4.0; CaCl<sub>2</sub>, 1.8; MgCl<sub>2</sub>, 1.0; NaHCO<sub>3</sub>, 24; NaH<sub>2</sub>PO<sub>4</sub>, 0.4; glucose, 6.1; Na pyruvate, 5.0; pH 7.4 with 5%CO<sub>2</sub>, 95%O<sub>2</sub>. ADP (30  $\mu\text{M}$ ) was added to the superfusate to augment spontaneous contractions in some preparations; actions described below were similar in the presence and absence of ADP. Portions of the preparation were heated with a heating coil placed immediately above the preparation. The temperature of the preparation was measured with a 100  $\mu\text{m}$  thermistor probe placed between the detrusor and mucosa layers. Temperature was raised to 40, 42, 44, 46, 48 and 50°C for 15-30 minutes followed in each case by return to 36°C. Contractile parameters measured were: base-line tension, magnitude of spontaneous contractions average over a ten minute period; frequency of spontaneous contractions. Samples of tissue were fixed in 4% formaldehyde, dehydrated and infiltrated with paraffin wax and embedded. Sections (5  $\mu\text{m}$ ) were cut, transferred onto slides, 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 preparations that had been heated or kept at 36°C. Data are recorded as mean $\pm$ SD values, differences between tested were tested with Student's *t*-tests and the null hypothesis was rejected when  $p<0.05$ .

### Results

Heating the bladder preparations had variable effects on spontaneous contractions and data are summarised on Figure 1. Heating to 42°C significantly reduced the amplitude of contractions. The frequency of contractions was increased as was the resting tension. Baseline tension did not return to control value, but other changes were reversible upon return to 36°C.

#### % control

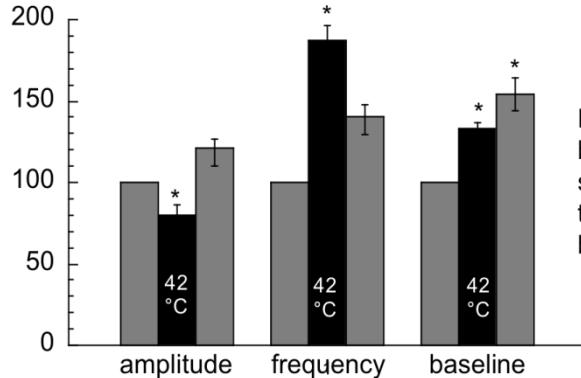


Figure 1. Effect of heating to 42°C (dark bar) on the amplitude and frequency of spontaneous contractions and baseline tension. The values at 36°C are shown before and after the intervention. \* $p<0.05$

Heating to 46°C generated different results. Spontaneous contraction amplitude was reversibly increased and there was a similar reversible increase of frequency. However, baseline tension increased greatly and was not reversible upon return to control temperature for up to 30 minutes (Figure 2). The increase of base-line tension was in turn reversed by addition of caffeine (10 mM) to the superfusate.

#### % control

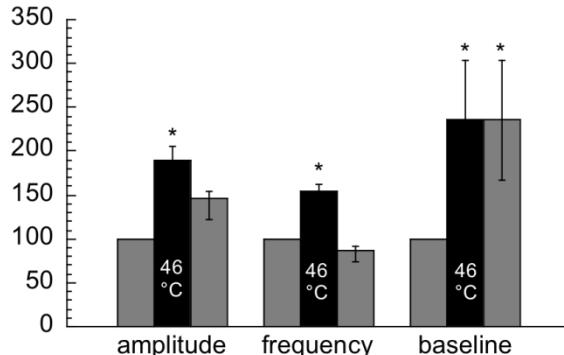


Figure 2. Effect of heating to 46°C (dark bar) on the amplitude and frequency of spontaneous contractions and baseline tension. The values at 36°C are shown before and after the intervention. \* $p<0.05$

At 50°C, spontaneous contraction amplitude was reversibly increased about three-fold and frequency also reversibly increased. In contrast to an increase to 46°C there was no significant change of base-line tension (Figure 3).

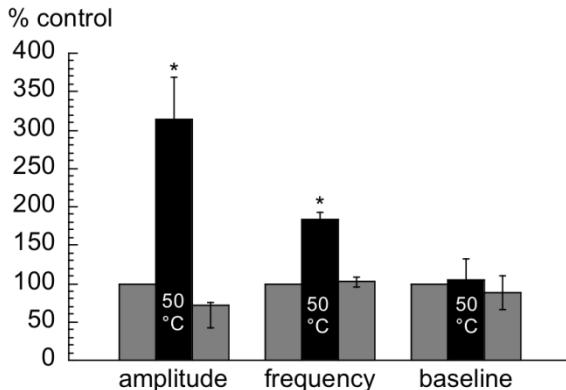


Figure 3. Effect of heating to 50°C (dark bar) on the amplitude and frequency of spontaneous contractions and baseline tension. The values at 36°C are shown before and after the intervention. \* $p<0.05$

There was no significant difference in the urothelium width, sub-urothelium cellular density or the muscle content between the heated tissues and those maintained at 37°C over similar time periods.

#### Interpretation of results

Increasing local heat over the range 36-50°C on the bladder wall exerted complex effects on the spontaneous contractility of the tissue. With relatively mild heating, up to 42°C, spontaneous contractions were reduced, but between 46 and 50°C there was progressive augmentation of contractility. However, heating over this range progressively increased base-line tension only between 42 and 46°C. The frequency of spontaneous contractions was increased by heating by the same rate over the entire temperature range. The different temperature-dependencies of these phenomena suggest that different mechanisms are altered by heating the tissue.

#### Concluding message

Heating the bladder wall may be a simple method to alter its contractile function. However, careful attention must be given to the temperature range as to whether contractile function is increased or decreased.

#### Disclosures

**Funding:** Boston Scientific **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Pig **Ethics not Req'd:** Experiments were conducted on isolated bladders obtained from the local abattoir, i.e. tissue was obtained from dead animals and no ethical committee approval or Home Office licence is required