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THE PHYSIOLOGICAL PROPERTIES OF THE DECOMPENSATED, SPINAL CORD INJURED BLADDER

Hypothesis / aims of study

Urinary retention is a significant clinical problem with a complex morbidity and is associated with bladder obstruction or neurological deficits. Retention is associated with high or low detrusor pressures [1], as co-morbidites such as urinary tract infections and more serious consequences such as renal failure are present, especially if pressure is high. However, low pressure retention is less amenable to surgical correction and persists longer. Voiding is difficult in the low pressure group and is commonly attributed to reduced detrusor contractility [1,2]. However the pathogenesis of low-pressure retention is unknown and it is important to determine if it is failure of muscle contractility or a remodelling of tissues within the bladder wall. The aim of this study was to address this question in a rat model where bladder obstruction through spinal cord injury was utilised to generate a large, low-pressure bladder.

Study design, materials and methods

Adult female Sprague-Dawley rats following spinal cord injury (SCI, T8 laminectomy) or sham-operated controls were used. Procedures were conducted under under isoflurane anaethesia (2%, in O_2, 0.6 l/min). Animals were assisted with bladder voiding for two weeks by abdominal compression twice daily until micturition reflexes recovered and injected daily, for the first week with gentamicin (2 mg/kg). After five weeks rats were weighed, sacrificed by cervical dislocation and exsanguination; the bladder was removed, weighed and stored in Ca^{2+}-free Krebs at 4°C. Whole bladders were catheterised, ex vivo, and ligated around the urethra and bladder neck. Bladders were filled with Krebs at 12 ml/h, up to an intravesical pressure of 30 mmHg, or to when leakage around the catheter was observed; pressure and volume were monitored and transformed into tension-length relationships, the slopes of which were a measure of bladder wall stiffness.

Bladder strips (≤1 mm diameter) were cut from the dorsal wall along the neck-dome axis after removal of the mucosa. Strips were mounted in a 5 ml organ bath under a tension of 10 mN and isometric contractions (spontaneous or agonist-evoked) recorded. Preparations were superfused with Ca-containing Krebs and equilibrated for 90 mins prior to each experiment. Contractile agonist was added to the superfusate from a stock aqueous solution. Strip weight was recorded at the end of the experiment. Strips were stretched rapidly (<0.5 s) to 10 mN and the decline of force analysed by a single exponential function with non-zero asymptote. Spontaneous contractions were analysed by measuring the average contraction magnitude and frequency over a 10-minute period using a custom-written macro. Fast-Fourier transforms of the data were also carried out to identify the dominant frequency based on a Lorentzian fit to an amplitude vs frequency plot. Data are quoted as medians [25%, 75% interquartiles, Q1 and Q3] and shown as values from SCI vs control groups respectively; differences between groups were tested using paired or unpaired non-parametric tests; the null hypothesis was rejected at $p<0.05$. Variability of spontaneous contraction amplitude was estimated by calculating the quartile deviation of the data, i.e. (Q3-Q1)/(Q3+Q1). Power calculations estimated n≥6 repeats sufficient to detect a 40% change with 80% power.

Results

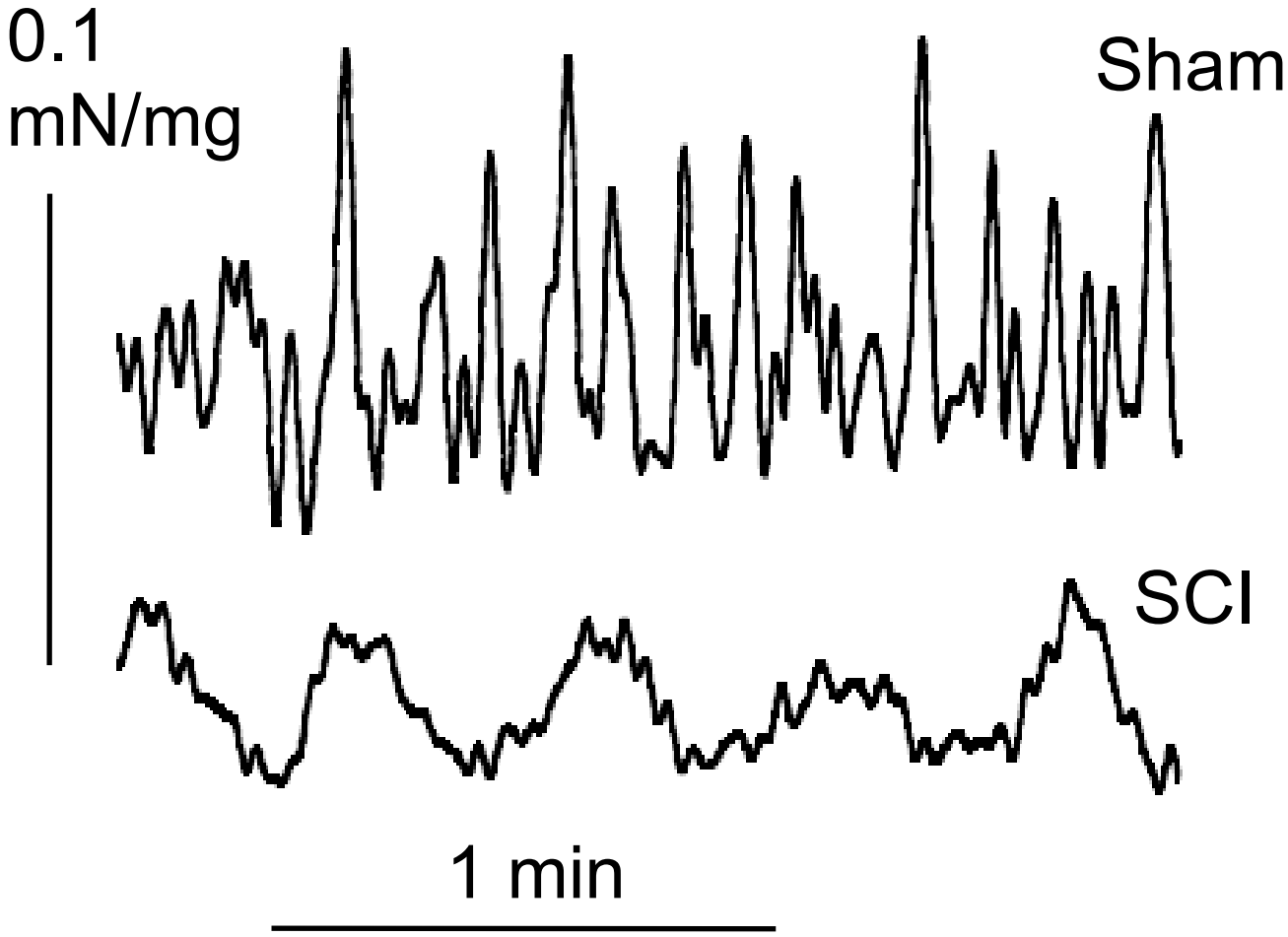
Spinal cord injury was associated with an increase of bladder-to-body weight ratio - 1.09 [0.94, 1.22] vs 0.34 [0.33, 0.35] mg/g, $P < 0.0001$. The tension-length relationship derived from the ex vivo pressure-volume relationship was significantly less in the SCI group ((161 [90, 198] vs 560 [277,764] N/m^2), interpreted as a decrease of passive bladder wall stiffness: i.e. bladders from SCI animals were decompensated. Rapid stretch of detrusor strips revealed a decline of tension, that was more rapid in those from SCI animals: the time constant of relaxation was (9.9 s [5.1,12.5] vs 29.5 s [16.7, 30.2]). The extent of relaxation was similar in both groups. Spontaneous contractions in strips from SCI bladders were characterised by slow, long-duration contractions (Figure 1A). The dominant frequency from the Fast-Fourier transform (Figure 1B, Table 1) of tension-time data showed the frequency of SCI strips was 0.051 Hz [0.039, 0.067] vs control (0.112 Hz [0.084, 0.139]). The amplitude of spontaneous contractions was smaller in the SCI group but less variable (Table 1).

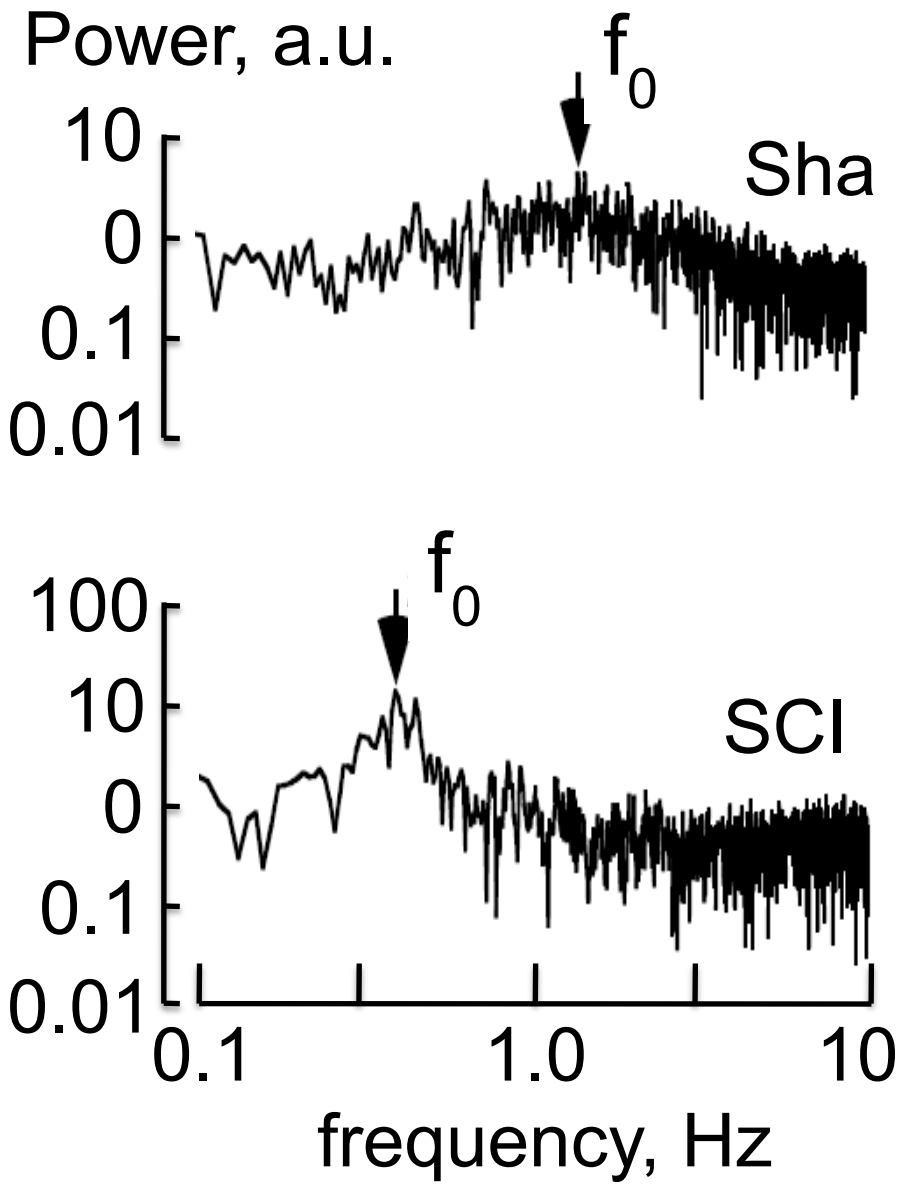
The reduced amplitude of spontaneous contractions may result either from i) impaired contractility or ii) an inability of the muscular tissue to generate force in the preparation due to the reduced passive stiffness of the tissue. The final column of Table 1 shows spontaneous contraction amplitude normalised to the passive stiffness of the tissue. In this case the SCI tissue demonstrated greater contraction strength compared to control. A further test for impaired contractility or otherwise was tested by exposure of strips to the contractile agonist GSK1016790A. SCI strips showed a significant augmentation of contraction amplitude (135% [68, 237], $p<0.01$). However, this was absent in control strips (24% [3, 129], $p>0.05$), a further indication that contractility was not impaired in tissue from SCI bladders.

Table 1. Contractile characteristics of spontaneous contractions of detrusor from SCI and control bladders *p<0.05

	Frequency, Hz	Amplitude, mN/mg	Amplitude variability	Amplitude/stiffness coeff, mN
Sham-operated	0.112 [0.084, 0.139]	0.092 [0.044, 0.124]	0.095 [0.052, 0.137]	8.5 [4.0, 10.5]
SCI	0.051 [0.039, 0.067] *	0.040 [0.032, 0.065] *	0.047 [0.028, 0.065] *	23.5 [17.5. 52.0] *

A B C


0.1
mN/mg
Sham
SCI
1 min


Power, a.u.
f0
10
0
0.1
0.01
Sha
f0
100
10
0
0.1
0.01
SCI
0.1
1.0
10
frequency, Hz

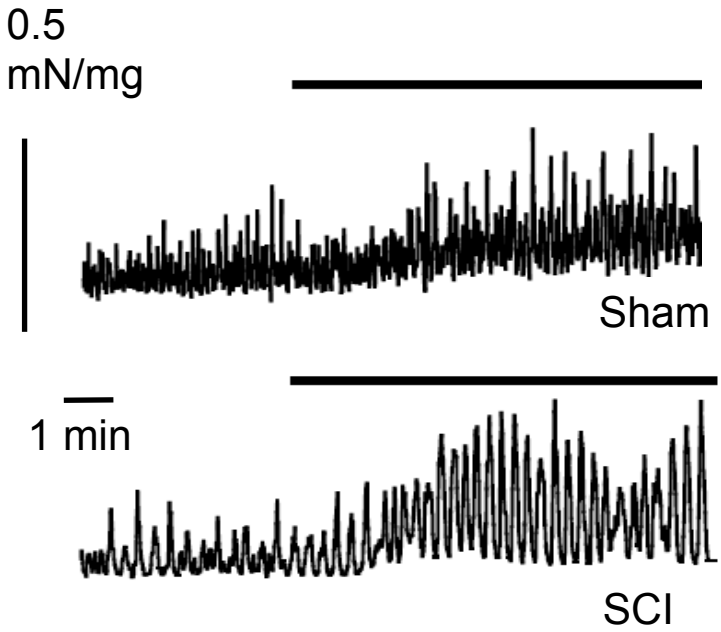

0.5
mN/mg
Sham
1 min
SCI

Figure 1 Contractile characteristics of SCI detrusor from SCI and sham-operated rat bladders. A: spontaneous activity. B: Fourier transform of the spontaneous activity data, the frequency, f_0, at which the spontaneous contractions most frequently occur is shown for each spectrum. C: Augmentation of spontaneous activity by the contractile agonist GSK1016790A (0.1 µM).

Interpretation of results

Bladders from rats subjected to spinal cord injury, and presumed to be obstructed, demonstrated a decompensated phenotype during ex vivo filling to determine the pressure-volume relationship. This would be equivalent to the low-pressure bladder exhibiting urinary retention in humans. The pattern of spontaneous activity in isolated preparations was indicative of an overactive phenotype of less frequent but prolonged spontaneous contractions, however contraction amplitude was reduced. The smaller contractions were unlikely to be due to impaired detrusor contractility as: i) the contractions were greater than control when normalised to the passive stiffness properties of the tissue and were able to respond more to a contractile agonist. We propose that the reduced contraction amplitude in SCI bladders is likely to be due to the inability of contracting muscle to develop force in a multicellular preparation due to the decreased stiffness of the extracellular matrix

Concluding message

The decompensated, low pressure phenotype resulting several weeks after spinal cord injury is not due to impaired contractility of the detrusor muscle but an inability of the tissue to distribute tension due to decreased stiffness of the non-muscular component.

References

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Pfizer (Sandwich) animal care committee**