The role of the urothelium in potassium sensitivity testing in the clinic.

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Introduction
Potassium sensitivity testing (high K+) for diagnosis in patients with bladder disorders is considered predictive of increased urothelial permeability. As such, high K+ as it diffuses across a leaky urothelium, is considered to directly depolarize sensory nerve endings. However, we hypothesised that high K+ may act at the level of the urothelium to release mediators that modulate afferent sensitivity indirectly.

Aim
The aim of this research was to understand the effect of urothelial mediator release on afferent nerve firing, with the hypothesis that stimulating the release of mediators from the urothelium will alter afferent nerve sensitivity.

Methods
Mouse bladder afferents were recorded (fig 1) during perfusion with isotonic NaCl or a high K+ solution (50mM KCl/100mM NaCl). The urothelium was chemically denuded with proteamine sulphate (PS, 10mg/ml, 1 hour) and the afferent response to bladder distension with high K+ was monitored. The response to high K+ was also examined in the presence of an inhibitory cocktail designed to block nitric oxide (L-name, 1mM), prostaglandins (indomethacin, 50µM), acetylcholine (atropine, 10µM), ATP (suramin, 500µM), and endocannabinoids (SR141716A, 10µM + SR144528, 10µM). Afferent firing was quantified in spikes/s. Statistical significance was verified using 1-way ANOVA.

2. Intravesical installation of high K+ inhibited afferent nerve firing.

![Image](image1.png)

A) Sample trace showing the attenuation of afferent firing in response to 30 minutes intravesical installation of high K+ solution. i) afferent nerve firing frequency histogram, ii) intravesical pressure of the bladder.

B) Intravesical application of high K+ solution for 30 minutes significantly inhibited mechanosensitivity (P<0.0001, 2-way ANOVA with Bonferroni post-test, n=8).

C) Spontaneous nerve firing was significantly decreased by 30 minutes high K+ solution installation (P=0.03, 1-way ANOVA with Bonferroni post-test, n=10).

D) Bladder compliance remained unchanged in the presence of high K+ solution (P=0.05, 2-way ANOVA with Bonferroni post-test, n=8).

3. Urothelial removal with proteamine sulphate abolishes the inhibitory effect of high K+ on mechanosensitivity.

![Image](image2.png)

A) Sample trace showing the excitation of nerve firing in response to intravesical installation of high K+ solution, following urothelial removal with PS, i) afferent nerve firing frequency histogram, ii) intravesical pressure of the bladder.

B) Intravesical application of high K+ solution following urothelial removal, significantly increased mechanosensitivity (P=0.003, 2-way ANOVA with Bonferroni post-test, n=6).

C) After urothelial removal, spontaneous nerve firing was significantly reduced by 30 minutes high K+ solution installation (P=0.003, 1-way ANOVA with Bonferroni post-test).

D) Bladder compliance remained unchanged in the presence of high K+ solution following removal of the urothelium (P=0.05, 2-way ANOVA with Bonferroni post-test, n=6).

4. The inhibitory effect of high K+ on mechanosensitivity is attenuated in the presence of the inhibitory cocktail.

![Image](image3.png)

A) Sample trace showing the small decrease in afferent nerve firing in response to intravesical installation of high K+ solution in the presence of the inhibitory cocktail, i) afferent nerve firing frequency histogram, ii) intravesical pressure of the bladder.

B) In the presence of the inhibitory cocktail, intravesical application of high K+ solution significantly decreased mechanosensitivity, but to a lesser extent than the inhibition seen by installation of high K+ solution alone (P=0.0076, 2-way ANOVA with Bonferroni post-test, n=6).

C) Spontaneous nerve activity was unaffected by the installation of high K+ solution plus the inhibitory cocktail (P=0.05, 1-way ANOVA with Bonferroni post-test, n=6).

D) Bladder compliance remained unchanged in the presence of high K+ solution plus the inhibitory cocktail (P=0.05, 2-way ANOVA with Bonferroni post-test, n=6).

Summary: Percentage inhibition of mechanosensitivity

<table>
<thead>
<tr>
<th>Factor</th>
<th>Inhibition (area under the curve)</th>
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<tbody>
<tr>
<td>Saline</td>
<td>0%</td>
</tr>
<tr>
<td>50mMKCl</td>
<td>100mM KCl</td>
</tr>
<tr>
<td>0%</td>
<td>40%</td>
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Conclusion
- High K+ stimulates the release of urothelial factor(s) that down-regulates bladder afferent sensitivity.
- Urothelial removal with PS abolishes this inhibitory response; unmasking an excitatory effect.
- Inhibition of inhibitory mediators, with the inhibitory cocktail reduces the inhibitory response of high K+ instillation.
- High K+ exposure does not affect bladder compliance.
- Spontaneous nerve firing is significantly inhibited by high K+ exposure, a phenomenon that is unaffected by denudation of the urothelium suggesting that spontaneous nerve firing and mechanosensitivity are not mediated by the same mechanisms.
- The potassium sensitivity test in patients may reveal information on the ability of the urothelium to modulate afferent sensitivity, rather than a simple test of permeability.
- Further exploitation of the urothelial/neuronal inhibitory pathways offers an exciting, new direction for therapy of bladder pathology.

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