

## HYDROGEN PEROXIDE ACTIVATES HIGH THRESHOLD INTRAMURAL VASCULAR AFFERENTS VIA TRPA1 BUT NOT TRPV1 CHANNELS IN THE GUINEA PIG BLADDER

### Hypothesis / aims of study

There is an increasing body of evidence that ischemia/reperfusion injury, which is largely attributable to effects of reactive oxygen species (ROS), contributes to the initiation of many bladder dysfunctions including overactive bladder induced by bladder outlet obstruction. Focal regions of hypoxia are well documented in animal models of bladder outlet obstruction (1). It has been also shown that intravesically applied hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, ~10 mM) evoked bladder overactivity, most likely acting on capsaicin-sensitive afferents in the bladder (2). However, it is still unclear which particular class of sensory neurons are activated by ROS and what are their major targets. The aim of this study was to determine the effect of oxidative stress induced by hydrogen peroxide on the major classes of bladder afferents and its mechanism of action on sensory neurons.

### Study design, materials and methods

'Close-to-target' single unit extracellular recordings were made from fine nerve trunks of pelvic nerves entering the guinea pig bladder in flat sheet preparations *in vitro* as previously described (3). The major classes of bladder afferents were distinguished on the basis of their responses to isotonic stretch (1-40 g), von Frey stroking (10-50 mg) and capsaicin sensitivity. All experiments were performed in the presence of nifedipine (4 μM). H<sub>2</sub>O<sub>2</sub> was applied either by adding into a small chamber, which was sealed above marked receptive field area of particular afferent, or by direct application into the bath. If an afferent unit was spontaneously active, its spontaneous firing rate was subtracted from overall responses evoked by H<sub>2</sub>O<sub>2</sub> and other drugs. Results are expressed as means ± SEM. The use of "n" numbers refers to the number of afferent units and N to the number of animals.

### Results

Four major classes of bladder afferents were recorded in this study: capsaicin-insensitive low threshold stretch-sensitive muscular and muscular-urothelial afferents and capsaicin-sensitive stretch-insensitive urothelial and high threshold intramural 'vascular' afferents, which were previously described in detail (3). Local application to the receptive field or bath application of H<sub>2</sub>O<sub>2</sub> (300-500 μM for 2 min) activate the majority of capsaicin-sensitive (70%, 24 out of 36 units, N=22) urothelial and high threshold vascular afferents but not low threshold muscular or muscular-urothelial afferents (N=14).

The mechanism of action H<sub>2</sub>O<sub>2</sub> on high threshold capsaicin-sensitive vascular afferents was studied in detail in urothelium-free bladder preparations. Bath application of H<sub>2</sub>O<sub>2</sub> induced concentration-dependent (30-1000 μM, N=7) activation of high threshold mechanoreceptors, with threshold between 30 and 100 μM. H<sub>2</sub>O<sub>2</sub> (300 μM for 2 min) evoked reproducible regular bursting firing of high threshold vascular afferents of  $0.78 \pm 0.2$  Hz (n=21, N=14). In most cases, both H<sub>2</sub>O<sub>2</sub> and TRPA1 agonist, mustard oil, activated the same afferents. Mustard oil produced concentration-dependent activation of high threshold vascular afferents with EC<sub>50</sub> = 55.2 μM (n=6, N=4).

Bath application of TRPA1 antagonist, HC-030031 (10 μM for 30 min), inhibited H<sub>2</sub>O<sub>2</sub> (300 μM)-induced activation of vascular afferents by  $65 \pm 8\%$  (n=11, N=7, P<0.05). In contrast, TRPV1 antagonist, capsazepine (10 μM for 30 min) did not affect the action of H<sub>2</sub>O<sub>2</sub> on vascular afferents ( $+1 \pm 15\%$ , n=5, N=3). Capsaicin application (0.5 μM for 30 s) evoked activation of high threshold vascular afferents with mean firing of  $3.6 \pm 0.7$  Hz (n=12, N=8). The activation of these afferents during second application of capsaicin (after 30 min of washing) was reduced by  $16 \pm 5\%$  (n=5, N=4, P<0.05). Capsaicin-induced activation of vascular afferents was also inhibited by HC-030031 by  $27 \pm 4\%$  (n=4, N=3, P<0.05). However, this inhibition was not different (P=0.1, NS) from the decline seen during second application of capsaicin alone. In contrast, application of capsazepine abolished the effect of second application of capsaicin (by  $99.6 \pm 1\%$ , n=7, N=4).

The role of other ROS (in particular, hydroxyl radical) in the activation of vascular afferents produced during H<sub>2</sub>O<sub>2</sub>-induced oxidative stress was determined by using dimethylthiourea (a hydroxyl radical scavenger) and deferoxamine (an iron-chelator that prevents formation of hydroxyl radical). Unexpectedly, dimethylthiourea (10 mM) itself evoked activation of capsaicin-sensitive vascular afferents with mean firing of  $0.63 \pm 0.29$  Hz (n=8, N=5). While in 5 out of 8 units dimethylthiourea reduced H<sub>2</sub>O<sub>2</sub> (300 μM)-induced activation, overall it did not significantly affect H<sub>2</sub>O<sub>2</sub> induced responses (n=8, N=5, P=0.34, NS). Deferoxamine (1 mM for 30 min) reduced the H<sub>2</sub>O<sub>2</sub>-produced responses by  $49 \pm 14\%$  (P<0.05, n=6, N=4). Prostaglandin synthesis inhibitor, dexametopfen (3 μM for 45 min) inhibited spontaneous activity and H<sub>2</sub>O<sub>2</sub> (300 μM)-induced activation of high threshold vascular afferents by  $68 \pm 13\%$  (n=4, N=3, P<0.05) and  $74 \pm 13\%$  (n=6, N=4, P<0.05), respectively. Application of superoxide anion radical generator, pyrogallol (1 mM for 2 min) produced activation (mean firing  $0.83 \pm 0.4$  Hz, n=4, N=3) of the same afferents that were activated by H<sub>2</sub>O<sub>2</sub> although with a longer latency.

### Interpretation of results

Our results indicated that H<sub>2</sub>O<sub>2</sub> strongly activates majority of capsaicin-sensitive urothelial and high threshold vascular afferents in the guinea pig bladder *in vitro* but not low threshold stretch-sensitive mechanoreceptors. The activation of high threshold intramural vascular afferents by H<sub>2</sub>O<sub>2</sub> is mediated via TRPA1 but not TRPV1 channels since TRPA1 but not TRPV1 channels antagonists reduced its effect. It is likely that during oxidative stress produced by H<sub>2</sub>O<sub>2</sub> application, in addition to direct activation of afferents by H<sub>2</sub>O<sub>2</sub> itself, release of both prostaglandins and formation of other ROS (in particular hydroxyl radical) can contribute to overall activation of high threshold vascular afferents in the bladder.

### Concluding message

The results of current study show that H<sub>2</sub>O<sub>2</sub>, in concentration range that have been detected in inflammation or reperfusion after ischemia, evoked long-lasting activation of majority of capsaicin-sensitive urothelial and high threshold vascular afferents. The data demonstrate that the TRPA1 channels on the endings of capsaicin-sensitive fibres are the major targets of ROS. Our data

support current hypothesis that increased production of ROS during ischemia/reperfusion injury seen in bladder outlet obstruction triggers the development of bladder overactivity via stimulation of capsaicin-sensitive afferents.

#### References

1. Scheepe et al (2011). J Urol 186: 1128-33
2. Masuda et al (2008). BJU Int 101: 775-80
3. Zagorodnyuk et al (2010). Aut Neurosci 153: 3-11

#### Disclosures

**Funding:** supported by grant #1046881 from the NH&MRC of Australia **Clinical Trial:** No **Subjects:** ANIMAL **Species:** guinea pigs **Ethics Committee:** Animal Welfare Committee at Flinders University