

Activation of Transient Receptor Potential Ankyrin 1 induces CGRP Release from Spinal Cord Synaptosomes

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Aim

TRP channels are polymodal cation channels which have well established roles in sensing chemical, thermal and mechanical stimuli in the periphery however the role played by these channels when expressed at the central terminals of sensory nerves remains ambiguous (Story *et al.*, 2003; Peier *et al.*, 2002; Caterina *et al.*, 1997). Recent studies have suggested that these channels, which are expressed on primary afferent terminals that enter the dorsal horn of the spinal cord, are novel therapeutic targets in nociceptive conditions due to their ability to modulate spinal synaptic transmission (Wrigley *et al.*, 2009; Jeffry *et al.*, 2009; Andersson *et al.*, 2011) Neurotransmitters released from primary afferent fibres relay nociceptive information in the spinal cord and play an important role in sensory signalling. Transmitter release can be used to profile functional TRP channels expressed at central terminals. *In vivo*, microdialysis has commonly been used to measure transmitter release however accurate estimations of drug concentrations reaching receptors cannot be made. Another approach is the measurement of transmitter release from tissue preparations *in vitro* using a high throughput (96-well format) release assay which has been developed and optimized (Anderson *et al.*, 2000; Mogg *et al.*, 2004). In the current study we measured CGRP release from a synaptosomal preparation of lumbar spinal cord in response to the administration of TRPA1 agonists and antagonists.

Methods

CGRP release was measured from a crude synaptosomal sample of native spinal tissue. The sample was prepared by hydro-extrusion of the lumbar spinal cord from adult male Sprague-Dawley rats (~250-500g) which were sacrificed by exposure to a rising concentration of CO₂ followed by cervical dislocation. The spinal cord was then homogenised allowing the formation of 'synaptosomes'. Using an enzyme immunometric assay the amount of CGRP released from the synaptosomes, after application of antagonists and agonists, was measured and compared. Compound stocks were prepared in DMSO and diluted to a maximum DMSO concentration of 1%. Antagonists were applied for 10 minutes prior to stimulation and the experiment was conducted at 37°C.

Results

The effects of various TRPA1 agonists, including the electrophilic exogenous activators, Allyl isothiocyanate (AITC) and Cinnamaldehyde, on CGRP release were studied. Both AITC (0.03-300µM) and Cinnamaldehyde (0.3-3000µM) evoked CGRP release in a concentration dependent manner (AITC, EC₅₀ ± SEM = 58.41 ± 13.01µM, n=11. Cinnamaldehyde, EC₅₀ ± SEM = 57.08 ± 9.54µM, n=14). The effect of a broad spectrum TRP antagonist, Ruthenium Red (RR) on these responses was assessed, 10µM RR reduced CGRP release induced by AITC from 123.75 ± 8.34% (n=6) to 84.50 ± 8.51%

of Basal Release \pm SEM (n=3) and Cinnamaldehyde-induced release from $125.63 \pm 6.72\%$ (n=6) to $89.92 \pm 7.58\%$ of Basal Release \pm SEM (n=3).

Conclusions

In vitro neurotransmitter release assays can be used to obtain detailed information about native signalling. These assays can more accurately represent the situation *in vivo* than recombinant cell-based assays yet escape limiting *in vivo* techniques. Moreover, synaptosomal preparations allow an exclusive look at the nerve-endings of primary afferent fibres and the ion channels which function at these central terminals. Information from these assays may be important for understanding modulation of nociceptive signalling at the level of the spinal cord.

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