

Cold thermal processing in the spinal cord

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Abstract

Two recently identified transient receptor potential (TRP) channels, TRPM8 and TRPA1, have been proposed to play an important role in mammalian cool and cold peripheral sensory transduction. When expressed in cell-lines the cloned TRPM8 and TRPA1 receptors have distinct pharmacological and temperature response characteristics. Although these receptors are also transported to the central terminals of primary afferents, little is known about their centrally mediated actions. In this thesis, I use an *in vitro* electrophysiological approach to investigate the dorsal horn processing of cool afferent modalities and the role of TRP ion channels. The results of this thesis provide further information on thermal processing, indicate direction for further research and suggest possible therapeutic targets for the management of abnormal cold sensory processing.

Initial experiments demonstrate that the cooling agents and known TRPM8 and TRPA1 agonists, menthol and icilin, inhibit primary afferent evoked excitatory postsynaptic currents (EPSCs) in rat spinal cord dorsal horn neurons. In addition, temperature reduction, menthol and icilin increase the frequency of miniature EPSCs without affecting amplitude distribution or kinetics. Little or no direct postsynaptic effect on dorsal horn neurons, GABAergic or glycinergic transmission was found. In combination, these observations demonstrate that temperature reduction, menthol and icilin act presynaptically to increase the probability of glutamate release from primary afferent fibres.

Further examination of the changes in glutamatergic synaptic transmission induced by temperature reduction, menthol and icilin reveals a subset of neurons sensitive to innocuous cool (< 29 °C) and low concentrations of icilin (3-10 µM) which closely match the temperature activation and pharmacological profile of TRPM8. In addition, the majority of lamina I and II neurons displayed characteristics partly consistent with TRPA1-activation, including a concentration-dependent response to icilin and blockade by ruthenium red. The present experiments did not allow thermal characterisation of these TRPA1-like responses. Together these observations indicate that the

effects of menthol and icilin on glutamatergic synaptic transmission in the superficial dorsal horn are mediated by TRPM8 and possibly by TRPA1.

Examination of the anatomical location of neurons activated by temperature reduction, menthol, icilin and capsaicin allowed the central termination pattern of thermoreceptive primary afferent fibres with specific TRP-like response characteristics to be determined. TRPM8-like presynaptic activation was confined to a subpopulation of neurons located in lamina I and outer lamina II, while the majority of neurons throughout laminae I and II received inputs sensitive to menthol, high concentrations of icilin and capsaicin. These findings suggest that innocuous cool sensation projects to a specific subpopulation of superficial dorsal horn neurons unlike other modalities (mediated by TRPV1, possibly TRPA1 and other receptors), which non-selectively engage circuits within the entire superficial dorsal horn. No morphological specificity was identified for recovered neurons after electrophysiological characterisation.

Finally, μ -opioids were shown to inhibit basal glutamatergic synaptic transmission as well as menthol- and icilin-induced transmission in the superficial dorsal horn. Of particular interest, δ -opioids selectively inhibited icilin-induced synaptic transmission within the same location. The selective effect of δ -opioids suggests a possible role in modulating receptors activated by icilin (TRPM8 and TRPA1).

Overall, this thesis provides further evidence that TRPM8 is responsible for the transduction of innocuous cold sensation in mammals and is a potential therapeutic target in humans with cold hyperaesthesia secondary to abnormal thermal processing. The use of δ -opioid agonists warrants further investigation in cold hypersensitivity states and potentially other forms of pain.

Statement

I hereby declare that this submission is my own work, and to the best of my knowledge and belief it contains no material previously published or written by another person or material which to a substantial extent has been accepted for the award of any other degree or diploma of a university or other institution or higher learning, except where due acknowledgement is made in the text.

Code of Ethics

All experiments were performed in accordance with the guidelines of the National Health and Medical Research Council 'Code of Practice for the Care and Use of Animals in Research in Australia' and with the approval of the Royal North Shore Hospital/University of Technology Sydney Animal Care and Ethics Committee.

Paul J Wrigley

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Abbreviations/Definitions

ACC - anterior cingulate cortex

ACSF - artificial cerebrospinal fluid

AIT - allyl iso-thiocyanate

Allodynia - pain arising from a stimulus which is not normally painful

AM 251 - (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide), a CB1 cannabinoid receptor antagonist

AMPA - α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor, a glutamate receptor subtype

CB - cerebellum

CB1 – a cannabinoid receptor subtype

cDNA – complementary deoxyribonucleic acid

CGRP – calcitonin gene-related peptide

CL - centrolateral nucleus of the thalamus

CNOX – a non-NMDA receptor antagonist

COOL – innocuous cold thermospecific neurons found in lamina I of the dorsal horn

CTAP - D-Phe-Cys-Tyr-D-Trp-Arg-Pen-Thr-NH₂, a selective μ -opioid antagonist

DAB - 3,3-diaminobenzidine

DAMGO - Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol enkephalin, a selective μ -opioid agonist

Deltorphin II – a selective δ -opioid agonist

dH₂O – distilled water

DMSO - dimethylsulfoxide

DRG – dorsal root ganglion

EC₅₀ – the concentration of an agonist that produces 50% of the maximal response for that agonist *in vitro*

EPSC – excitatory postsynaptic current

FRAP – fluoride-resistant acid phosphatase

GABA – γ -amino-n-butyric acid

Gd³⁺ - gadolinium, a lanthanide and non-specific calcium channel-blocker

GlyR – glycine receptor

HC icilin – high micromolar concentrations of icilin (>30 μ M)

HPC – lamina I dorsal horn neuron receiving convergent heat, pinch and cold sensory inputs

12-HPETE - 12-hydroperoxy-eicosatetraenoic acid, a lipoxygenase metabolite of arachidonic acid

HT – high threshold

Hyperaesthesia – excessive sensitivity to a sensory stimulus

Hyperalgesia – excessive sensitivity to a painful stimulus

I-RTX – iodoresiniferotoxin

IB4 – *Bandeiraea simplicifolia* I- isolectin B4

IC - insular cortex

ICI-174,864 – a selective δ -opioid antagonist

IPSC – inhibitory postsynaptic current

I-RTX - iodoresiniferotoxin

LC icilin – low micromolar concentrations of icilin (<30 μ M)

LT – low threshold

LTB4 - leukotriene B4, a lipoxygenase metabolite of arachidonic acid

MDvc - ventrocaudal part of medial dorsal nucleus of the thalamus

NADA - *N*-arachaonoyl-dopamine, an anandamide analogue

NMDA - *N*-methyl-D-aspartate receptor, a glutamate receptor subtype

nor-BNI - nor-binaltorphimine dihydrochloride, a selective κ -opioid antagonist

NS – nociceptive specific

P2X3 – a purinergic receptor subtype

PB - Phosphate buffer

PET - positron emission tomography

Pf - parafascicular nucleus of the thalamus

PFC - prefrontal cortex

Primary afferent convergence – the meeting of different primary afferent sensory modalities onto one dorsal horn neuron

Primary afferent divergence – widespread primary afferent projection to spinal cord dorsal horn neurons

rCBF - regional cerebral blood flow

RR - ruthenium red, a non-specific TRP receptor antagonist

SP – substance P

SR 95531 – a GABA_A receptor antagonist

STT – spinothalamic tract

TG – trigeminal ganglion

THC - Δ^9 -tetrahydrocannabinol, a non-selective cannabinoid receptor agonist

ThermoTRPs – temperature-activated transient receptor potential ion channels

TrkA – neurotrophic tyrosine kinase receptor, type 1

TRP – transient receptor potential

TTX – tetrodotoxin, a sodium channel blocker

U-69593 - a selective κ -opioid agonist

VGCC - voltage-gated-Ca²⁺-channels

VMpo - posterior ventral medial nucleus of the thalamus

VPI - ventral posterior inferior nucleus of the thalamus

VPL - ventral posterior lateral nucleus of the thalamus

VPM - ventral posterior medial nucleus of the thalamus

WDR – wide dynamic range