AuPS/ASB Meeting - Adelaide 2010

Free communications: Sensory

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Chair: Nick Spencer

Selective loss of visceral pain in the aganglionic rectum of lethal spotted mutant mice

V.P. Zagorodnyuk, M. Kyloh, S. Nicholas, S.J.H. Brookes and N.J. Spencer, Department of Human Physiology, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia.

Aims. Mutations in the gene coding for endothelin 3 each account for approximately 5% of human cases of Hirschsprungs disease. Mice with deletions of endothelin 3, lethal spotted (ls/ls) mouse, show comparable defects - loss of both enteric neurons and ganglia (aganglionosis) in the distal bowel. We have previously established that many extrinsic sensory neurons have transductions sites in enteric ganglia, including low threshold, wide-dynamic range mechanoreceptors (Lynn *et al.*, 2003; Spencer *et al.*, 2008). Preliminary results showed that the visceromotor responses (VMRs) to noxious levels of rectal distension were reduced or absent in ls/ls mice. The aim of this study was to investigate in details extrinsic innervation of colorectum and VMRs in ls/ls mice.

Methods. In anaesthetized mice (200-300 μl of 6 mg/ml of pentobarbital sodium, s.c.), electromyogram recordings were made from the transverse oblique abdominal muscles during noxious rectal distensions (up to 120 mmHg) to activate VMRs. Extrinsic spinal innervation of the mouse colorectum in wild type and ls/ls mice was investigated by retrogradly labelling of DRG neurons with DiI tracer injected into the rectum, by immunohistochemistry to sensory neurons marker, calcitonin gene related peptide (CGRP) and by extracellular recordings from fine rectal nerve trunks *in vitro*.

Results. Intraluminal distension (15-20 s, increments of 20 mmHg), applied to the colorectum of anaesthetized wild type mice, consistently evoked VMRs with a threshold of approximately 20 mmHg, which increased linearly with pressure up to 120 mmHg (n=9). When the same incremental distensions were applied to the aganglionic colorectum of ls/ls mice, no detectable visceromotor responses were elicited (n=11). VMRs evoked by intraluminal distension (20-100 mmHg) of the bladder (n=6) or by somatic stimuli (calibrated pinch to the tail or hind limb, n=14) were not different between wild type and ls/ls mice. We tested whether there was a complete loss of functional pain pathways from the colorectal region of the gut in ls/ls mice. Electrical stimulation (1-20 Hz, 0.4 ms, 60 V, 10 s) applied to the exposed rectum consistently evoked VMRs in both wild type (n=14) and ls/ls mice (n=12). However, responses in mutant mice were significantly smaller ($p<0.001$) than controls. In control mice (n=4), the greatest number of DiI-labelled neurons were located in dorsal root ganglia of S1 and S2, with a small proportion of neurons labelled in L3. In ls/ls mice (n=6), significantly fewer neurons (60-80% loss) were labelled in S1 and S2 than in wild type controls ($p<0.001$). In ls/ls mice (n=4), the aganglionic rectum had a significant reduction in immunoreactivity to CGRP compared with controls (n=4). Stretch-induced firing of low threshold stretch-sensitive afferents in ls/ls mice (n=27) was approximately half that of control mice (n=25, $p<0.0001$) while stretch-induced firing of serosal high threshold afferents did not differ significantly between control (n=14) and mutant (n=17) mice.

Conclusions. The current study has identified that, in addition to colorectal aganglionosis, mice deficient in endothelin 3 also have a selective deficiency in nociception from the aganglionic colorectum. The results revealed a significant reduction in density of spinal sensory innervation of aganglionic rectum and impairment of mechanosensitivity of low threshold, wide-dynamic range mechanoreceptors which together may account for a loss of VMRs in ls/ls mice.

Lynn PA, Olsson C, Zagorodnyuk V, Costa M & Brookes SJ. (2003) Rectal intraganglionic laminar endings are transduction sites of extrinsic mechanoreceptors in the guinea pig rectum. *Gastroenterology* **125**, 786-794.

Spencer NJ, Kerrin A, Singer CA, Hennig GW, Gerthoffer WT & McDonnell O. (2008) Identification of capsaicin-sensitive rectal mechanoreceptors activated by rectal distension in mice. *Neuroscience* **153**, 518-534.

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Purinergic signalling via ATP-gated ion channels mitigates noise-induced hearing loss

G.D. Housley,[1,2] Y. Sivakumaran,[1] T.L. Loh,[1] S.F. Tadros,[1] A.C.Y. Wong,[1] P.R. Thorne,[2,3] S.M. Vlajkovic[2] and A.F. Ryan,[4] [1]Department of Physiology & Translational Neuroscience Facility, School of Medical Sciences, University of New South Wales, NSW 2052, Australia, [2]Department of Physiology, Faculty of Medical and Health Sciences, The University of Auckland, New Zealand, [3]Audiology Section, Faculty of Medical and Health Sciences, The University of Auckland, New Zealand and [4]Departments of Surgery & Neuroscience & VA Medical Center, University of California, San Diego, La Jolla, CA 92093, USA.

We tested the hypothesis that activation of $P2X_2$ receptor-mediated signal transduction in the cochlea mitigates noise-induced hearing loss (NIHL). ATP-gated ion channels assembled from $P2X_2$ receptor subunits are expressed by the cochlear sensory hair cells, associated epithelial supporting and secretory cells, and by the spiral ganglion neurons. These sites of expression may be activated by noise-induced release of ATP to affect sound transduction, cochlear electrochemical homeostasis, and auditory neuron excitability (see Housley, Bringmann & Reichenbach, 2009, for a review). This $P2X_2$ receptor signalling is up-regulated by sustained exposure to high noise levels (Wang *et al.*, 2003), suggesting a potential relationship. Wildtype (WT) and $P2X_2$ receptor knockout (KO) mice (C57BL/6J background strain) were exposed to two noise conditions: acute - high level noise, and long-term – medium level (environmental) noise. In the case of the acute study (30 minutes, 95 dB SPL, 1 octave (8 – 16 kHz) white noise), hearing sensitivity was measured by auditory brainstem response (ABR) before, immediately after, and then two weeks after the noise exposure, to determine temporary (TTS) and permanent (PTS) threshold shifts.* The WT and KO mice groups had comparable TTS within the noise band, however, the KO mice sustained high frequency PTS. In the second study, WT and KO mice were born into either an acoustically attenuated “quiet” environmental chamber, or an environmental chamber providing exposure to moderate ambient noise (75 dB white noise). After four months, both WT and KO mice in the “noise chamber” had significantly worse hearing than the mice in the “quiet chamber”. However, as seen in the acute noise study, hearing loss in the KO mice extended to higher frequencies than in the WT mice.

Conclusion: In the absence of $P2X_2$ receptor signalling (KO mice), NIHL in the cochlea is exacerbated for both high-level, short-term noise exposure and long-term moderate noise levels. Thus $P2X_2$ receptor signalling is oto-protective, providing intrinsic reduction of high-frequency NIHL.

*The mice were anaesthetized using ketamine (40 mg/kg); xylazine (8 mg/kg); acepromazine (0.5 mg/kg) (i.p.) during the ABR measurements following a protocol approved by the UNSW Animal Care and Ethics Committee.

Housley GD, Bringmann A, Reichenbach A (2009) *Trends in Neurosciences* **32:** 128-141.

Wang J C-C, Raybould NP, Luo L, Ryan AF, Cannell MB, Thorne PR, Housley GD (2003) *NeuroReport* **14:** 817-823.

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Extracellular recording of viscerofugal neurons in guinea-pig colon

T.J. Hibberd, N.J. Spencer and S.J.H. Brookes, Human Physiology and Centre for Neuroscience. School of Medicine. Flinders University, Bedford Park, SA 5042, Australia.

Viscerofugal neurons have cell bodies in the myenteric plexus of the gut wall and project out of the gut to synapse with postganglionic sympathetic neurons in the abdominal prevertebral ganglia. Noradrenergic sympathetic neurons in turn project back into the gut, where they inhibit transmitter release from enteric neurons. This reflex circuit, when activated by mechanical and chemical stimulation of the intestine, causes inhibition of gut motility and secretion. Much of our current understanding of viscerofugal neurons has been deduced from intracellular recordings of cholinergic synaptic input onto symapthetic nerve cell bodies, where many viscerofugal terminals synapse. Direct extracellular recordings from axons of viscerofugal neurons, from mesenteric nerves, would make possible more detailed investigation of their physiology. However, this has not been possible to date because of the presence of spinal sensory neurons within the same nerve trunks. Previously, we have reported that maintaining preparations of guinea pig distal colon in organotypic culture for a period of 3-5 days causes degeneration of extrinsic nerve fibres which have been severed from their cell bodies. In these preparations, viscerofugal neurons and their axons survive and we were able to make direct extracellular electrophysiological recordings from identified viscerofugal axons. Our current aim was to determine whether axons of viscerofugal neurons could be recorded in mesenteric nerves in acute preparations (that had not been organ-cultured).

Methods: Close extracellular recordings from colonic nerve trunks were made from flat sheet preparations of guinea pig distal colon freshly removed from humanely killed guinea pigs. Preparations were studied *in vitro*, after removal of the mucosa, sub-mucosa and circular muscle layers. The nicotinic receptor agonist, DMPP, was ejected onto myenteric ganglia through a micropipette (5-10μm tip) using nitrogen pulses (100kPa, 10-40ms). Putative viscerofugal nerve cell body locations were identified when DMPP-stimulation of a ganglion evoked a burst of action potentials in the recorded colonic nerve. Ganglia were classified as responsive or non-responsive (to DMPP) and the results were mapped onto a printed micrograph of the preparation. The recorded nerve trunk was then filled with biotinamide and locations of viscerofugal nerve cell bodies projecting into the recorded nerve trunk were mapped.

Results: DMPP-sensitive sites were significantly associated with the presence of viscerofugal nerve cell bodies. In ten mapped preparations, 16 of 24 ganglia containing viscerofugal nerve cell bodies were DMPP-sensitive. Conversely, 158 of 162 ganglia without viscerofugal nerve cell bodies were non-responsive to DMPP. This association was highly significant ($p<0.001$). In responsive ganglia, spritzes of DMPP evoked bursts of action potentials from 100-1000ms in duration, typically of small amplitude (<200μV peak-to-peak). In many cases, single units could be discriminated with firing rates at up to 50Hz. None of the DMPP-responsive units were activated by capsaicin.

Conclusion: Single unit recordings of viscerofugal neurons can be made using standard intracellular recording techniques *in vitro*. Identified axons of viscerofugal neurons are abundant in colonic nerves and can be distinguished with high reliability by their responses to localised application of a nicotinic agonist. This result paves the way for detailed characterisation of viscerofugal neuron activity.