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

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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.

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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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