Extracellular cyclic GMP (cGMP), the downstream mediator released in response to linaclotide-induced activation of guanylate cyclase-C (GC-C), reduces excitability of murine and human dorsal root ganglion neurons

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BACKGROUND

- Linaclotide, an investigational GC-C agonist, improves abdominal pain and bowel symptoms in humans with Irritable Bowel Syndrome with Constipation (IBS-C).1
- Pain from the colon and rectum is signalled via two distinct anatomical spinal afferent pathways.2
- Linaclotide inhibits colonic nociceptors in vitro 1.

METHODS

- We performed whole cell patch clamp recordings in current clamp mode from retrogradely traced thoracolumbar (T10-L1) colonic DRG neurons.
- We compared the effect of extracellular cGMP (100nM-50µM) on the rheobase, or threshold for action potential firing, of DRG neurons from healthy C57BL/6 mice and mice with CVH, 28 days post-TNBS administration 1,6.
- We performed calcium-imaging studies and compared the effects of cGMP (10µM-300µM) on-calcium (Ca²⁺) influx in response to hyposmotic stimuli of DRG neurons from healthy donors.

RESULTS

- Cyclic GMP (cGMP) is a second messenger produced in guanylate cyclase cells when activated by guanylate-cyclase C (GC-C) agonists.

AIMS

- We investigated the effects of exogenous extracellular cGMP on sensory neuron function in both healthy and CVH mice.

METHODS

- Mouse DRG studies
  - We performed whole cell patch clamp recordings in current clamp mode from retrogradely traced thoracolumbar (T10-L1) colonic DRG neurons.
- CVH mice: Exogenous extracellular cGMP application causes potent inhibition of colonic DRG neurons

SUMMARY

- Mouse DRG studies
  - Colonic DRG neurons from CVH mice displayed a significantly reduced rheobase, firing significantly more action potentials compared with healthy mice at baseline.
  - In a subpopulation of colonic DRG neurons, cGMP inhibited the neuronal excitability of putative nociceptors, significantly increasing their rheobase and reducing action potential discharge.
  - This effect was evident in both healthy and CVH DRG neurons, was most apparent in CVH DRG neurons, and occurred at concentrations as low as 100nM cGMP.

- Human DRG studies
  - In human DRG neurons, cGMP induced an overall reduction in the number of cells responding to hypo-osmotic stimulation.
  - In addition in human DRG neurons cGMP caused, in a concentration-dependent manner, up to 60% inhibition of the Ca²⁺ influx induced by hypo-osmotic stimulation.

CONCLUSIONS

- Exogenous cGMP directly decreases the excitability of sensory DRG neurons isolated from both mice and humans.
- These results complement our previous findings in mice, which demonstrated that cGMP inhibited the peripheral endings of nociceptors within the wall of the colon.
- These current findings also provide further mechanistic insight into how linaclotide, through GC-C agonism and the release of cGMP from mucosal epithelial cells, reduces nociceptive signaling from the colon.

ACKNOWLEDGEMENTS

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REFERENCES

1 Castro et al., and Brierley 2011, Gastroenterology.