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Facilitation of murine enteric cholinergic neurotransmission by 5-HT$_4$ receptor activation: control by phosphodiesterases

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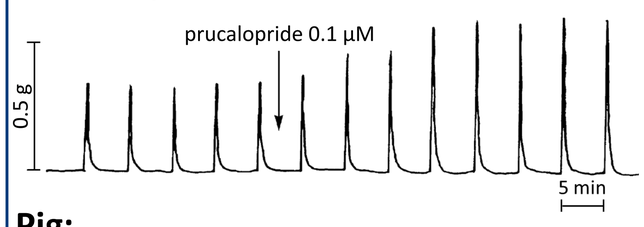
Background

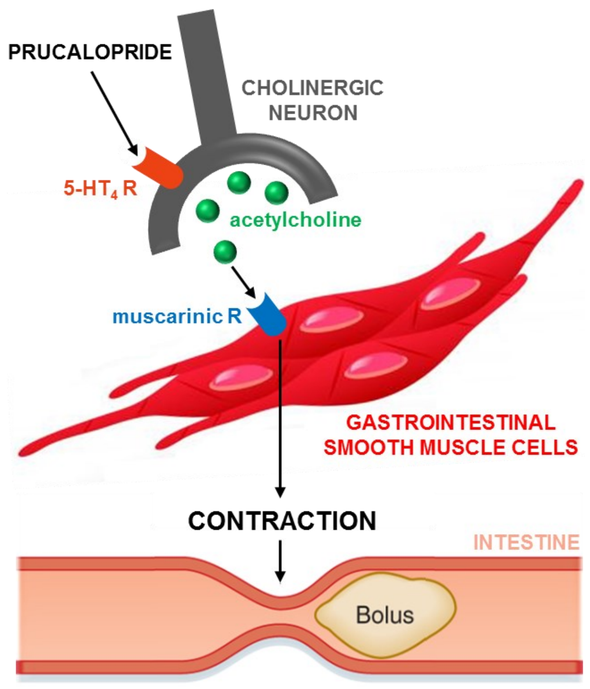
Man, dog, pig:

- 5-HT$_4$ receptors present on enteric cholinergic neurons innervating smooth muscle cells
- activation of those 5-HT$_4$ receptors by a 5-HT$_4$ receptor agonist (e.g. prucalopride)
  => ↑ ongoing acetylcholine release
  => ↑ smooth muscle contraction



Pig:

- 5-HT$_4$ receptor pathway in enteric cholinergic neurons is controlled by phosphodiesterase (PDE) 4
- PDE4 inhibition
  => contractions, facilitated by prucalopride, are further enhanced



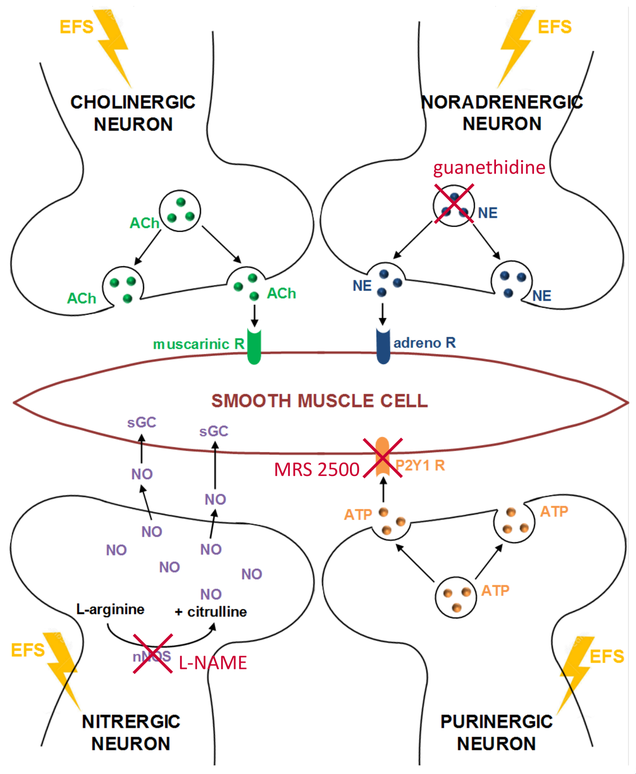
Aim:

Mouse: 5-HT$_4$ receptors on enteric cholinergic neurons innervating smooth muscle cells + control by phosphodiesterases?

Methods

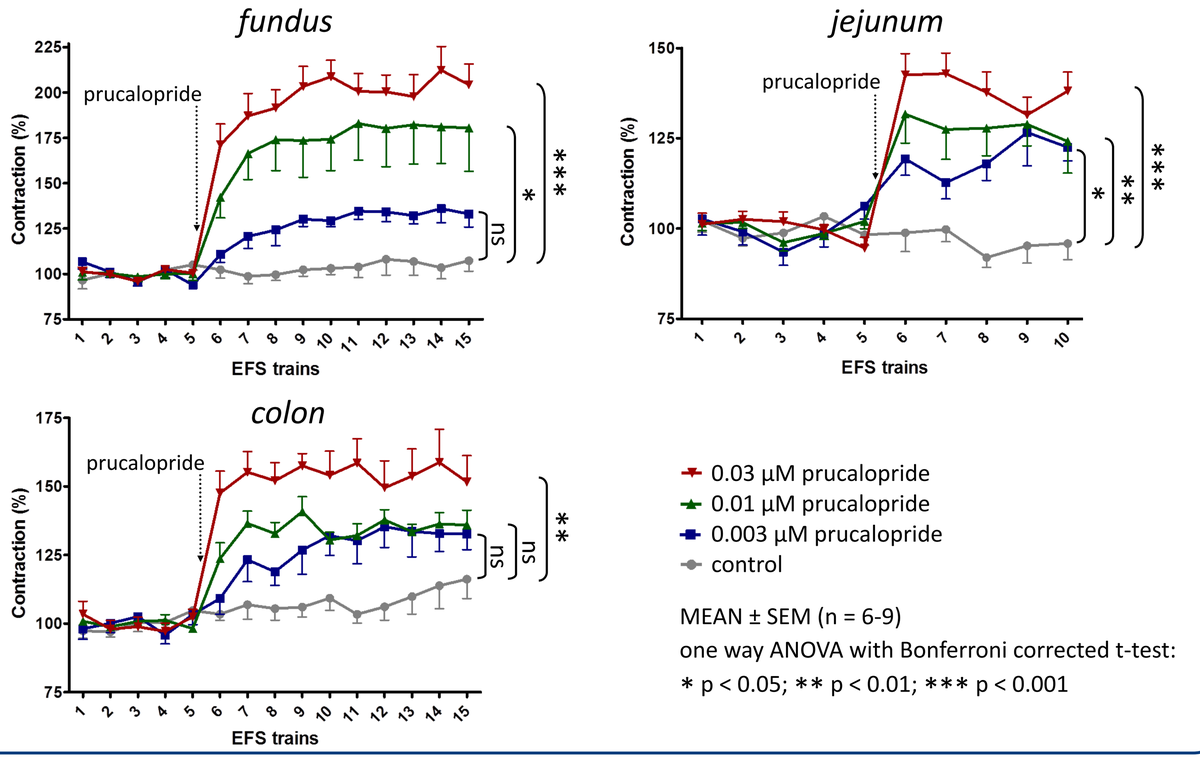
- circular smooth muscle strips from murine fundus, jejunum and colon
- organ bath with oxygenated Krebs solution:
  + guanethidine (4 µM)
  + L-NAME (300 µM)
  + MRS 2500 (1 µM; only for colon)
- isometric tension recording
- electrical field stimulation (EFS):
  - 10 s train
  - 500 µs pulse duration
  - 4 (fundus) or 8 Hz (jejunum + colon)
  - 5 (fundus + colon) or 10 min (jejunum) intertrain interval
  - V_{max} = 30 V → voltage reduced till a response of 50%

  => EFS-induced submaximal neurogenic cholinergic on-contractions
- contractions expressed as % of the mean amplitude of 5 contractions before prucalopride, GR113808 or IBMX



Results

1. **PRUCALOPRIDE** = 5-HT$_4$ receptor agonist



- 0.03 µM prucalopride
- 0.01 µM prucalopride
- 0.003 µM prucalopride
- control

MEAN ± SEM (n = 6-9)
one way ANOVA with Bonferroni corrected t-test:
* p < 0.05; ** p < 0.01; *** p < 0.001

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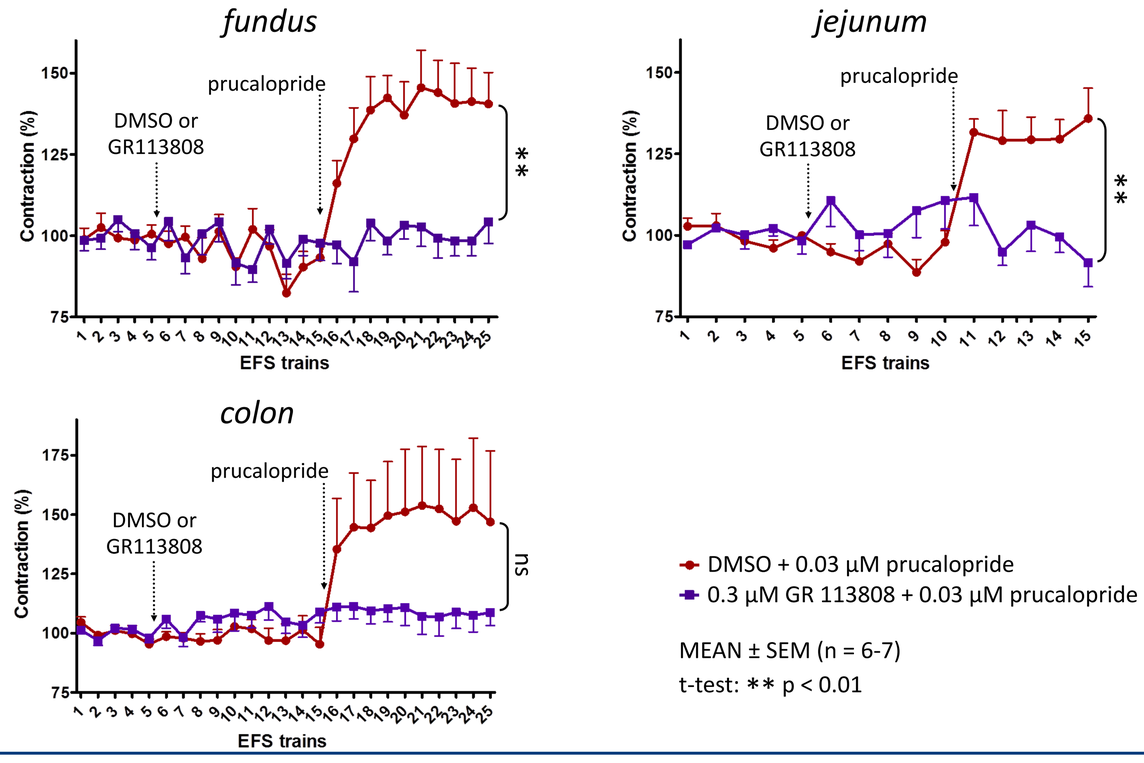
2. **GR113808** = 5-HT$_4$ receptor antagonist + **PRUCALOPRIDE**



- DMSO + 0.03 µM prucalopride
- 0.3 µM GR 113808 + 0.03 µM prucalopride

MEAN ± SEM (n = 6-7)
t-test: ** p < 0.01

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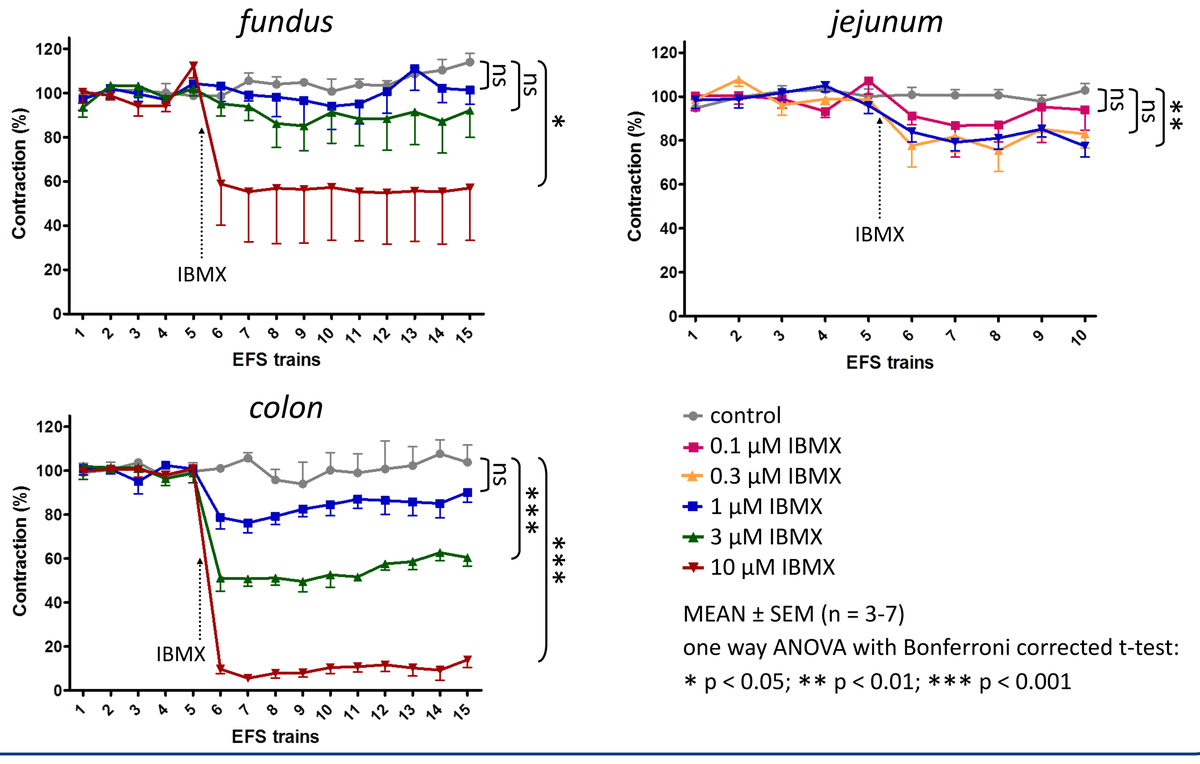
3. **IBMX** = non-selective phosphodiesterase inhibitor



- control
- 0.1 µM IBMX
- 0.3 µM IBMX
- 1 µM IBMX
- 3 µM IBMX
- 10 µM IBMX

MEAN ± SEM (n = 3-7)
one way ANOVA with Bonferroni corrected t-test:
* p < 0.05; ** p < 0.01; *** p < 0.001

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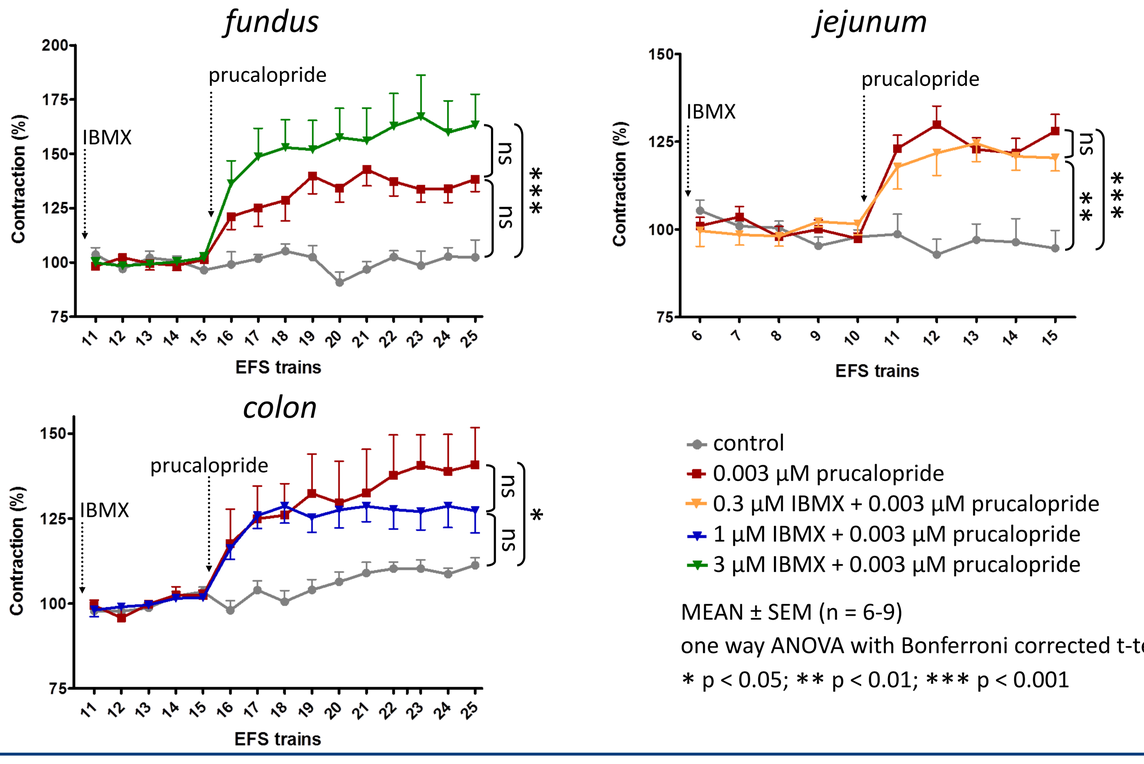
4. **IBMX + PRUCALOPRIDE**



- control
- 0.003 µM prucalopride
- 0.3 µM IBMX + 0.003 µM prucalopride
- 1 µM IBMX + 0.003 µM prucalopride
- 3 µM IBMX + 0.003 µM prucalopride

MEAN ± SEM (n = 6-9)
one way ANOVA with Bonferroni corrected t-test:
* p < 0.05; ** p < 0.01; *** p < 0.001

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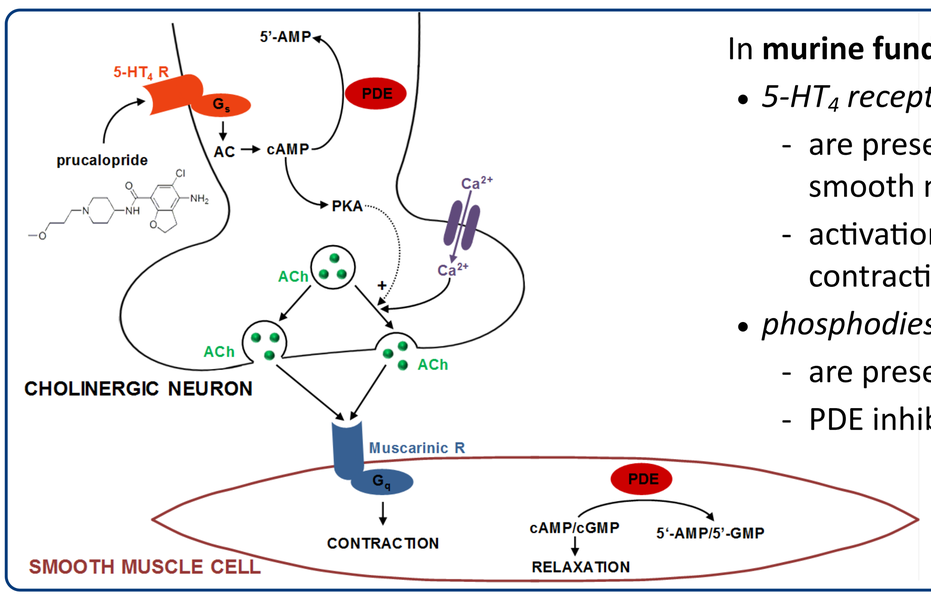
Conclusion



In **murine fundus**, **jejunum** and **colon**:

- *5-HT$_4$ receptors*
  - are present on cholinergic neurons innervating circular smooth muscle cells
  - activation enhances electrically induced cholinergic contractions
- *phosphodiesterases (PDEs)*
  - are present in circular smooth muscle cells
  - PDE inhibition induces relaxation

In **murine fundus**:

- 5-HT$_4$ receptor pathway in enteric cholinergic neurons is controlled by PDEs
- mild PDE inhibition enhances the facilitating effect of prucalopride

In **murine jejunum** and **colon**:

- no evidence for PDE-mediated control of the 5-HT$_4$ receptor pathway in enteric cholinergic neurons was yet obtained
- further investigation with selective PDE inhibitors is necessary