Differential effects of ATP-sensitive P2X4 and P2X7 on vagal neuromodulation in TNBS-induced post-inflammatory rat ileitis

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Introduction
- Cholinergic neurotransmission is severely affected in post-inflammatory ileitis, despite no depletion of vagal parasympathetic efferent neurons was still observed (Vieira et al., 2014).
- It has been demonstrated that this feature results from an unbalance between adenosinergic neuromodulation under physiological conditions and excessive ATP release (“danger molecule”) from inflammation-induced proliferating enteric glial cells (Vieira et al., 2017).
- Activation of pre-junctional P2X purinoceptors by ATP transiently facilitates the release of [3H]-ACH from non-stimulated myenteric nerve terminals (Duarte-Araujo et al., 2009), but the subtype of the receptor involved is still a matter of speculation.

Aim
- This study was designed to investigate the role ATP-sensitive P2X4 and P2X7 receptors, which are often operating neuro-immune interactions in rats with post-inflammatory ileitis caused by intraluminal injection of TNBS.
- Sub-diaphragmatic vagotomy (VGX) was used to assess the influence of the vagal tone on inflammatory-induced molecular, morphological and functional alterations.

Methodology
- Inflammation of the ileum was induced by intraluminal injection of 2,4,6-trinitrobenzenesulfonic acid (TNBS, 40 mM) in 2 months old Wistar rats.
- For sub-diaphragmatic vagotomy, each vagus nerve trunk was carefully isolated and cut (2-3 mm apart) below the diaphragm.
- 7 days after surgical procedures, longitudinal muscle-myenteric plexus (LM-MP) strips of the ileum were isolated from control (CTRL), vagotomised (VGX), inflamed (TNBS), and inflamed plus vagotomised (TNBS + VGX) animals.
- [3H]-ACH release experiments were performed at 37°C on isolated longitudinal muscle-myenteric plexus (LM-MP) preparations of the rat ileum loaded with 2.5 μCi/mL. [3H]-ACH release was evoked by electrical field stimulation (EFS, 5 Hz, 200 pulses). The tritium content of collected samples was evaluated by liquid scintillation spectrometry.
- Co-localization experiments were performed by immunofluorescence confocal microscopy (Olympus FV1000).

Results

**VGX decreased [3H]ACH release from electrically-stimulated longitudinal muscle/myenteric plexus of the ileum of control and TNBS-treated rats.**

**Inflammation increased the TTX-resistant component of [3H]ACH release compared to healthy controls.**

**Selective blockade of the P2X7 receptor with A438079 decreased [3H]ACH release from myenteric neurons of TNBS-treated rats, but not of their control littermates.**

**The P2X4 receptor antagonist, 5-BDBD reduced [3H]ACH release in control rats, while increasing the amount of [3H]ACH release in TNBS-treated rats.**

**Conclusion**

In control rats, ATP facilitates the release of [3H]-ACH from stimulated cholinergic myenteric neurons through the activation of 5-BDBD-sensitive P2X4 receptors. Conversely, high ATP amounts released from proliferating enteric glial cells in TNBS-induced post-inflammatory ileitis play a role to sustain TTX-resistant [3H]-ACH outflow from myenteric neurons via the activation of ATP-affinity/low-desensitizing P2X7 receptor (cf. Vieira et al., 2014), whereas a shift towards inhibition was observed for the P2X4 receptor under such experimental conditions. The vagus nerve seems to be critical to P2X4 and P2X7 receptors neuromodulation as their effects were abrogated by sub-diaphragmatic vagotomy.

**Drugs/Actions**
- **TTX**: Nerve action potential blocker; **5-BDBD**: P2X4 antagonist; **A438079**: P2X7 antagonist

**References**
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