Benzamil, but not triamterene, reduces mechanosensitive 5-HT release from EC cells of guinea pig intestine

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Introduction. Serotonin (5-hydroxytryptamine; 5-HT) is a gastrointestinal (GI) hormone which is produced by the enterochromaffin (EC) cells present in the epithelium of the GI lumen. A variety of lumenal stimuli are known to release 5-HT, including tastants, nutrients and mechanical stimulation (Bertrand & Bertrand, 2010). Released 5-HT then activates enteric sensory neurons which control GI reflexes, although there is debate about the exact contribution of 5-HT in the initiation or modulation of mechanically evoked motor patterns (Bertrand, 2012; Keating & Spencer, 2010; Grider & Jin, 1994). Despite the controversy, the mechanosensitive release of 5-HT from the epithelium is well-documented; however, the mechanoreceptors are yet to be identified. We hypothesize that acid sensing ion channels (ASICs), which are known to be present in the ENS, may play a key role as we have previously shown that the non-selective ASIC blocker amiloride reduces 5-HT release (Polglaze *et al.*, 2010). Thus, our aim was to demonstrate that blockade of ASICs and not of epithelial sodium channels (ENaC) decreases mechanically evoked release of 5-HT from EC cells.

Methods. Segments of guinea pig ileum, proximal colon, and distal colon (both sexes: 434 ± 26 g) were opened, pinned flat mucosal side up and superfused with warm (37°C) physiological saline solution. Mechanical stimulation was carried out with a carbon fibre electrode which simultaneously recorded mechanically evoked 5-HT release (Bertrand, 2004). Recordings of 5-HT levels were taken in control conditions, after 20 minutes exposure with the ASIC blocker benzamil (100 μM) or the specific ENaC blocker triamterene (30 μM) and compared using a paired t-test with significance at $P < 0.05$.

Results. Benzamil caused a significant decrease in peak levels of compression evoked 5-HT release from all regions. In ileum, mechanically evoked 5-HT release decreased from 12.5 ± 2.1 μM in control to 7.6 ± 1.3 μM in the presence of benzamil (n = 7; $P < 0.05$). Benzamil also reduced 5-HT release from proximal colon (control: 14.5 ± 2.4 μM; benzamil: 5.0 ± 0.9 μM; n = 6; $P < 0.05$) and distal colon (control: 16.6 ± 2.4 μM; benzamil: 7.9 ± 1.4 μM; n = 7; $P < 0.05$). In order to investigate any contribution of ENaCs, we also tested an ENaC only blocker triamterene. Consistent with our previous data, triamterene had no effect on mechanically evoked 5-HT from any region of intestine tested (ileum - control: 24.2 ± 7.6 μM; triamterene: 24.1 ± 3.8 μM; n = 7), (proximal colon - control: 15.9 ± 3.7 μM; triamterene: 17.6 ± 3.8 μM; n = 7), (distal colon - control: 20.8 ± 5.4 μM; triamterene: 20.1 ± 2.9 μM; n = 7).

Conclusion. The reduction of mechanically evoked 5-HT release in ileum and colon by benzamil suggests that ASICs may be involved in 5-HT release from EC cells in the intestinal mucosa. The lack of effect of triamterene suggests that benzamil and our previous results with amiloride are likely acting at ASICs and demonstrates that ENaCs do not play a significant role in mechanically evoked 5-HT release.

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