5-HT2$_B$ receptors regulate enteric neurotransmission in the submucosal plexus of rat colon

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Introduction: Previously we demonstrated a role for 5-HT$_{2B}$ receptors in the modulation of colonic visceral hypersensitivity in anxiety-prone rats (O'Mahony et al., 2010). Furthermore, *in vivo* treatment with a selective 5-HT$_{2B}$ receptor antagonist, RS127445 decreased rat faecal output (Bassil et al., 2009); however faecal fluid content was not recorded. As functional gastrointestinal disorders, such as irritable bowel syndrome, present as a combination of visceral pain and altered bowel habit our aim was to determine, using calcium imaging, a role for 5-HT$_{2B}$ receptors in submucosal neurotransmission, the functional effects of which may impact on colonic seccretomotor function.

Methods: Whole-mount preparations of the submucosal plexus from adult male Sprague Dawley rats were prepared by excising the colon and removing the muscular and mucosal layers. The exposed submucosal plexus was loaded with the ratiometic calcium indicator, Fura 2AM (7μM) in Krebs solution. Real-time calcium imaging experiments were conducted using a standard epifluorescence imager. Ganglionic neurones were identified based on morphology and responsivity to 75 mM KCl (made up in normal Krebs solution). n values represent the number of neurones from which recordings were obtained, and at least three animals were used to obtain submucusal preparations. A Student's paired t-test or One-way ANOVA with Bonferroni's post-test were used for statistical analysis as appropriate, and a $P < 0.05$ was considered significant. Data are presented as mean +/- s.e.m.

Results: BW723C86 (BW; 100nM), a putative 5-HT$_{2B}$ receptor agonist, induced a significant increase in intracellular calcium compared to baseline (baseline 1.18 ± 0.07 F340/F380 ratio, n=17 vs. BW, 1.37 ± 0.10 F340/F380 ratio, n=17, P<0.001).At higher concentrations the BW723C86-induced increase in calcium was significantly less than that induced by 100nM BW7the 23C86 (1μM, P<0.01). In total, 44.1% of submucosal neurones responded to BW723C86 (100nM). However, only 44.5% of 5HT (10uM)-responsive neurones, which itself significantly increased the F340/F380 ratio, also responded to BW723C86. Furthermore, we identified BW723C86-rsponsive neurones which were also capsaicin (100nM)-sensitive. Notably, a significant increase in intracellular calcium was also observed following addition of the 5HT$_{2B}$ receptor antagonist RS127445 (baseline, 0.77 ± 0.06 F340/F380 ratio, n=12 vs. RS, 0.92 ± 0.05 F340/F380 ratio, n=12, P<0.01).

Conclusion: Our data suggest that a proportion of submucosal neurones, including sensory nerves, are responsive to BW723C86. However, as BW723C86 may also activate 5HT$_{2A}$ and 5HT$_{2C}$ receptors the precise subtype involved in mediating the BW723C86 effect warrants further pharmacologic investigation. Nonetheless, the response elicited by RS127445 implies a role for 5HT$_{2B}$ receptors in the regulation of submucosal neurotransmission. Therefore, our data implicate 5HT$_{2B}$ receptors, and potentially related subtypes, in mediating colonic submucosal neurotransmission, which functionally could influence colonic fluid and electrolyte transport.

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