

5-HYDROXYINDALPINE, THE PUTATIVE 5-HT_{1P} RECEPTOR AGONIST, ACCELERATES DISTENSION-INDUCED PERISTALTIC REFLEX IN MOUSE ISOLATED COLON

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Activation of the putative 5-HT_{1P} receptors on the submucosal intrinsic primary afferent neurons (IPANs) has been suggested to initiate the peristaltic reflex (Pan & Gershon, 2000). Current knowledge on these receptors has been derived from electrophysiology and binding studies; the experiments on the peristalsis reflex have been limited to flat-bed preparations (Grider *et al.*, 1996; Foxx-Orenstein *et al.*, 1996). We have therefore investigated the effect of the putative 5-HT_{1P} receptor agonist, 5-hydroxyindalpine (5-OHIP) on distension-induced peristalsis in mouse isolated intact colon.

A 4 cm length of proximal colon was dissected from male CB57BL6/J mice (25-30g) and cannulated in a 100 ml bath containing Krebs' buffer (NaCl 121.5, CaCl₂ 2.5, KH₂PO₄ 1.2, KCl 4.7, MgSO₄ 1.2, NaHCO₃ 25.0, glucose 5.6 mM; 37°C; 5% CO₂ in O₂). The colon was perfused at the oral end with Krebs' buffer at 250 µl/min. The perfusate was collected from the caudal end into a reservoir on an adjustable platform. The intraluminal pressure was recorded by pressure transducers at both oral and caudal ends. The peristaltic reflex was initiated by increasing the intraluminal pressure by raising the reservoir at the caudal end. The effect of compounds on frequency (Hz), time to reach threshold pressure (s), maximum pressure (mmH₂O), basal pressure (mmH₂O) and threshold pressure (mmH₂O) were recorded.

A regular peristaltic reflex which lasted over 2 hours was achieved by increasing the intraluminal pressure to 35 mmH₂O. The average frequency, time to reach threshold, maximum pressure, basal pressure and threshold pressure of peristaltic strokes were 0.0119±0.0001 Hz, 58.4±0.6 s, 5210±40 mmH₂O, 35.0±0.1 mmH₂O and 38.3±1.5 mmH₂O respectively (all recordings from oral end; n=10). Peristaltic activity was completely abolished by scopolamine (10 µM), hexamethonium (100 µM) or tetrodotoxin (1 µM) (n=4 each). Addition of 5-OHIP (0.1 nM-10 µM) into the bath caused acceleration of peristaltic activity; observed as a significant decrease in time to reach threshold and an increase in frequency. Maximum effect was observed at 100 nM (35.0±6.7% increase in frequency; 70.1±3.9% decrease in time to reach threshold; n=5, P<0.05 vs basal values). At high concentrations (10 µM), 5-OHIP caused 32.5±8.1% decrease in the frequency of the peristaltic strokes (n=5; P<0.05 vs basal). 5-OHIP (0.1 nM-10 µM) did not alter maximum pressure, basal pressure and threshold pressure (5430±200 mmH₂O, 45.0±10.1 mmH₂O and 45.0±13.2 mmH₂O respectively in the presence of 100 nM 5-OHIP; n=5, P>0.05 vs basal values).

These results suggest that the mouse isolated colon is a reproducible model for distension-induced peristalsis. The peristaltic reflex is mediated by neuronal activity and by activation of muscarinic receptors. The accelerating effect of 5-OHIP further supports the need to investigate the actions of this compound and other putative 5-HT_{1P} receptor ligands on the peristaltic reflex.

Foxx-Orenstein A.E. *et al.* (1996) *Gastroenterol.* **111**, 1281-1290.

Grider J.R. *et al.* (1996) *Am. J. Physiol.* **270**, G778-G782.

Pan H. & Gershon M.D. (2000) *J. Neurosci.* **20**, 3295-3309.

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