

Supplementary Data File 3.

Concentration-response curves for carbachol in circular muscle from adult and elderly ascending and descending colon.

Methods

After approval by the local ethics committee (REC 10/H0703/71), written informed consent was obtained for use of macroscopically-normal ascending and descending/sigmoid (referred to hereon as descending) colon (5-10 cm from tumour) from patients undergoing elective surgery for non-obstructing bowel cancer. Patient records were examined for use of ongoing medication and evidence of comorbidity but a systematic survey of bowel functions (e.g. stool output/frequency) was not conducted. No patient had previous chemo- or radiotherapy or a diagnosis of inflammatory bowel disease. The time between surgery and immersion of tissue into pre-oxygenated Krebs solution was between 60 and 120 min. Tissues were separated into two discontinuous age groups: Adult (35-60 years) and Elderly (≥ 70 years).

After removing the mucosa, strips were cut parallel to the circular muscle (~5mm wide, 10-15mm long; 3-31 from each patient) and mounted in warmed tissue baths containing Krebs solution (mmolL⁻¹: NaCl 121.5, CaCl₂ 2.5, KH₂PO₄ 1.2, KCl 4.7, MgSO₄ 1.2, NaHCO₃ 25, glucose 5.6, equilibrated with 5% CO₂/95% O₂) for measuring isometric muscle tension.

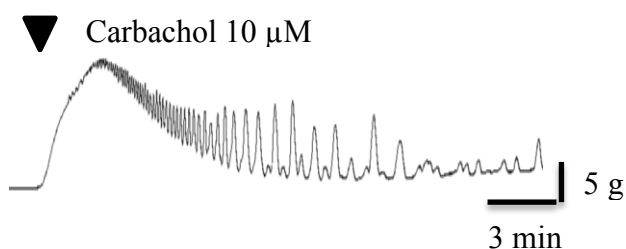
Non-cumulative concentration-response curves for carbachol (muscarinic M₁-M₅ receptor agonist) were established by applying single concentrations for 15 min or until maximum effect was observed. Carbachol was freshly prepared prior to use (Sigma, UK). Data are expressed as means \pm standard error of the mean (S.E.M) with N values indicating number of patients. Differences between means were determined using the Student's t-test for unpaired observations. P<0.05 represented statistical significance.

Results

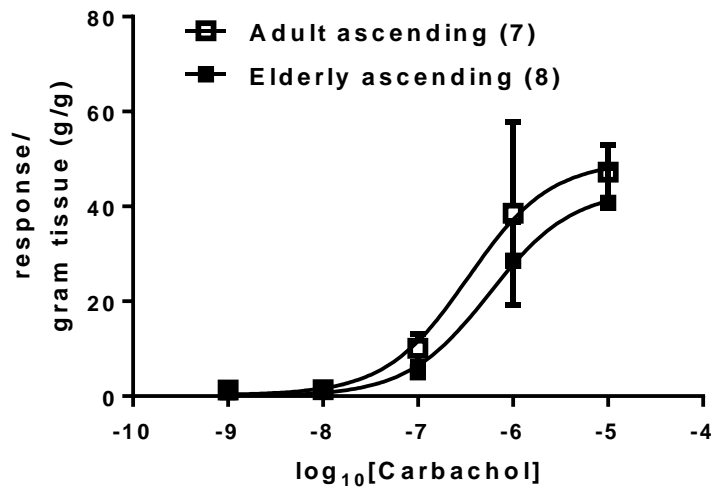
Concentration-response curves for carbachol were unaffected by advancing age in both the ascending and descending colon.

The figure below shows (A) Trace showing a typical contraction evoked by carbachol 10 μ M, (B) Concentration-response curves for carbachol in adult and elderly ascending colon and (C), Concentration-response curves for carbachol in adult and elderly descending colon. N (number of patients studied; one strip tested per patient) = Adult ascending: 7 (5 Female 2 Male), Elderly Ascending: 8 (3F, 5M), Adult Descending: 7 (4F, 3M), Elderly Descending: 12 (5F, 4M)

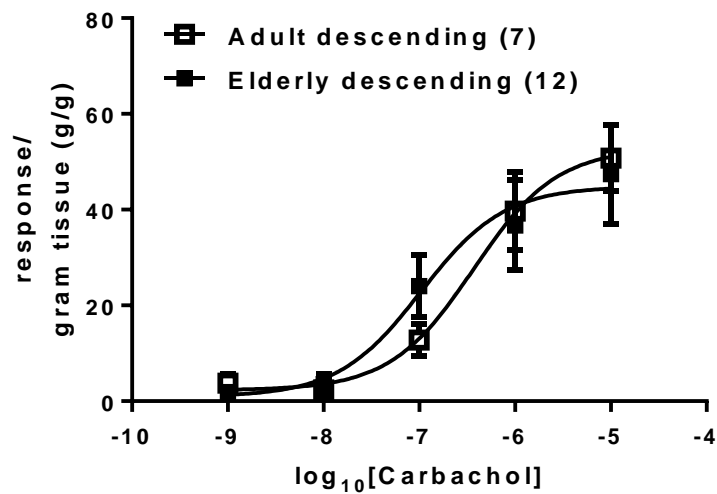
(A)



(B)



(C)



Conclusion

The amplitude of contractions evoked by carbachol were unaffected by increasing age in either region of colon. There was no evidence for differences between responses generated in tissues from male or female patients.