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Impaired colonic motility and reduction in tachykinin signalling in the aged mouse

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ABSTRACT

Ageing is associated with an increased incidence of constipation in humans. The contribution that the ageing process makes to this condition is unclear. The aim of this study was to determine the effects of age on faecal output and colonic motility in male C57BL/6J mice and to determine the role that altered tachykinin signalling plays in this process. Total faecal output recorded over a 24 h period decreased with age due to a reduction in the number of pellets produced and their water content. These changes occurred in the absence of any significant change in food and water intake. There was an increase in the amount of faecal matter stored in the isolated colon with age which caused a proportional increase in colonic length. Analysis of colonic motility using an artificial pellet demonstrated that pellets moved in a stepwise fashion through the colon. There was an age-related increase in pellet transit time due to decreases in the step distance, velocity, and frequency of stepwise movements. These changes were reversed using the neurokinin 2 (NK_2) receptor agonist neurokinin A. Addition of the NK_2receptor antagonist GR159897 significantly increased transit time in the young animals by decreasing step distance, velocity and frequency, but was without effect in the aged colon. In summary, the ageing C57BL/6J mouse shows an impaired motility phenotype. These effects appear, at least in part, to be due to an attenuation of tachykinin signalling via NK_2 receptors.

1. Introduction

Ageing is frequently associated with chronic constipation which can predispose sufferers to faecal impaction and overflow incontinence (Obokhare, 2012). Chronic constipation is a major cause of morbidity in older people and affects around 30–40% of community dwelling adults over the age of 65 years and up to 74% of the institutionalised elderly (Gallagher and O'Mahony, 2009; Rao and Go, 2010). The impact of chronic constipation on quality of life and health care costs is very large (Tariq, 2007). The causes of chronic constipation in the elderly are likely to be multi-factorial and include the effects of age on gastrointestinal (GI) tract physiology, co-morbidities, increased medication use, loss of mobility, reduced caloric intake and ano-rectal sensory changes (Rao and Go, 2010).

Studies using rodents have provided a wealth of information about the physiological mechanisms that regulate GI motility. However, there are limited studies investigating the effects of age on motility. To date only a single study has detailed the changes in faecal output in wild type aged rodents (Smits and Lefebvre, 1996). This study showed a decrease in total faecal output between 12 and 24 month rats, due to a significant reduction in both the number of pellets produced and the wet mass of the pellets. The reasons for these changes are unclear, but the authors suggested that this may include age-related decreases in colonic transit/pellet propulsion and/or reduced tissue excitability, although these were not examined.

In humans, one factor proposed to contribute to age-related constipation is a decrease in the number of myenteric neurons (reviewed in Camilleri et al., 2008; Saffrey, 2013). Moreover a decrease in choline acetyltransferase positive neurons and a relative increase in the percentage of nitric oxide synthase positive neurons have been demonstrated in middle-aged aged adults suffering from slow-transit constipation (Wattchow et al., 2008). However, a reduction in myenteric neuronal number is also seen in humans with no detectable GI disorders and therefore their involvement in age-related constipation is questionable (Bernard et al., 2009). Similarly in animal models the loss of myenteric neurons has also been observed in many of the studies of rodent species although whether the losses are functionally significant is currently unclear (Saffrey, 2013). Reductions in neuronal number have been linked to decreases in migrating motor complex activity in

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the heterozygous piebald mouse, although the effects of age in this model were not examined (Ro et al., 2006).

Irrespective of whether neuronal loss is a determinant of altered function in the ageing GI tract, alterations in neuronal signalling processes are likely to play a major role in the direct effect of age on GI motility. Tachykinins are one of the important regulators of colonic motility in mice (Brierley et al., 2001; Deiteren et al., 2011; Mulè et al., 2007). Tachykinins regulate colonic motility via NK_1 and NK_2 receptors. NK_2 receptors are predominantly located on longitudinal and circular smooth muscle cells in both mice (Dickson et al., 2010; Matsumoto et al., 2009) and humans (Giuliani et al., 1991; Jaafari et al., 2007) and provide the main receptor through which electrical field stimulation (EFS)-evoked release of tachykinins causes smooth muscle contraction. In human colons, application of NK_2 receptor agonists induced contractions, while selective NK_2 antagonists were capable of almost completely blocking EFS-evoked colonic contractions (Giuliani et al., 1991; Nakamura et al., 2011). These data are consistent with NK_2 agonists having a pro-kinetic effect in this part of the GI tract. In addition, both a reduction in the density of tachykinin immunoreactive nerve fibres in human colonic circular muscle (Porter et al., 1998) and decreases in tachykinin signalling have been observed in patients with slow transit constipation consistent with tachykinins having a pro-kinetic effect (King et al., 2010; Stanton et al., 2003).

The contribution that changes in NK_2-mediated signalling makes to age-related changes in colonic motility has not previously been examined. This study therefore examined the effects of age on food and water intake, faecal output, artificial pellet propulsion and percentage of the colon full with faecal matter in 3, 12, 18 and 24 month old C57BL/6 mice and the possible role played by altered NKA signalling in these changes.

2. Methods

All procedures were carried out according to U.K. Home Office regulations and were approved by the University of Brighton Ethics Committee. Male C57BL/6J mice were obtained from Harlan UK at 8 weeks of age and housed in individual ventilated cages in groups of 3–4, under barrier-reared conditions until required. Animals were maintained at 19.0 ± 1 °C, 55% humidity and fed on a maintenance diet (irradiated RM1 (E) 801002 chow, Special Diet Services) and had free access to irradiated water. The animals were kept on a 12 h light/dark cycle and studied at 3, 12, 18 and 24 months of age. With the exception of experiments designed to assess 24 h faecal output, all experiments were carried out between 09.00 and 12.00 h.

2.1. Assessment of faecal output

Faecal output was analysed by conducting measurements in a metabolic cage for a period of 24 h on animals from each of the four age groups under the same environmental conditions described above (n = 6 per group). For each individual animal, the weight of the food and volume of water consumed during the 24 h period were calculated. Faecal pellets were removed at the end of the 24 h period and the total wet weight obtained. Pellet counts were made and the pellets were then left to dry at 50 °C for 24 h. Total wet and dry faecal outputs were recorded. For the average faecal pellet weight, the mean weights from 25 dry faecal pellets, sampled at random, were taken from each animal group.

2.2. Expulsion of pellets

The entire colon and any faecal pellets it contained were placed in a Sylgard (Corning, UK)-lined organ bath and continuously perfused with oxygenated (95% O_2 and 5% CO_2) Krebs buffer solution, pH 7.4 (117 mM NaCl, 4.7 mMKCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$ and 11 mM glucose) at 37 ± 1 °C at a flow rate of 8 ml min^{-1}. The proportion of the colon containing faecal matter was determined in animals of each age group. Images of the colon were taken using Ethovision tracking software (Ethovision XT vs7). The images were analysed using Image J, and the ratio of the area of the colon occupied by faecal matter to the total area of the colon determined. The number of faecal pellets within the colon and the length of the full colon were also recorded. Colon length was also measured following the evacuation of the faecal pellets.

2.3. Artificial pellet propulsion

The whole colon was harvested from 3, 12, 18 and 24 month old animals and placed in ice cold Krebs buffer solution. The mesentery was trimmed using fine scissors and the whole colon was then loosely pinned in a Sylgard-lined flow bath, allowing a lateral movement of approximately 0.5 cm about the mid-line and perfused with oxygenated Krebs buffer solution at 37 ± 1 °C at a flow rate of 8 ml min^{-1}. A small (2 mm) incision was made in both ends of the colon and the openings pinned flat to facilitate pellet insertion and its expulsion at the distal end. If spontaneous evacuation was not achieved, the faecal pellets were removed from the isolated colon after 30 min, by gently flushing the lumen of the colon with warmed Krebs buffer solution. The colon was then left to stabilise for 15 min, prior to recordings of pellet motility.

Measurements of motility were carried out using an epoxy-coated artificial faecal pellet. A different sized artificial pellet was used for each age group. The choice of faecal pellet utilised for each of the 4 age groups was based on image analysis of multiple pellets using Image J.20 random pellets from each animal of each age group were analysed for their area, length and width and the average parameters for each age group used to identify the faecal pellets used for colonic motility measurements. These 'average' faecal pellets were coated with 3 coats of epoxy prior to in vitro monitoring. The artificial faecal pellet was inserted 3–4 mm into the proximal end of the bowel using a fire-polished glass capillary and the movement of the pellet was monitored using a video camera. Pellet motility was tracked using Ethovision tracking software. Following a successful trial, the experiment was repeated two further times and the average response was utilised. Measurements were conducted on all age groups, and the maximum time that a trial was conducted was 45 min. The total transit time of the artificial faecal pellet was recorded along with the distance, velocity and frequency of stepwise pellet movements for all age groups. The threshold for a step was defined as the time point when the pellet first moved ≥2 mm. The end of a step was defined by a period of time ≥ 10 s in which there were no movements of ≥2 mm.

Measurements were also carried out to study the changes in pellet motility in the presence of the NK_2 antagonist, 1 μM GR159897 and the agonist, 1 μM neurokinin A. Briefly, following control recordings, preparations were perfused for a minimum of 20 min with either NKA or GR159897 and then a maximum of three pellet motility trials were carried out using the same epoxy-coated faecal pellet utilised for the control recordings.

2.4. Measurements of colonic muscle thickness

Tissues were fixed in 4% paraformaldehyde in phosphate-buffered saline and then embedded in paraffin. Sections were deparaffinised in Histoclear and rehydrated in graded ethanol solutions. Sections were viewed and images were acquired using an Olympus BX-UCB microscope.

2.5. Statistical methods

Statistical comparison of data was carried out using either a one-way or two-way ANOVA with post hoc Tukey or Bonferroni test and $p < 0.05$ was considered statistically significant. All graphical data was presented