COLON

Molecular and functional studies of electrogenic Na⁺ transport in the distal colon and rectum of young and elderly subjects

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Background: Human distal nephron and distal colon both exhibit mineralocorticoid sensitive electrogenic Na⁺ absorption and make significant contributions to Na⁺ homeostasis. Na⁺ resorption in the distal nephron diminishes with age but it is unclear whether a similar change occurs in the distal colon.

Aims: To evaluate the effect of age on expression of apical Na⁺ channels and basolateral Na⁺, K⁺-ATPase, and on the responsiveness of electrogenic Na⁺ absorption to mineralocorticoid stimulation in human distal colon and rectum.

Materials and methods: Mucosal biopsies were obtained from healthy sigmoid colon and proximal rectum in "young" (aged 20–40 years) and "old" (aged 70 years or over) patients during routine colonoscopy/flexible sigmoidoscopy. Na⁺ channel subunits and Na⁺, K⁺-ATPase isoforms were studied at the mRNA level by in situ hybridisation and northern blotting, and at the protein level by immunocytochemistry and western blotting. The mineralocorticoid responsiveness of electrogenic Na⁺ absorption was evaluated in the two groups by measuring amiloride sensitive electrical potential difference (PD) in the proximal rectum before and 24 hours after oral administration of 1 mg of fludrocortisone.

Results: Na⁺ channel subunit and Na⁺, K⁺-ATPase isoform expression at the level of mRNA and protein was similar in "young" and "old" patients. Both basal and the fludrocortisone stimulated amiloride sensitive rectal PDs were similar in the two groups.

Conclusions: In contrast with the distal nephron, mineralocorticoid sensitive electrogenic Na⁺ absorption in the human distal colon does not diminish with age, and may be particularly important in maintaining Na⁺ homeostasis in the elderly.

The kidneys and colon are critically important for maintaining Na⁺ homeostasis. Although elderly people conserve Na⁺ during dietary Na⁺ restriction, the renal response takes almost twice as long to achieve in subjects over the age of 60 years than in those below the age of 30 years.¹ This reflects lower plasma concentrations of renin and aldosterone, and a diminished renal tubular response to aldosterone in the elderly compared with young adults.^{1–5} Basal secretion of atrial natriuretic peptide (ANP) is also fivefold greater in the elderly and, compared with younger individuals, the elderly show an exaggerated renal response to ANP.^{6–8} ANP decreases plasma aldosterone concentrations by inhibiting the renin-angiotensin-aldosterone axis and aldosterone secretion by the adrenal cortex.^{9–10} In addition, Na⁺ resorption by the distal nephron diminishes with age due in part to a decrease in Na⁺, K⁺-ATPase mediated Na⁺ extrusion from renal tubular cells.^{11–12}

Compared with renal Na⁺ handling, little is known about the effects of aging on Na⁺ absorption in the colon. Senescence leads to mucosal atrophy in the human colon¹³ and delayed transit (but not decreased faecal water content) in rat colon.^{14–15} By contrast, faecal water content is decreased in the elderly,^{16–17} and prolonged intestinal transit with lower faecal weight occurs in otherwise healthy elderly subjects compared with young individuals.¹⁸

Na⁺ absorption in the human colon is mediated by electroneutral and electrogenic processes.¹⁹ Electrogenic Na⁺ absorption occurs mainly in the sigmoid colon and proximal rectum, reflecting apical Na⁺ channel and basolateral Na⁺, K⁺-ATPase activities in surface colonocytes.¹⁹ The Na⁺ channel is a heteromeric protein composed of α , β , and γ subunits.²⁰ Age related changes in colonic Na⁺ channel and

Na⁺, K⁺-ATPase expression have never been explored, despite decreased Na⁺, K⁺-ATPase activity in aging rat kidney,¹¹ and in the erythrocytes and lymphocytes of elderly people.^{21–22} The present study was designed to evaluate the effect of age on Na⁺ channel and Na⁺, K⁺-ATPase expression and function in human distal colon and rectum.

MATERIALS AND METHODS

Volunteers

Written informed consent was obtained from two groups of patients ("young", aged 20–40 years; "old", aged 70 years or over) undergoing diagnostic colonoscopy or flexible sigmoidoscopy. Studies were approved by the local ethics committees in Salford and Leeds. "Young" patients had functional abdominal or bowel symptoms and normal colonic histology. "Old" patients were undergoing investigation of altered bowel habit or anaemia. Patients taking corticosteroids, digoxin, or diuretics, and those with mucosal inflammation, carcinoma, or multiple polyps were excluded, irrespective of age. Those with a single polyp were not excluded if biopsies were taken at least 10 cm away from the polyp. Ethics committee approval was dependent on limiting the number of biopsies to five per patient, and these were taken from the sigmoid colon and proximal rectum of 25 "young" and 23 "old" patients. Thus different sets of subjects provided biopsies for in situ hybridisation, immunocytochemistry,

Abbreviations: ANP, atrial natriuretic peptide; PBS, phosphate buffered saline; PD, potential difference

and northern and western blotting, although mucosal morphology was assessed in all subjects.

In situ hybridisation

Biopsies were fixed in 10% formaldehyde in phosphate buffered saline (PBS; pH 7.2) for 24–48 hours, dehydrated in ethanol and then Histoclear (National Diagnostics Ltd, Atlanta, Georgia, USA) for four hours, and embedded in paraffin wax. Sections of 6 µm were mounted on aminopropyltriethoxysilane coated glass slides, dewaxed in xylene for 20 minutes and then an ethanol series, and rehydrated by immersion in PBS for two minutes. After incubating in proteinase K solution (10 µg/ml in 20 mmol/l Tris buffer containing 2 mmol/l CaCl₂, pH 7.4) at 37°C for 15 minutes, then washing in PBS at room temperature for two minutes, sections were incubated in 4% paraformaldehyde at room temperature for 10 minutes to promote cross linking of proteins and RNA retention. Further incubation in triethanolamine solution (containing 1.85 g triethanolamine, 0.9 g NaCl, and 0.25 g acetic anhydride per 100 ml of diethylpyrrolidone treated water) acetylated basic groups and reduced non-specific background binding of digoxigenin labelled cDNA probes (see below). After washing in PBS, sections were dehydrated using methanol, chloroform, and finally 100% methanol, and then air dried and stored at –20°C.

Full length digoxigenin labelled cDNA probes were used for the in situ hybridisation studies. Human renal apical Na⁺ channel α , β , and γ subunit cDNAs^{23–24} and cDNAs for the α_1 and β_1 isoforms of human HeLa cell Na⁺, K⁺-ATPase^{25–26} have previously been characterised. Probes were labelled using the DIG-High Prime random primed labelling kit (Boehringer Mannheim GmbH, Mannheim, Germany). Hybridisation and detection of probe with antidigoxigenin-alkaline phosphatase conjugated Fab fragments was conducted according to the manufacturer's recommendations.

Northern blot analyses

Total RNA was extracted from colonic biopsies (4–5 from each patient) using the RNAzol B method (AMS Biotechnology, Oxon, UK) based on the guanidium thiocyanate/phenol/chloroform extraction method.²⁷ Purified RNA pellets were dissolved in 30 µl DEPC treated water and stored at –70°C. Northern blot analyses of RNA samples (10 µg RNA per track) were performed as described previously.²⁸ Washed membranes were either exposed to X-OMAT x ray film (Kodak IBI Ltd, Cambridge, UK) for 1–7 days or placed on a phosphorimager plate for 4–48 hours. After processing, x ray film was analysed using the Sigmagel analysis program. Processed phosphorimager plates were evaluated using the TINA imaging system.

Immunocytochemistry

Colonic biopsies were processed as described for the in situ hybridisation studies. Mucosal morphology was assessed by staining some sections with haematoxylin and eosin. For immunocytochemistry, sections were incubated at 37°C overnight, and at 60°C for one hour immediately before use.

Na⁺ channel subunits

The α subunit antibody raised in rabbit against a full length recombinant fusion protein generated from the α subunit of the bovine renal papilla Na⁺ channel^{29–30} was used at 1:40 dilution. The β subunit antibody raised in rabbit against a synthetic peptide corresponding to aminoacids 411–420 of the cloned human Na⁺ channel β subunit was used at a 1:60 dilution. The γ subunit antibody raised in rabbit against apical Na⁺ channel protein from bovine renal papilla^{29–31} was applied at a 1:60 dilution. Although this antibody immunoprecipitates only in vitro translated Na⁺ channel γ

subunit, it may also bind to the α subunit protein at the tissue level (DJ Benos, personal communication). Na⁺ channel antibodies were detected using the avidin-biotin complex technique.³² The secondary antibody was biotinylated swine antirabbit antibody (Dako Ltd, High Wycombe, UK) diluted 1:300 with TBS, and applied for 30 minutes at room temperature.

Na⁺, K⁺-ATPase α_1 isoform

The primary antibody was an IgG₁ monoclonal antibody cloned from hybridoma culture supernatant (used at a 1:5 dilution) raised in mouse against the Na⁺, K⁺-ATPase α_1 isoform extracted from rat kidney,³³ and was localised using IgG₁ containing complexes of calf intestinal alkaline phosphatase and mouse monoclonal antialkaline phosphatase.³⁴

Na⁺, K⁺-ATPase β_1 isoform

The Na⁺, K⁺-ATPase β_1 isoform was localised using a mouse monoclonal antibody (Affinity Bioreagents, Golden, USA) raised against the β_1 isoform extracted from lamb kidney,³⁵ and a secondary peroxidase conjugated rabbit antimouse IgG (Dako Ltd; diluted 1:200 with TBS).

Western blot analyses

Colonic biopsies were stored in liquid N₂. Weighed biopsies from each patient were homogenised (Polytron, Kinematica AG, Lucerne, Switzerland) in isotonic homogenising buffer (containing sorbitol 274.4 mmol/l, HEPES 5 mmol/l, Na₂EDTA 0.5 mmol/l, and the protease inhibitors phenylmethylsulphonyl fluoride 0.1 mmol/l, pepstatin 1.5 µmol/l, and leupeptin 2.1 µmol/l), centrifuged at 7500 *g* for 10 minutes to remove large fragments, and the supernatant centrifuged again at 20 000 *g* for 30 minutes at 4°C. The weighed pellet was resuspended in isotonic homogenising buffer (~250 mg/0.5 ml), and the protein concentration measured.³⁶ The resuspended pellet was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis.³⁷ Gels were blotted onto nitrocellulose membranes which were placed in PBS containing 5% fat free dried milk and 0.2% Tween 20 for one hour at room temperature. Na⁺ channel α , β , and γ subunit and Na⁺, K⁺-ATPase α_1 isoform antibodies were applied at dilutions of 1:100, 1:200, 1:200, and 1:500, respectively. Na⁺ channel antibodies required incubation overnight at 4°C and were detected by incubating for one hour at room temperature with horseradish peroxidase conjugated swine antirabbit antibody (Dako Ltd) at a 1:3000 dilution. Na⁺, K⁺-ATPase α_1 isoform antibody required three hours of incubation at room temperature, and was detected by incubating for one hour at room temperature with horseradish peroxidase conjugated rabbit antimouse antibody (Dako Ltd) at a 1:4000 dilution. Membranes were washed in PBS, and the bands detected by enhanced chemiluminescence (ECL western blotting system; Amersham Biosciences Ltd, Little Chalfont, Bucks, UK). Band intensity was quantified by Sigmagel image analysis.

Rectal electrical potential difference

Rectal transmucosal electrical potential difference (PD) was measured in five “young” (one male, four females) and five “old” (four males, one female) patients using dialysis bags incorporating a 3 mm polyvinyl tube containing 4% agar in 0.9% saline, and a 1 mm polyvinyl tube that allowed the bag to be filled with a solution (containing Na⁺ 120 mmol/l, K⁺ 30 mmol/l, Cl[–] 120 mmol/l, and HCO₃[–] 30 mmol/l), after being placed in the upper rectum at 09.00 h on the first day of the study.³⁸ With the distal end of the bag 8 cm from the anus, PD was monitored in the left lateral position using a portable voltmeter.³⁸ The bag was filled with 10 ml of the solution, the tube clamped, and PD monitored over

30 minutes at five minute intervals. The bag was then emptied and refilled with 10 ml of the solution containing 1 mmol/l amiloride, which decreased PD to a stable lower value after 15–20 minutes. This solution was then withdrawn and replaced with 10 ml of fresh solution containing 1 mmol/l amiloride, after which PD was monitored over 30 minutes. Immediately after removing the bag, 0.5 mg of fludrocortisone was administered orally followed by another 0.5 mg dose 12 hours later, and rectal PD measurements ($\pm$ amiloride) repeated at 09.00 h the next day. Values of PD during the study periods were averaged to obtain mean values before and after placing amiloride in the rectal lumen. It should be noted that PD was the product of the total mucosal resistance and the net current flow across the mucosa. Although amiloride increased apical membrane resistance by inhibiting apical Na⁺ channels, the increase in total mucosal resistance would have been much smaller than the decrease in net (Na⁺) current flow, owing to the relatively high paracellular shunt conductance of this epithelium.^{39–41} We therefore took changes in PD in response to amiloride to reflect amiloride sensitive electrogenic Na⁺ transport.

Statistical analysis

Numerical data are shown as mean values (SEM). The Student's *t* test (unpaired) was used to compare differences in mean values between “young” and “old” patients, *p* < 0.05 (two tailed) being taken to indicate statistical significance.

RESULTS

Mucosal morphology

Postmortem studies of colonic morphology in healthy individuals dying of trauma have revealed age related mucosal atrophy and cellular infiltration of the lamina propria.¹³ The present study revealed no differences in crypt length or cellularity of the lamina propria between 25 “young” and 23 “old” patients when sections were assessed “blindly” by two of the authors (fig 1).

Apical Na⁺ channel expression

Na⁺ channel expression at the RNA level was determined in three “young” and three “old” patients by in situ hybridisation using digoxigenin labelled full length cDNA probes for the Na⁺ channel α , β , and γ subunits. As shown in fig 2, hybridisation with each probe was restricted almost entirely to surface cells, there being no staining in negative controls (not shown). Taken together, results from all subjects indicated that age has no effect on either the density of staining or its distribution along the surface cell-crypt cell

axis. Identical colocalisation of Na⁺ channel α , β , and γ subunit mRNAs within surface cells occurs in the distal colon of Na⁺ depleted rats.⁴² Because levels of all three Na⁺ channel subunit mRNAs were low in human distal colon biopsies, attempts to quantify mRNAs by northern analysis were unsuccessful.

Localisation of Na⁺ channel subunit proteins was evaluated by immunocytochemistry. Four “young” and four “old” patients were studied with the α subunit antibody, four “young” and four “old” with the β subunit antibody, and eight “young” and eight “old” with the γ subunit antibody. As reported previously in rat colon,^{42–44} staining with all three antibodies was restricted to the apical membrane of surface cells, while negligible staining in crypt cells was localised around the crypt openings (fig 3). With each antibody, there appeared to be no difference in the depth of staining or its distribution along the surface cell-crypt cell axis in the two groups. Apical membrane staining was patchy with all three antibodies (fig 3), owing to the low copy number of Na⁺ channels (10²–10³ per cell).⁴⁵ Individual Na⁺ channel subunit proteins were therefore quantified by western blot analysis. Figure 4 shows that the α subunit antibody detected bands of 70 kDa, 55 kDa, and 40 kDa in the distal colon from three “young” and three “old” patients.³⁰ In rat kidney (positive control), a 90–100 kDa band was detected in addition to the 70 kDa and 55 kDa bands. The 70 kDa band correlates with the full length functional Na⁺ channel α subunit³⁰ although the 55 kDa band was the dominant one in human colon. The 55 kDa band could reflect partial degradation of the 70 kDa protein but it is noteworthy that the β subunit antibody detected a single well defined band when applied to the same protein samples (fig 4). Both bands detected with the α subunit antibody were therefore subjected to densitometry, which indicated that expression of the 70 kDa and the 55 kDa proteins was similar in “young” and “old” subjects (fig 4). The β subunit antibody detected a single 58 kDa protein (fig 4) in the distal colon from three “young” and three “old” patients, which was also rather smaller than predicted (72 kDa),⁴⁶ but there was no evidence of protein degradation. Furthermore, the β subunit antibody detects a single similarly sized band on western blots of distal colon of dietary Na⁺ depleted rats but not control animals,⁴⁷ which provides a strong indication that it is β subunit specific. Densitometry indicated that the 58 kDa protein was expressed at similar levels in “young” and “old” patients. Blots using the γ subunit antibody had a high level of “background” despite prolonged incubation with blocking agents, and are not shown.

Figure 1 Representative sections (stained with haematoxylin and eosin; $\times 100$ magnification) of distal colon from a “young” patient (A) (less than 40 years of age) and an “old” patient (B) (over 70 years of age).

Figure 2 Representative sections ($\times 100$ magnification) of distal colon from “young” (') and “old” (') patients, demonstrating localisation of apical Na^+ channel α (A), β (B), and γ (C) subunit mRNAs by in situ hybridisation. Haematoxylin was used throughout as a counterstain. In negative controls (not shown), there was no staining in the absence of cDNA probes.

Basolateral Na^+ , K^+ -ATPase expression

Expression of basolateral Na^+ , K^+ -ATPase mRNA was evaluated in five “young” and five “old” patients by northern blot analyses using ^{32}P labelled full length cDNA probes for the α_1 and β_1 isoforms of human Na^+ , K^+ -ATPase. Bands of 3.7 kb and 2.7 kb were identified (fig 5), corresponding to the α_1 and β_1 isoform mRNAs, respectively,^{25 26} and densitometry revealed that Na^+ , K^+ -ATPase mRNA levels were similar in “young” and “old” patients.

Immunocytochemical localisation of basolateral Na^+ , K^+ -ATPase along the surface cell-crypt cells axis was performed

in 25 “young” and 23 “old” patients using the specific α_1 isoform antibody. Figure 6 shows uniform expression of α_1 isoform protein along the surface cell-crypt cells axis, with similar density of staining in both groups. Western blot analyses revealed a doublet of ~ 57 kDa,⁴⁸ with an additional faint band of about 90 kDa in rat kidney but to a lesser and variable extent in human colon (fig 7). The 90 kDa protein is the usual size of the α_1 isoform in the rat⁴⁸ but in view of its variability in human colon, only the 57 kDa band was quantified, and its expression was similar in 14 “young” and 14 “old” patients (fig 7).

Figure 3 Representative sections ($\times 100$ magnification) of distal colon from “young” (') and “old” (') patients, demonstrating apical membrane localisation of Na⁺ channel α (A), β (B), and γ (C) subunit proteins by immunocytochemistry (indicated by arrows). Haematoxylin was used throughout as a counterstain. In negative controls (not shown), there was no staining in the absence of specific Na⁺ channel antibodies.

Electrogenic Na⁺ transport in the proximal rectum

Table 1 summarises rectal PD measurements in the absence and presence of luminal amiloride in five “young” and five “old” patients, before and 24 hours after treatment with 1 mg fludrocortisone. Before fludrocortisone treatment, pre (basal) and post amiloride PD values were similar in both groups. The significant decreases in PD elicited by amiloride indicated blockade of apical Na⁺ channels and inhibition of electrogenic Na⁺ transport, and were similar in both groups (“young”: Δ PD -14.6 (3.3) mV; “old”: Δ PD -18.9 (7.4) mV; NS). Although fludrocortisone had no effect on pre amiloride (basal) PD after 24 hours, amiloride produced substantial and similar decreases in PD in both groups (“young”: Δ PD -27.1 (2.4) mV; “old”: Δ PD -33.4 (6.4) mV; NS), which were significantly greater than those seen before fludrocortisone ($p < 0.05$ for both groups). These data show that age has no effect on either normal or mineralocorticoid stimulated

levels of amiloride sensitive electrogenic Na⁺ transport in the proximal rectum.

As reported previously,^{49–51} and irrespective of fludrocortisone treatment, a substantial lumen negative PD remained in the presence of amiloride. This amiloride insensitive component of the rectal PD in vivo may reflect a background “tone” of electrogenic Cl[−] secretion, despite there being overall net Cl[−] absorption. In “young” patients at least, post amiloride PD was significantly lower after fludrocortisone than before fludrocortisone (-7.5 (1.7) mV versus -22.3 (2.8) mV; $p < 0.01$), raising the possibility that the mineralocorticoid altered electrogenic Cl[−] secretion. These findings fit with our recent studies showing that aldosterone has a rapid non-genomic inhibitory effect on intermediate conductance basolateral K⁺ channels in human colonic epithelial cells, which decreases the capacity of the colon for Cl[−] secretion.⁵²

Figure 4 (A) Western blots of protein (40 μ g per lane) from distal colonic biopsies from individual "young" (Y) and "old" (O) patients, using antibodies raised against apical Na⁺ channel α and β subunits (see methods). Rat kidney (K; 10 μ g protein per lane) served as a positive control. (B) Mean (SEM) band densities, using rat kidney as the comparator. Results from three "young" and three "old" patients are depicted. In each case, results from "young" and "old" patients were not significantly different.

Figure 5 (A) Northern blots of mRNA (10 μ g per lane) from distal colonic biopsies from individual "young" (Y) and "old" (O) patients using full length cDNA probes against the α_1 and β_1 isoforms of basolateral Na⁺, K⁺-ATPase (see methods). β -Actin RNA was used as an internal control. Rat kidney was used as the comparator (data not shown). (B) Mean (SEM) band densities from five "young" and five "old" patients. In both cases, results from "young" and "old" patients were not significantly different.

Figure 6 Representative sections ($\times 100$ magnification) of distal colon from (A) “young” and (B) “old” patients, demonstrating basolateral membrane localisation of Na⁺, K⁺-ATPase along the surface cell-crypt cells axis by immunocytochemistry, using the specific α_1 isoform antibody (see methods). Haematoxylin was used throughout as a counterstain. In negative controls (not shown), there was no staining in the absence of the specific α_1 isoform antibody.

DISCUSSION

Advancing age leads to hormonal changes that decrease the ability of the kidneys to conserve Na⁺.^{1-5 7 8} Human descending colon, sigmoid colon, and rectum possess the same aldosterone sensitive electrogenic Na⁺ absorptive process as

the renal distal tubule and cortical collecting duct. The capacity of the distal colon for Na⁺ conservation might also be diminished in the elderly, particularly after sigmoid colectomy for cancer or diverticular disease. The present study explored the effect of age on expression of key proteins required for electrogenic Na⁺ transport, and compared basal and mineralocorticoid stimulated electrogenic Na⁺ absorption in “young” and “old” patients.

Unlike studies on autopsy material,¹³ we found age had no effect on distal colonic epithelial morphology. Human distal colon normally exhibits a modest level of electrogenic Na⁺ transport, which is readily enhanced by corticosteroid hormones.^{19 38} Aldosterone sensitive Na⁺ channels located in the apical membrane of surface colonocytes control Na⁺ diffusion from faecal water into surface cells, whereas Na⁺, K⁺-ATPase mediated exchange of three Na⁺ ions for two K⁺ ions at the basolateral membrane accounts for the electrogenic nature of this process. Using in situ hybridisation, specific Na⁺ channel α , β , and γ subunit mRNAs were localised to surface cells of the distal colon, their levels of

Figure 7 (A) Representative western blots of protein (50 μ g per lane) from distal colonic biopsies from three “young” (Y) and three “old” (O) patients, using the specific Na⁺, K⁺-ATPase α_1 isoform antibody (see methods). Rat kidney (K; 10 μ g protein per lane) served as a positive control. (B) Mean (SEM) band densities, using rat kidney as the comparator. Results from 14 “young” and 14 “old” patients are depicted. Results from the two groups of patients were not significantly different.

Table 1 Effect of fludrocortisone on amiloride sensitive rectal electrical potential difference (PD) in “young” and “old” patients			
Rectal PD (mV) “Young” patients (n=5)		Rectal PD (mV) “Old” patients (n=5)	
Pre fludrocortisone		Pre fludrocortisone	
Basal −36.9 (3.8)		Basal −41.8 (7.9)	
+Amil −22.3 (2.8)		+Amil −22.9 (5.0)	
Δ −14.6 (3.3)		Δ −18.9 (7.4)	
p<0.015		p<0.03	
Post fludrocortisone		Post fludrocortisone	
Basal −34.6 (3.0)		Basal −48.1 (7.9)	
+Amil −7.5 (1.7)		+Amil −14.7 (5.4)	
Δ −27.1 (2.4)*		Δ −33.4 (6.4)*	
p<0.0005		p<0.01	

+Amil, 1 mmol/l amiloride present in the rectal dialysis bag;
 Δ , difference between basal and +Amil value.
*p<0.05 compared with pre fludrocortisone value.

expression being similar in the "young" and "old" patients. Immunocytochemistry and western blot analyses also revealed similar levels of Na⁺ channel α , β , and γ subunit proteins localised mainly in surface cells in both groups. The apparently patchy distribution of all three Na⁺ channel subunit proteins within the apical membrane of surface cells probably reflected relatively low levels of basal electrogenic Na⁺ transport and the presence of mucus secreting cells within the surface epithelium. In the case of Na⁺, K⁺-ATPase, northern blot analyses demonstrated similar levels of α_1 and β_1 isoform mRNAs in the "young" and "old" patients. Immunocytochemistry indicated a uniform distribution of the Na⁺, K⁺-ATPase α_1 isoform protein within the basolateral membrane along the surface cell-crypt cell axis, its level of expression appearing to be independent of age. Western blot analyses of the α_1 isoform, which is the most important isoform in terms of epithelial Na⁺ transport, confirmed this impression.

Our studies indicate that apical Na⁺ channel and basolateral Na⁺, K⁺-ATPase expression in human distal colon and rectum remains constant during the aging process. To evaluate the effects of age on Na⁺ transport proteins at a functional level, we measured rectal PD before and 24 hours after treatment with the mineralocorticoid agonist fludrocortisone in "young" and "old" patients, equating the amiloride sensitive PD with electrogenic Na⁺ transport. Before fludrocortisone treatment, luminal amiloride elicited similar decreases in rectal PD in the two groups. After fludrocortisone, the amiloride induced changes in PD were substantially greater but nevertheless were similar in the "young" and "old" patients. Our data therefore provide a clear indication that both basal and mineralocorticoid enhanced electrogenic Na⁺ absorptive function in human rectum (and presumably within the rest of the distal colon) is unaffected by age. Studies in rat distal colon have shown that nanomolar concentrations of aldosterone stimulate amiloride sensitive electrogenic Na⁺ transport after eight hours, an effect which coincides with increased expression of Na⁺ channel β and γ subunit mRNAs.⁵³ This leads to enhanced localisation of the Na⁺ channel $\alpha\beta\gamma$ heteromeric complex in the apical membrane of surface cells.⁴² It therefore seems likely that the ability of fludrocortisone to stimulate similar levels of amiloride sensitive electrogenic Na⁺ transport in the "young" and "old" patients reflected increased expression of the Na⁺ channel β and/or γ subunits. It is highly unlikely that basolateral Na⁺, K⁺-ATPase levels would have increased so quickly after fludrocortisone, as this change is not seen for seven days in rats fed a Na⁺ free diet to induce secondary hyperaldosteronism.⁵⁴ However, we cannot exclude the possibility that fludrocortisone may have stimulated the turnover rate of pre-existing Na⁺, K⁺-ATPase mediated Na⁺ pumps.

The distal nephron and distal colon share key proteins involved in electrogenic Na⁺ transport and are target epithelia for aldosterone. Renal Na⁺ conservation decreases with age due, at least in part, to a diminution of the renin-angiotensin-aldosterone axis and probably also impaired aldosterone sensitivity of aging cells in the distal tubule and cortical collecting duct. By contrast, age has no effect on basal expression of Na⁺ transport proteins (Na⁺ channels and Na⁺, K⁺-ATPase) in the distal colon and rectum, or their response to mineralocorticoid stimulation. This may reflect constant renewal of aldosterone sensitive surface cells in the distal colon. In any event, retention of mineralocorticoid responsive electrogenic Na⁺ transport in the distal colon and rectum is likely to play an important role in maintaining Na⁺ homeostasis in the elderly, in whom renal Na⁺ handling is declining.

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