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Original article

Sodium transport across the caecal and colonic epithelium of germfree and specific-pathogen free rats

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Summary — It was assumed that the enlarged caecum and the accumulation of semiliquid contents in germfree rats is accompanied by changes in sodium absorption. Transepithelial sodium fluxes were studied under Ussing chamber conditions across epithelial sheets of the caecum and colon of germfree (GF) and specific-pathogen free (SPF) rats. Net sodium transport was highest in the proximal colon and in the proximal segment of the distal colon; it was considerably lower in the caecum and in the distal segment of the distal colon. In the caecum and proximal colon of the GF rats, the electroneutral sodium absorption was increased as compared to the SPF rats. In the proximal segments of the distal colon, no differences were seen. In the distal segment of the distal colon, the mainly electroneutral sodium transport in the SPF rats was changed into electrogenic transport in the GF rats. These differences may be due to the increased aldosterone levels of the GF rats.

germfree / specific-pathogen free / rat / caecum / colon / sodium transport / in vitro

Résumé — Transport du sodium à travers l'épithélium cæcal et colique chez des rats axéniques ou sans germes pathogènes. *L'hypothèse initiale du présent travail était que des modifications de l'absorption du sodium accompagnent l'élargissement du cæcum et l'accumulation de contenus semiliquides chez des rats axéniques. Les flux transépithéliaux de sodium ont été étudiés, dans les conditions de chambre d'Ussing, à travers des parois épithéliales de cæcum et de côlon de rats axéniques (GF) ou sans germes pathogènes (SPF). Le transport net du sodium était le plus élevé dans le côlon proximal et dans le segment proximal du côlon distal ; il était considérablement plus faible dans le cæcum et dans le segment distal du côlon distal. L'absorption électroneutre du sodium dans le cæcum et le côlon proximal était augmentée chez les rats GF par rapport aux rats SFP. Aucune différence n'a été observée dans les segments proximaux du côlon distal. Dans le segment distal du côlon distal, le transport du sodium, surtout électroneutre chez les rats SFP, devenait électrogénique chez les rats GF. Ces différences pourraient être dues aux niveaux accrus d'aldostérone chez les rats GF.*

axénique / sans germe pathogène / rat / cæcum / côlon / transport du sodium / in vitro

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INTRODUCTION

The most obvious differences between germfree (GF) and conventional animals relate to the gastrointestinal tract. In the gastrointestinal tract of the GF animals a marked distension of the lower bowel, reduced muscle tone, accumulation of semiliquid contents and a constant mild diarrhea are common characteristics (Gordon and Bruckner, 1984). This is particularly evident in the GF rodents where an enlarged caecum is found, which together with its contents may attain 30% of the animal's body weight (Loesche, 1969). After the introduction of either a conventional bacterial flora or a single bacterial species into the intestinal tract, these anomalies disappeared within a few weeks (Asano, 1967; Große-Siestrup, 1991).

Inhibition of water absorption from the lower bowel appears to play a major role in the development of GF anomalies. An accumulation of nondegraded mucus and food carbohydrate leads to a high colloid osmotic pressure in the GF intestinal contents, and this may be responsible for the inhibition of water absorption (Gordon and Nakamura, 1975). Due to the presence of unabsorbable anions (primarily acid mucopolysaccharides), the diffusible cations, mostly sodium, are retained in the intestinal lumen. This in turn diminishes the solute coupled water absorption (Asano, 1969). When the natural contents of the GF animal's caecum are replaced with saline during in an in vivo experiment, water absorption becomes normal or is even greater than in conventional controls (Gordon and Wostmann, 1973; Donowitz and Binder, 1979). In the GF rats the turnover of bile acids and steroid hormons is reduced to half the normal level (Gustafsson, 1982), and a considerable excess of kallikrein-like peptides are present in the hindgut contents (Gordon, 1967). These substances are known to have large effects on transepithelial electrolyte transport (Binder and Sandle, 1987). In the caecum of the GF rats, sodium is absorbed to a greater extent and against a higher transepithelial sodium gradient than in conventional rats (Nakamura and Gordon, 1973). An increase in basolateral Na^+/K^+-ATPase activity may be responsible for this (Simonetta et al, 1975).

To date, no detailed evaluation of transepithelial electrolyte transport in the GF rat intestine has been reported. It was the aim of this study to compare sodium transport across the caecal and segments of the colonic epithelium of the GF and conventional rats, and factors and mechanisms that may affect differences in sodium transport were considered.

METHODS

Animals and preparations

The experimental animals were male germfree (GF) rats and specific-pathogen free (SPF) rats of the inbred rat strain AS-Ztm (Zentrales Tierlaboratorium der Medizinischen Hochschule, Hannover, Germany), weighing between 182–393 g. The GF rats were raised in plastic isolators (modifications of the apparatus described by Trexler and Reynolds [1957]). Supplies were brought into the isolators after vapour autoclaving in steel cylinders. The collected faeces of the GF rats were tested for bacterial contamination every 4 weeks. The SPF rats were free from pathogens and parasites stated in the 'list of specified pathogens in SPF laboratory animals' (Kunstyr, 1988). All rats were fed a vapour autoclaved standard diet (Altromin No 1314 fortified, Altromin, Lage, Germany). Water and food were available ad libitum. The animals were maintained on a 12 h light:12 h dark photoperiod. For transport of the GF rats, an autoclaved glass isolator was used. Both the GF and SPF rats were killed by decapitation within 40 min after being removed from their cages. This occurred between 0800 and 0900 hours.

The caecum, proximal and distal colon were removed. The distal colon corresponded to a gut segment about 3 cm long located proximal to a large lymphoid plaque that was, on average,

3.25 cm ahead of the anus. The gut segments were flushed with cold Ringer solution to remove luminal contents and placed in ice-cold Ringer solution that was gassed continuously with a mixture of 95% O_2 and 5% CO_2. Since prostaglandins are known to influence electrolyte transport across the hindgut epithelium (Smith et al, 1981; Diener and Rummel, 1990), indomethacin (10^{-6} M) was routinely added to all solutions to inhibit endogenous prostaglandin formation.

The proximal colon (starting from the caecum) and the distal colon (starting from the rectum) were cut into two 1.5 cm-long pieces, respectively, and then opened along the mesenteric border. The caecum was opened along the mesenteric border as well and then cut into pieces. Two adjacent tissues from the caecum, proximal and distal colon were mounted for each animal. In the distal colon, findings from the two adjacent tissues were significantly different and were therefore evaluated separately. The two tissues are designated as being either the proximal and distal segment of distal colon.

The muscle layers were manually dissected with forceps ('partial mucosal strip'). The mucosal sheets were mounted in Ussing chambers with an exposed surface area of 1.13 cm^2 for the caecum and proximal colon, and 0.50 cm^2 for the distal colon. A thin layer of silicon grease (Baysilon, Bayer AG, Leverkusen, Germany) on the chamber reduced edge damage. The tissues were incubated with 10 ml Ringer solution (37 °C) on both sides. The solution was circulated by a gas lift system using the 95% O_2 –5% CO_2 mixture. In order to diminish bacterial contamination of the GF epithelium, vapour autoclaved instruments and disposable products were used for the tissue preparation. In addition, surgical masks and disposable gloves were used.

Electrical measurements

Each chamber was connected to an automatic, computer-controlled voltage-clamp amplifier (AC Copy, Aachen, Germany). Fluid resistance and self-potential of the electrodes were determined before mounting the tissues and automatically corrected during the experiment. Transepithelial potential difference (V_t) was measured with Ringer-agar bridges connected to Ag^-/AgCl electrodes in 3 M KCl and referenced to the mucosal solution. Short-circuit current (I_{sc}) was passed through Ringer-agar bridges connected to Ag^-/AgCl electrodes in 3 M KCl. I_{sc} was considered positive for cation flow from the mucosal to the serosal side.

Initially, the mounted tissues were left under open-circuit conditions for about 30 min. The transepithelial conductance (g_t) was determined each minute by bipolar current pulses of 100 $\mu A.cm^{-2}$ and 500 ms duration. All electrical parameters (V_t, I_{sc} and g_t) were printed out at intervals of 1 min.

Isotopic measurements

All isotope experiments were carried out under short-circuit conditions. Pairs of tissues of similar g_t from the caecum, proximal colon and distal colon were selected to measure mucosal-to-serosal (J_{ms}) and serosal-to-mucosal (J_{sm}) fluxes. In the distal colon, pairing was done within the proximal and distal segments separately. Following this, 5 µCi of [$^{22}Na^+$] (Amersham Buchler, Braunschweig, Germany) was added to either the mucosal or serosal solution. After an equilibrium period of 30 min, 0.5 ml aliquots were taken at 10 min intervals from the solution in which the isotopes had not been initially added. The sample volume was replaced by an equal volume of unlabelled solution. This was taken into account in the flux calculations.

Solution and drugs

All chemicals were of analytical grade (Merck, Darmstadt, Germany). The standard Ringer solution contained (mM) 140 Na^+, 124 Cl^-, 21 HCO_3^-, 5.4 HPO_4^{2-}, 0.6 $H_2PO_4^-$, 1.2 Mg^{2+}, 1.2 Ca^{2+} and 10 glucose. Osmolality was adjusted in all solutions to 300 $mosmol.L^{-1}$ with mannitol; pH was adjusted to 7.4. In the case of the GF rats, the Ringer solution was prepared with sterile aqua destillata and contained 2.5 $mg.L^{-1}$ penicillin G (potassium salt) [1 570 $E.mg^{-1}$] and 6 $mg.L^{-1}$ streptomycinsulfate [750 $E.mg^{-1}$] (Serva, Heidelberg, Germany). The Ussing chambers were rinsed with this solution prior to beginning the experiments.

Amiloride hydrochloride and ouabain were successively added to the mucosal or serosal solution. Before and after the addition of amiloride, three samples were taken and after addition of ouabain, six samples were taken. All drugs were purchased from Sigma, Deisenhofen, Germany.

Statistics

Differences were estimated using Student's paired or unpaired two-sided *t*-test, where appropriate.

RESULTS

Sodium fluxes and electrical parameters under control conditions

Under control conditions, J^{Na}_{ms} exceeded J^{Na}_{sm} in all segments of both test groups, resulting in a net sodium absorption. The highest absorptive rates were measured in the proximal colon and in the proximal segment of the distal colon.

Caecum and proximal colon

In the caecum (table I) of the GF rats, J^{Na}_{ms} was 79% higher and J^{Na}_{net} was three times larger than in the caecum of the SPF rats, while the electrical parameters were the same. In the proximal colon (table II), J^{Na}_{ms} and J^{Na}_{net} values of the GF rats were also increased by 46 and 79%, respectively, in comparison to the SPF rats. At

Table I. Effects of amiloride and ouabain, successively added to the mucosal (m) or serosal (s) solution, on unidirectional sodium fluxes (mucosal to serosal, J_{ms}; serosal to mucosal, J_{sm}) and net sodium fluxes (J_{net}), on short-circuit current (I_{sc}) and transepithelial conductance (g_t) across caecal mucosa of specific-pathogen free (SPF) rats in comparison to germfree (GF) rats.

	Caecum	
	*SPF rats (14) **	*GF rats (25) **
Control		
J^{Na}_{ms}	6.83 ± 0.25 ^{aA}	12.20 ± 0.44 ^{aB}
J^{Na}_{sm}	4.65 ± 0.21 ^{aA}	5.47 ± 0.16 ^{aB}
J^{Na}_{net}	2.18 ± 0.16 ^{aA}	6.73 ± 0.37 ^{aB}
I_{sc}	0.45 ± 0.10 ^{aA}	0.63 ± 0.04 ^{aA}
g_t	6.38 ± 0.29 ^{aA}	7.07 ± 0.23 ^{aA}
m Amiloride (10^{-3} mol.L^{-1})		
J^{Na}_{ms}	4.96 ± 0.20 ^{bA}	6.72 ± 0.28 ^{bB}
J^{Na}_{sm}	3.90 ± 0.19 ^{bA}	4.35 ± 0.16 ^{bA}
J^{Na}_{net}	1.06 ± 0.14 ^{bA}	2.37 ± 0.21 ^{bB}
I_{sc}	0.50 ± 0.08 ^{bA}	0.66 ± 0.03 ^{bB}
g_t	5.76 ± 0.23 ^{bA}	6.48 ± 0.21 ^{bB}
s Ouabain (10^{-3} mol.L^{-1})		
J^{Na}_{ms}	5.64 ± 0.28 ^{cA}	7.47 ± 0.25 ^{cB}
J^{Na}_{sm}	5.66 ± 0.35 ^{cA}	6.89 ± 0.19 ^{cB}
J^{Na}_{net}	−0.03 ± 0.26 ^{cA}	0.58 ± 0.18 ^{cA}
I_{sc}	0.55 ± 0.06 ^{abA}	0.49 ± 0.02 ^{cA}
g_t	9.64 ± 0.53 ^{cA}	12.88 ± 0.40 ^{cB}

Means ± SEM are given. * Number of tissues used. Rows with the same superscripts are not significantly different ($P \leq 0.05$): ^{abc} comparison of drug effects within the individual parameters J_{ms}, J_{sm} and J_{net}; ^{AB} difference between SPF and GF rats.

Table II. Effects of amiloride and ouabain, successively added to the mucosal (m) or serosal (s) solution, on unidirectional sodium fluxes (mucosal to serosal, J_{ms}; serosal to mucosal, J_{sm}) and net sodium fluxes (J_{net}), on short-circuit current (I_{sc}) and transepithelial conductance (g_t) across the mucosa of the proximal colon of specific-pathogen free (SPF) rats in comparison to germfree (GF) rats.

	Proximal colon	
	*SPF rats (14) **	*GF rats (23) **
Control		
J^{Na}_{ms}	12.51 ± 0.43 ^{aA}	18.25 ± 0.40 ^{aB}
J^{Na}_{sm}	4.91 ± 0.24 ^{aA}	4.68 ± 0.27 ^{aA}
J^{Na}_{net}	7.60 ± 0.55 ^{aA}	13.58 ± 0.36 ^{aB}
I_{sc}	0.68 ± 0.03 ^{aA}	0.95 ± 0.04 ^{aB}
g_t	9.82 ± 0.68 ^{aA}	7.49 ± 0.44 ^{aB}
m Amiloride (10^{-3} mol.L^{-1})		
J^{Na}_{ms}	10.21 ± 0.40 ^{bA}	12.60 ± 0.34 ^{bB}
J^{Na}_{sm}	5.28 ± 0.36 ^{aA}	4.34 ± 0.26 ^{bB}
J^{Na}_{net}	4.94 ± 0.42 ^{bA}	8.26 ± 0.25 ^{bB}
I_{sc}	0.45 ± 0.03 ^{bA}	0.84 ± 0.03 ^{bB}
g_t	10.83 ± 0.76 ^{bA}	7.69 ± 0.43 ^{bB}
s Ouabain (10^{-3} mol.L^{-1})		
J^{Na}_{ms}	9.92 ± 0.33 ^{bA}	10.25 ± 0.36 ^{cA}
J^{Na}_{sm}	8.79 ± 0.31 ^{bA}	8.62 ± 0.34 ^{cA}
J^{Na}_{net}	1.13 ± 0.44 ^{cA}	1.63 ± 0.35 ^{cA}
I_{sc}	0.41 ± 0.03 ^{bA}	0.56 ± 0.03 ^{cB}
g_t	15.47 ± 0.63 ^{cA}	13.05 ± 0.42 ^{cB}

Means ± SEM are given. * Number of tissues used. Rows with the same superscripts are not significantly different ($P \leq 0.05$): ^{abc} comparison of drug effects within the individual parameters J_{ms}, J_{sm} and J_{net}; ^{AB} difference between SPF and GF rats.

the same time, the I_{sc} of the GF rats was 41% higher and g_t was 24% lower than in the SPF rats.

Distal colon

In the distal colon (tables III and IV), no differences in sodium fluxes were observed between both groups. In the proximal segment, I_{sc} was not significantly different between the SPF and GF rats. In the distal segment of the distal colon, however, I_{sc} was 2.5 times higher in the GF rats compared to the SPF rats. I_{sc} accounted for 24% (SPF rats) and 46% (GF rats) of the net absorptive sodium fluxes, respectively. In both groups, J^{Na}_{net} of the distal segment of distal colon made up only 32–40% of the proximal segment values. At the same time, I_{sc} of the distal segment exceeded the value of the proximal segment, particularly in the GF rats.

Effects of amiloride on sodium fluxes and electrical parameters

Amiloride (10^{-3} M) was added to the luminal solution to inhibit the apical electrogenic and electroneutral sodium transport.

Table III. Effects of amiloride and ouabain, successively added to the mucosal (m) or serosal (s) solution, on unidirectional sodium fluxes (mucosal to serosal, J_{ms}; serosal to mucosal, J_{sm}) and net sodium fluxes (J_{net}), on short-circuit current (I_{sc}) and transepithelial conductance (g_t) across the mucosa of the proximal segment of the distal colon of specific-pathogen free (SPF) rats in comparison to germfree (GF) rats.

	Distal colon, proximal segment	
	*SPF rats (7) **	*GF rats (11) **
Control		
J^{Na}_{ms}	14.98 ± 1.17 ^{aA}	14.66 ± 1.83 ^{aA}
J^{Na}_{sm}	4.42 ± 0.80 ^{aA}	3.89 ± 0.45 ^{aA}
J^{Na}_{net}	10.56 ± 1.58 ^{aA}	10.77 ± 1.63 ^{aA}
I_{sc}	0.52 ± 0.06 ^{aA}	0.45 ± 0.08 ^{aA}
g_t	7.34 ± 1.24 ^{aA}	6.37 ± 0.69 ^{aA}
m Amiloride (10^{-3} mol.L^{-1})		
J^{Na}_{ms}	9.07 ± 1.01 ^{bA}	7.56 ± 0.88 ^{bA}
J^{Na}_{sm}	2.74 ± 0.52 ^{bA}	2.88 ± 0.34 ^{bA}
J^{Na}_{net}	6.33 ± 1.31 ^{bA}	4.69 ± 0.73 ^{bA}
I_{sc}	0.55 ± 0.07 ^{aA}	0.20 ± 0.02 ^{bB}
g_t	5.88 ± 0.80 ^{bA}	5.98 ± 0.58 ^{bA}
s Ouabain (10^{-3} mol.L^{-1})		
J^{Na}_{ms}	5.30 ± 0.23 ^{cA}	5.55 ± 0.43 ^{cA}
J^{Na}_{sm}	4.16 ± 0.62 ^{aA}	4.38 ± 0.46 ^{aA}
J^{Na}_{net}	1.14 ± 0.61 ^{cA}	1.17 ± 0.38 ^{cA}
I_{sc}	0.62 ± 0.10 ^{aA}	0.24 ± 0.02 ^{bB}
g_t	6.18 ± 0.79 ^{abA}	7.12 ± 0.57 ^{aA}

Means ± SEM are given. * Number of tissues used. Rows with the same superscripts are not significantly different ($P \leq 0.05$): ^{abc} comparison of drug effects within the individual parameters I_{sc} and g_t; ^{AB} difference between SPF and GF rats.

Caecum and proximal colon

In the caecum and proximal colon (tables I and II), amiloride diminished J^{Na}_{ms} in the GF rats almost twice as much as in the SPF rats. J^{Na}_{ms} of the GF rats decreased by 45 and 31%, the J^{Na}_{ms} of the SPF rats by 27 and 18%, respectively. Except for the proximal colon of the SPF rats, a small decrease of J^{Na}_{sm} was apparent. As a result, J^{Na}_{net} dropped to 35–49% (caecum) and 61–65% (proximal colon) of control values. In addition, amiloride diminished I_{sc} of the proximal colon by 33% (SPF rats) and 12% (GF rats).

Distal colon

Amiloride reduced J^{Na}_{ms} in both test groups and in both segments of distal colon by 37–48% (tables III and IV). Only in the proximal segment did J^{Na}_{sm} decrease by 26–38%. This led to a decrease of J^{Na}_{net} by 40% (SPF rats) and 57% (GF rats) in the proximal segment and by 77–80% in the distal segment, respectively; J^{Na}_{net} was not significantly different from zero in the distal segment of the SPF rats.

Furthermore, amiloride had pronounced effects on I_{sc} in the distal segment of the distal colon in the GF rats. The compara-

Table IV. Effects of amiloride and ouabain, successively added to the mucosal (m) or serosal (s) solution, on unidirectional sodium fluxes (mucosal to serosal, J_{ms}; serosal to mucosal, J_{sm}) and net sodium fluxes (J_{net}), on short-circuit current (I_{sc}) and transepithelial conductance (g_t) across the mucosa of the distal segment of the distal colon of specific-pathogen free (SPF) rats in comparison to germfree (GF) rats.

	Distal colon, distal segment	
	SPF rats (7) *	*GF rats (11)* *
Control		
J^{Na}_{ms}	6.78 ± 0.81 ^{aA}	7.15 ± 0.71 ^{aA}
J^{Na}_{sm}	3.39 ± 0.52 ^{aA}	2.89 ± 0.28 ^{aA}
J^{Na}_{net}	3.39 ± 0.69 ^{aA}	4.26 ± 0.55 ^{aA}
I_{sc}	0.80 ± 0.09 ^{aA}	1.95 ± 0.32 ^{aB}
g_t	6.28 ± 0.88 ^{aA}	7.09 ± 0.69 ^{aA}
m Amiloride (10^{-3} mol.L^{-1})		
J^{Na}_{ms}	4.30 ± 0.47 ^{bA}	4.24 ± 0.35 ^{bA}
J^{Na}_{sm}	3.61 ± 0.40 ^{aA}	3.25 ± 0.34 ^{bA}
J^{Na}_{net}	0.69 ± 0.38 ^{bA}	0.99 ± 0.27 ^{bA}
I_{sc}	0.53 ± 0.06 ^{bA}	−0.11 ± 0.07 ^{bB}
g_t	6.62 ± 0.87 ^{aA}	6.76 ± 0.58 ^{aA}
s Ouabain (10^{-3} mol.L^{-1})		
J^{Na}_{ms}	3.39 ± 0.35 ^{cA}	3.61 ± 0.27 ^{cA}
J^{Na}_{sm}	4.22 ± 0.39 ^{bA}	3.94 ± 0.32 ^{cA}
J^{Na}_{net}	−0.83 ± 0.41 ^{cA}	−0.33 ± 0.24 ^{cA}
I_{sc}	0.51 ± 0.05 ^{bA}	0.05 ± 0.04 ^{cB}
g_t	6.08 ± 0.70 ^{aA}	6.76 ± 0.47 ^{aA}

Means ± SEM are given. * Number of tissues used. Rows with the same superscripts are not significantly different ($P \leq 0.05$): ^{abc} comparison of drug effects within the individual parameters I_{sc} and g_t; ^{AB} difference between SPF and GF rats.

tively high I_{sc} in the distal segment of the GF rats was completely blocked by amiloride. In the distal segment, the amiloride sensitive I_{sc} accounted for 48% of the control J^{Na}_{net} in the GF rats and for 8% in the SPF rats. In the proximal segment of the GF rats and in the distal segment of the SPF rats, a slight reduction of I_{sc} was seen. Amiloride had no marked effects on g_t in all segments of the large intestine, except for a modest decline in the proximal segment of the SPF rats.

Effects of ouabain on sodium fluxes and electrical parameters

Finally, ouabain (10^{-3} M) was added to the serosal solution.

Caecum and proximal colon

In the caecum and proximal colon (tables I and II) of both test groups, the addition of ouabain led to a drastic increase in g_t and to

an increase in J^{Na}_{sm}. In the caecum, J^{Na}_{sm} was only slightly elevated in both groups. In the proximal colon, J^{Na}_{ms} remained unchanged in the SPF group, while it further decreased by 19% in the GF group. As a result, J^{Na}_{net} dropped to zero in the caecum of the SPF rats. In the caecum of the GF rats and in the proximal colon of both groups after addition of ouabain, only a small sodium net absorption remained. I_{sc} reacted upon ouabain with a modest decline in the caecum and in the proximal colon of the GF rats. It remained unchanged in the SPF rats.

Distal colon

In the distal colon (tables III and IV), ouabain led to a further decrease of J^{Na}_{ms} by 27 and 42% in the proximal and 15 and 21% in the distal segment of the SPF and GF rats, respectively. At the same time, J^{Na}_{sm} increased slightly. Except for the proximal segment of the GF rats, ouabain reduced J_{net} to a value not significantly different from zero. In contrast to the caecum and the proximal colon, in the distal colon no or only a modest increase in g_t was observed after the addition of ouabain.

DISCUSSION

Sodium transport across the hindgut epithelium in conventional rats

In the proximal colon of rats, sodium is transported across the apical membrane by an electroneutral Na^+/H^+ exchange (Binder et al, 1986; Foster et al, 1986a, b). In the rat caecum, Escobar et al (1990) suggested a similar mechanism. Values for the caecum and the proximal colon of our SPF rats agree with those previously reported (Foster et al, 1986a; Escobar et al, 1990). The significant reduction of J^{Na}_{ms} and J^{Na}_{net} after adding amiloride without there being a major change in I_{sc} supports the idea of the presence of an apical Na^+/H^+ exchanger. These data do not, however, exclude the possibility of an amiloride nonsensitive electrogenic sodium transport in the rat caecum as has been observed in the rabbit caecum (Sellin et al, 1988). J^{Na}_{sm} in the rat colon is assumed to be due to passive paracellular transport (Gazitúa and Robinson, 1982; Sweiry and Binder, 1990). Consequently, in our experiment increasing g_t values occurred at the same time as increased J^{Na}_{sm} values.

In the proximal and distal segments of the distal colon, similar absorption rates for sodium have been observed in conventionally raised rats (Perrone and Jenks, 1984). However, in our SPF rats, J^{Na}_{net} was three times higher in the proximal segment as compared to the distal segment. Compared with other studies (Foster et al, 1983; Perrone and Jenks, 1984; Turnamian and Binder, 1989), sodium absorption in the proximal segment of the distal colon was higher in our SPF rats. The sodium absorption in the SPF rats was completely inhibited by mucosal amiloride and serosal ouabain, without there being corresponding changes in I_{sc}. We assume that the higher level of sodium absorption was most likely due to an increased activity of the Na^+/H^+ exchanger.

In the distal segment of the distal colon of the SPF rats, a slight amiloride sensitivity was observed. In other experiments with normally fed rats, no indications of sodium channels in the distal colon were observed (Sandle and McGlone, 1987; Rajendran et al, 1989). After an incubation of the rat 'colon descendence' for 7 h with dexamethasone, the level of electroneutral sodium absorption in the proximal section was enhanced, and in the distal section electrogenic sodium absorption was seen (Bridges et al, 1987). In the AS rat line, renal sodium absorption under an acute stress was more pronounced as compared to Wistar rats; in AS rats under control conditions, renal corticosterone

excretion was already high (Karstens, 1989). Corticosterone does not, however, induce electrogenic sodium absorption in the rat colon (Hierholzer et al, 1990). We thus assume that in our AS rats under control conditions, the sodium transport already had been influenced by a strain-specific higher plasma aldosterone level.

Sodium transport across the hindgut epithelium in GF rats

Findings on sodium absorption in the enlarged caecum of the GF rats are controversial. When an isotonic NaCl solution was introduced into the caecum of the GF rats, water, sodium and chloride absorption was similar (Donowitz and Binder, 1979) or double the rate (Loeschke and Gordon, 1970; Gordon and Wostmann, 1973; Nakamura and Gordon, 1973) in conventional rats. Donowitz and Binder (1979) explained the similar absorption rates with the comparatively small and equal volume that was introduced into the caecum of both groups.

The higher J^{Na}_{ms} and J^{Na}_{net} in the caecum and in the proximal colon of our GF rats as compared to SPF rats were significantly reduced by amiloride, without there being a major change in I_{sc}. We thus concluded that the higher sodium absorption was due to an increased activity of the apical Na^+/H^+ exchanger. The driving force for the apical Na^+/H^+ exchange is Na^+/K^+-ATPase located in the basolateral membrane. Inhibition of Na^+/K^+-ATPase finally abolished sodium netfluxes in the GF as well as in the SPF rats. This may indicate that in the caecum and in the proximal colon in the GF rats, the Na^+/K^+-ATPase activity may have increased. This possibility was already suggested in earlier observations of the ileum, caecum and colon in GF rats (Reddy, 1972; Simonetta et al, 1975).

A higher electroneutral sodium absorption in the proximal colon of the GF rats could be due to an increased glucocorticoid concentration as well as a mineralocorticoid one. Both are capable of inducing similar changes in the sodium transport levels in the proximal colon of rats (Turnamian and Binder, 1990). In our GF rats, I_{sc} in the distal segment of the distal colon was up to 2 $\mu Eq.cm^{-2}.h^{-1}$ higher than it was in the SPF rats. In sodium-depleted rats, however, I_{sc} increased up to 16 $\mu Eq.cm^{-2}.h^{-1}$ (Will et al, 1980). In the distal segment of the distal colon of the GF rats, sodium transport was mainly electrogenic; in the SPF rats, the electrogenic transport was considerably lower. It is known that in the distal colon, glucocorticoids stimulate the electroneutral sodium transport, whereas mineralocorticoids induce a change from electroneutral to electrogenic transport (Turnamian and Binder, 1989). Furthermore, in adrenalectomiced rats, Bastl et al (1992) have demonstrated that aldosterone and glucocorticoid had an antagonistic effect on sodium transport in the rat colon. Further indications of an increased plasma-aldosterone concentration in GF rats are a reduced urine flow, a high urine concentration (Lev et al, 1970) and heavier adrenals (Gordon et al, 1966).

Our studies underline the marked segmental differences in the magnitude of sodium transport along the hindgut of conventional and germfree rats. We conclude that the higher J^{Na}_{ms} and J^{Na}_{net} in the caecum and in the proximal colon in the GF rat are due to a stimulation of the electroneutral sodium transport, most likely itself due to an increased plasma-aldosterone level. In the proximal segment of the distal colon, sodium transport in both the GF and SPF rats was similar. Also in the distal segment of the distal colon, unidirectional as well as net sodium fluxes were not affected by GF conditions. In the distal segment, however, the mainly electroneutral sodium transport was changed into an electrogenic transport in the GF

rats, and this also may be due to an increase of aldosterone concentrations.

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