Circadian rhythm of apical Na-channels and Na-transport in rabbit distal colon

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Summary. In vivo and in vitro studies showed that electrogenic sodium transport in rabbit distal colon is modulated by aldosterone. It varies in a circadian rhythm; the external synchronizer is the light-dark cycle. The site of regulation was found to be in the apical membrane of colonic epithelial cells, in which the number of conducting sodium-channels is increased by aldosterone.
Key words. Rabbit; colon; in vivo; in vitro; potential difference; isotopes; noise analysis; sodium transport; apical sodium channels; plasma aldosterone.

Rabbit distal colon has served as a model for the investigation of the mechanisms of Na-transport across large intestinal epithelia[1]. Na-transport in this epithelium is entirely electrogenic, and can be measured by the short circuit current generated during the translocation of the ions. Recently it has been demonstrated that rabbit colonic function undergoes a circadian rhythmic process in elaborating a special nutrient-rich fecal fraction (cecotrophe). With a 2–3-fold higher Na-concentration, this fraction differs considerably in ion concentration from the normal colonic contents, and is reingested by the animal[2,3]. In addition, it was observed that several functional properties (fecal Na/K-ratio, rectal potential difference, plasma aldosterone concentration) vary in a rhythmic manner[2,4]. In order to search for a mechanism which may regulate colonic Na-absorption, we investigated under in vivo conditions the circulating aldosterone level and the transmural electrical potential difference in the distal colon and established that changes in these parameters show a circadian rhythm. The rhythm could be modified by use of hormonal agonists and antagonists or by changing the light-dark regime.

To verify possible differences in Na-transport, and in order to look for the possible site of regulation, we conducted in vitro studies on epithelial sheets of rabbit distal colon. They were isolated at certain times of the day, and mounted in Ussing chambers. In these experiments the specifity of the current was confirmed by measuring Na-transport directly with isotopes (^{22}Na) and by current fluctuation analysis. By shifting the light-dark cycle, and by the use of hormonal stimulation or blockage, we can readily show that Na-absorption in rabbit distal colon varies in a circadian rhythm. This depends on the light-dark regime of the environment, and is presumably modulated by endogenous changes in the level of adrenal steroids. Moreover, by the use of noise analysis, we have located the cellular site of regulation in the apical cell membrane, where the density of operational Na-channels varies with time over a 2-fold range.

For the in vivo experiments, the animals were maintained on a constant light-dark cycle (L:D = 12:12) with light on at 06.00 h, and free access to food and water. For the time shift experiments, the beginning of the light period was shifted from 06.00 to 02.00 h. In certain periods (usually every 2 h) blood was sampled from an i.v. catheter (which served also as connection to the reference electrode). The transepithelial electrical potential (PD) was measured via an agar/Ringer bridge inserted into the rectum. D-aldosterone (128 µg/kg, Ciba-Geigy) was injected i.m., and spironolactone (93 mg/kg, Boehringer Mannheim) was given orally. Plasma aldosterone concentration was measured by radioimmunoassay.

For the in vitro experiments, the experimental technique was similar to that described previously[5,6]. The epithelial layer of rabbit distal colon was removed from the underlying muscle layers by blunt dissection. For the flux studies four pieces of tissue were mounted in modified Ussing chambers with an area of 1.32 cm^2 exposed to the recirculating bathing solutions, and short-circuited by a microcomputer-controlled voltage clamp. A standard colon Ringer solution was used[5,7]. Isotopic Na-fluxes were measured and calculated by standard procedures[5]. For the noise analysis, the tissue was mounted in a chamber with 0.5 cm^2 area. Both types of chambers had special silicone seals to avoid edge damage. Series and tissue resistance were determined either by voltage deflections and Ohm's law or by AC-impedance analysis[8]. All experiments were carried out with solutions warmed to 37 °C and aerated by carbogen or in some experiments (where bicarbonate-free Ringer solution was used) by pure oxygen.

For noise analysis, a special low-noise voltage clamp was used[6,9]. The transepithelial voltage was clamped to zero, and the fluctuating component of the short circuit current was amplified, sampled and analyzed by a computer[6]. Current fluctuations were induced by low doses of mucosal amiloride (0.15–50 µM), generating a Lorentzian component in the current power density spectrum. A two-state model was assumed where the apical Na-channel is either fully open or totally closed by amiloride. From the characteristics of the Lorentzian component (plateau value, corner frequency) single channel current and Na-channel density could be calculated[10].

Figure 1 shows an example of the time dependency of the transmural electrical potential difference in the distal colon (PD) and the plasma aldosterone concentration (PA) obtained from one rabbit. Both parameters show a clear 24-h rhythm with low values at the beginning of the light period (06.00–10.00 h) and high values at the end of the light period (15.00–18.00 h). The PD varied between 30 and 50 mV (serosal side positive), and the PA level varied between 5 and

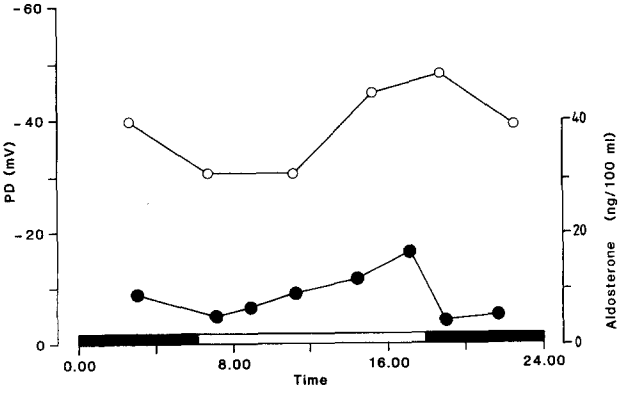

Figure 1. Representative example of the circadian rhythm of transmural electrical potential difference of the rabbit distal colon (○) (colonic lumen – blood, blood side = reference), and the plasma-aldosterone-concentration (●) over 24 h. L:D = 12:12, light on at 06.00 h.

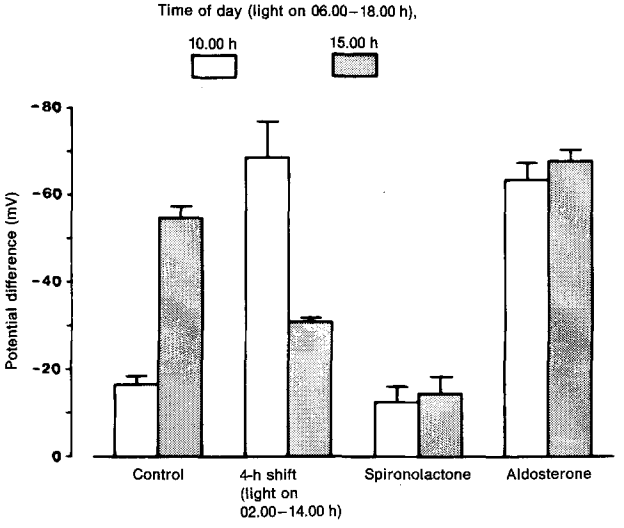

Figure 2. Transmural electrical potential difference (colonic lumen–blood, blood side = reference) of the rabbit distal colon under various experimental conditions. Numbers represent mean values ± SEM of 5–9 rabbits in each group.

18 ng/100 ml. The PA-rhythm preceeded the PD-rhythm by approximately 4 h. Similar variations of PA and PD have been obtained in 4 (PA) and 9 (PD) other rabbits under investigation.

Figure 2 shows the mean PD-values for all rabbits, obtained at 10.00 h and 15.00 h under the various experimental conditions. Under the normal light-dark cycle (light on 06.00–18.00 h), all rabbits had low PD-values (mean = − 16.2 ± 2.0 mV, n = 9) at 10.00 h. In the afternoon at 15.00 h, however, the PD of all rabbits had increased by a factor of three (mean value − 54.5 ± 3.2 mV, n = 9). A time shift of 4 h (light on 02.00–14.00 h) clearly shifted also the minima and maxima of the PD accordingly. Peak values were now observed in the morning (mean value − 68.0 ± 8.4 mV, n = 5), whereas in the afternoon the mean PD had already decreased to − 30.6 ± 1.8 mV (n = 5). These findings clearly demonstrate that the transmural electrical PD of rabbit distal colon varies in a circadian rhythm, with the external synchronizer being the light-dark cycle.

The following experiments were then designed to determine whether this PD-rhythm was dependent on the observed variations of the PA-levels (see fig. 1). In a first approach we used spironolactone, a well known aldosterone antagonist [11]. In order to obtain a complete blockage of the endogenous aldosterone-mediated effects on the PD, we loaded the rabbits orally with 93 mg/kg spironolactone, at 18.00 h the day before the PD-measurements, and again at 06.00 h at the day of measurements. This treatment resulted in a complete disappearance of the PD-rhythm. Mean PD-values at 10.00 h (− 12.3 ± 3.7 mV, n = 7) were no longer significantly different from measurements at 15.00 h (− 13.7 ± 5.1 mV, n = 7).

In a second series of experiments, we used exogenous aldosterone (2 times 64 µg/kg, i.m.), to stimulate the PD continuously. This aldosterone dose was chosen to ensure a maximal stimulatory effect [7]. The injections were given to rabbits under the normal light dark schedule, with the first injection at 06.00 h, and the second at 11.00 h. Figure 2 shows that aldosterone significantly increased the morning values 4-fold to − 63.3 ± 3.3 mV (n = 7), and also increased the afternoon values slightly to a mean of − 67.3 ± 3.4 mV (n = 7). Both aldosterone values are not significantly different from the control value for 15.00 h. From these two series of experiments we conclude that aldosterone modulates the electrogenic transport processes in rabbit distal colon in a time-dependent fashion.

To evaluate the nature of the electrogenic transport processes, we isolated colonic epithelial sheets of rabbits sacrificed at either 10.00 h or 15.00 h, and measured the electrical properties and unidirectional Na-fluxes under controlled conditions in a Ussing chamber. As shown in the table, two groups of epithelia can clearly be distinguished under in vitro conditions. Tissues taken at 10.00 h had a low potential difference (V_T), a low short circuit current (Isc) and a low net Na-absorption. In contrast, tissues taken at 15.00 h had a doubled Isc and net Na-absorption, and a 5-fold V_T.

With the noise analysis it was possible to trace the site of regulation to the apical membrane. Tissues with high Na-transport had twice the channel-density as compared to low transporting tissues, whereas the single channel current was not significantly different (table). This indicates that aldosterone modulates in a regular circadian rhythm the number of Na-conducting channels in the apical membrane of rabbit distal colonic cells. Additional experiments were conducted in both groups with mucosal amphotericin B in order to eliminate the apical membrane functionally, and to investigate the maximal transport capacity of the basolateral Na/K-pump [12]. These experiments gave similar maximal transport rates for low and high transporting tissues: Isc-L = 4.8 ± .4, Isc-H = 4.5 ± 0.6 µeq/cm²h (n = 6). No increase in pump capacity was seen. We therefore conclude that the basolateral Na/K-pump seems to operate in both groups within its normal physiological transport capacity, which is dependent on the rate of apical Na-entry into the cell [1, 12].

Although we have not yet investigated possible alterations of other basolateral transport systems, the apical membrane is obviously one major target site for the regulatory process of transepithelial Na-absorption. This is in accordance with the current transport model for this epithelium [1], and with the present knowledge about aldosterone action on transepithelial Na-transport [13–15]. However, the new and important finding is that the number of operational Na-channels in this membrane varies in a circadian rhythm in synchrony, and the variation is causally related to the circulating aldosterone concentration. As the time course of this on- and off-regulation is very short, it might be possible that the cellular metabolic mediators of the hormonal stimulus are not only synthesis and degradation of new channels [15], but may involve more rapid activation and inactivation of preexisting channels, as has been described recently [13]. The physiological rationale of this regulatory process could be the retention of sodium during periods when feces are normally discarded (period of high Na-transport), and a recycling of sodium

Electrical properties and Na-transport in low- and high-transporting tissues

	n	V_T (mV)	I_{sc} (µeq/cm²h)	J^{Na}_{net} (µeq/cm²h)	n	i (pA)	N (mill./cm²)
Low	51	− 7.1 ± 0.6	1.6 ± 0.6	1.6 ± 0.6	4	1.5 ± 0.1	25.2 ± 2.4
High	54	− 36.1 ± 1.3*	3.9 ± 0.1*	3.6 ± 0.9*	6	1.1 ± 0.1	59.8 ± 6.7*

Values are mean ± SEM, n = number of tissues, * = significantly different ($p < 0.001$) from low transporting tissues. V_T = transepithelial potential difference, I_{sc} = short circuit current, J^{Na}_{net} = net sodium absorption (measured by isotopes), i = single channel current, N = number of channels.

together with the products of cecal microbial digestion during the period of low Na-transport for a second passage through the gastrointestinal tract.

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Stimulation by D-glucose of mitochondrial respiration

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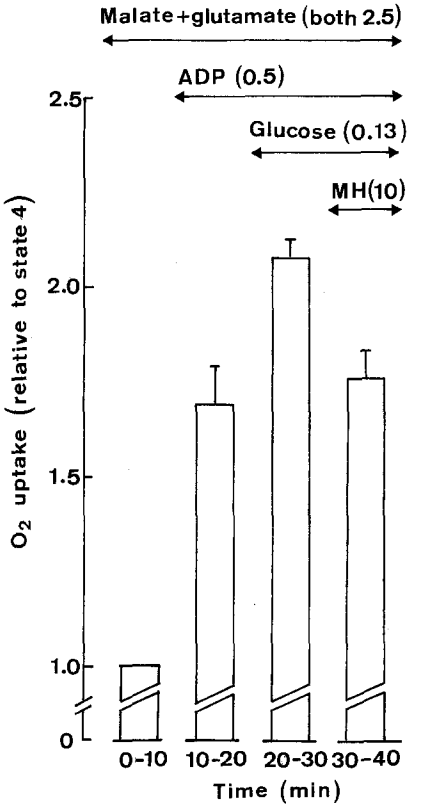
Summary. D-glucose increases O_2 uptake by cerebellum mitochondria. This effect is abolished by D-glucose-6-phosphate and D-mannoheptulose. It is proposed that the phosphorylation of D-glucose as catalyzed by bound hexokinase directly affects mitochondrial respiration.
Key words. Mitochondria; respiration; D-glucose.

In several cell types, e.g., in brain cells, a large fraction of hexokinase is bound to mitochondrial porin[1-3]. Bound hexokinase may use mitochondrial rather than cytosolic ATP as a substrate for D-glucose phosphorylation[4,5]. It was recently proposed that such a process may represent a novel pathway for the direct coupling of hexose phosphorylation to mitochondrial respiration[6]. In the present study, the validity of the latter proposal was assessed by examining the effect of D-glucose on O_2 uptake by isolated brain mitochondria.

Groups of two cerebella removed from fed albino rats were weighed (400–500 mg wet weight), minced and homogenized in Potter-Elvehjem tubes (20 strokes) with 2.2 ml of a Hepes-KOH buffer (10 mM, pH 7.4) containing sucrose (250 mM), KCl (20 mM) and EDTA (0.2 mM). After a first centrifugation for 5 min at 1000 × g and 4 °C, an aliquot (2.0 ml) of the supernatant was removed and centrifuged for 20 min at 4300 × g and 4 °C[7]. After removal of the supernatant, the pellet was washed twice and eventually resuspended in 0.5 ml of the same buffer. Aliquots (usually 0.1 ml each) were placed in the incubation chamber of a Gilson oxygraph in 1.6 ml of a Hepes-KOH buffer (40 mM, pH 7.4) containing KCl (100 mM), $MgCl_2$ (5 mM) and KH_2PO_4 (10 mM). The oxygraph was equipped with a YSI Clark oxygen electrode (Gilson Medical Electronics, Middleton, WI). Respiratory activity in the mitochondrial suspension was measured in the closed and magnetically stirred chamber at 37 °C, and was found to be proportional to the volume of mitochondrial suspension with a reproducibility of 4.6% (n = 12). Metabolites were added by introducing small volumes (20–40 µl) of appropriately concentrated solutions and the O_2 uptake measured over the ensuing 10 min. Calibration was performed over 30–60 s at 37 °C using freshly dissolved β-D-glucose in the presence of glucose oxidase, as described elsewhere[8]. All results are expressed as the mean (± SEM) together with the number of individual observations (n). The



Time course for O_2 uptake by rat cerebellum mitochondria. The effect of ADP, D-glucose and mannoheptulose (MH) were judged from paired comparison (n = 3 or more) of measurements performed over 10-min incubation in each case. The concentration of each metabolite is expressed as mM (in parentheses).