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Colonic water and ion transport under the influence of deoxycholic acid, theophylline and ethylenediaminetetraacetate in rats

Wpływ kwasu dezoksycholowego, teofiliny i EDTA na transport jonów i wody w okrężnicy szczurów

SUMMARY

The experiments (*in situ*) on and isolated colonic loop in rats aimed at indicating the changes in the secretion/absorption of electrolytes and water under the influence of such factors as deoxycholic acid (DCA), theophyline (T) and EDTA, in various ways changing the transport mechanisms in colon. Temporary changes of water volume in the studied loop and ion movement were calculated after finding the marker concentration of PEG 4000). Contrary to the control solution, both DCA and T caused the secretion of water and electrolytes to the colon. Combining the two applied compounds decreased the secretive response of colon. After applying EDTA only a decrease of water absorption was observed instead of secretion. DCA caused increased excretion of Na^+ and the greatest, of all the used substances, increase of Cl^- secretion, while T caused the greatest secretion of sodium, decreasing the secretion of chloride. On the other hand, EDTA caused a considerable increase of Na^+ and a slight increase of Cl^-. The received secretive responses point out that the applied substances affect various transport processes in colon. The effect of EDTA results from its properties chelating Ca^{2+} ions inside the cells. It should be supposed that only non-conjugated bile acids, including DCA, increase the filtration to the colon beside the cells, giving rise of microscopic injuries of mucosa.

Key words: deoxycholic acid, theophylline, EDTA, isolated colonic loop, sodium, potassium, calcium, chloride

INTRODUCTION

A wide range of causative agents like augmented level of bile salts [Nell *et al.* 1976, Ammon *et al.* 1983, Argenzio and Whipp 1983], chemicals [Karbach and Wanitschke 1984, Heinke *et al.* 1998] and endotoxins [Yates *et al.* 2001] from infection agents provokes intestinal barrier failure and diarrheal fluid secretion into the colonic lumen with the partition of different mechanisms and

influence on epithelium. Taking into account such variable reasons leading to the development of colonic diarrhea, in this study we sought the factors which are the most influential in development of diarrhogenic state in this part of intestine. Ethylenediaminetetraacetic acid (EDTA) as a chelator is capable of binding a wide variety of metals and only a small fraction of EDTA is absorbed [Pantzar *et al*. 1994]. The second factor deoxycholic acid (DCA) provokes colonic injury, both functionally and morphologically [Breuer *et al.* 1983].

The aim of the present study was to examine colony water movement under the influence of such solutes as DCA, theophylline (T) and EDTA and to examine electrolytes flux in the loop of rat colon subjected to the action of these solutes. To assess whether there are changes in the ion movement across colon during the exposition of colonic mucosa, we performed two kinds of experiments. In the beginning we introduced control saline solution to the colon four hours after introducing DCA or other modifiers.

MATERIAL AND METHODS

Experiments were performed on mature Wistar rats weighing 300–350 g. They were given a pellets ration and water *ad libitum* under a 14/10 hour light/dark cycle. Under continuous ketamine 90 mg/kg – xylasine 10 mg/kg anesthesia, a median laparotomy was made. After exteriorization and opening of the proximal portion of the colon, the lumen was cleansed with a warmed solution of 0.9% NaCl and infused at a rate 1 ml/min until the rectal effluent was clear. Then 10 cm long colon loop was prepared by instillation on proximal and distal part of colon two purse-string sutures. Each loop was filled with 6 ml one of four experimental solution in a random sequence. An initial sample was withdrawn after the solutions had been mixed (usually after 3 min) in the loop. The colon was then replaced and the abdomen temporally closed. Blood samples were taken, by cardiac puncture, at the beginning and at the end of each experiment. One hour after inoculation of the loop the rats were exanguinated by decapitation and fluid was recovered from the loops.

Experimental design

The experiment comprises five schedules of different solutions with the same 6 ml volumes:
1) control solution contained $NaCl/NaHCO_3$,
   (75% 100 mM NaCl + 25% 50 mM $NaHCO_3$) + PEG 4000,
2) 2 mM DCA + PEG 4000,
3) 2 mM DCA + 20 mM theophylline + PEG 4000,
4) 20 mM theophylline + PEG 4000,
5) 25 mM EDTA + PEG 4000.

Each solution contained equal quantity of PEG 4000 (0.635 mg/ml) as a volume marker [Hyden 1955]. There was a mean PEG 4000 recovery of 97% for the buffered saline solutions in control loops.

Calculations of net ion and water fluxes

The net change in loop volume during 1 h period was calculated with the formula

$$X = \left[\left(\frac{PEG_i \cdot V_i}{PEG_0} - V_0 \right) \cdot \frac{PEG_0}{PEG_f} \right] - 1$$

where:

X – net volume absorption or secretion;

V_0 – volume of 3 min time samples;

V_i – initial volume placed in the loop;
PEG_i, PEG_0 and PEG_f – PEG concentrations in the initial solution, 3 min time sample and final sample, respectively.

Net ion fluxes were calculated from the change in volume and ion concentrations between the zero-time and final samples.

The net change in loop volume during 1 h period was calculated with a simplified form of this formula:

$$X = 10 \cdot \frac{PEG_i}{PEG_f}$$

where:
10 – initiale volume placed in the loop;
PEG_i and PEG_f – PEG concentrations in the initial solution and final sample, respectively.

Secretion (positive values) or absorption (negative values) of ions were calculated according to formula:

$$F\ (flux) = ([ion]_i\ xv_i) - ([ion]_f\ xv_f)$$

where:
$[ion]_i$ – initial concentration of given ion;
v_i – initial concentration of colon volume;
$[ion]_f$ – final concentration of a given ion;
v_f – final volume of the colon.

Results are expressed as the mean ± SE. Statistical significance was assessed with Student's t-test for paired or unpaired observations. All the results discussed below are significant at the $P<0,05$ level.

Chemical analyses

Concentrations of PEG 4000 were determined with the method of Hyden [1955]. Concentrations of Na^+, K^+, Cl^- and Ca^{2+} were determined by jonoselective electrodes coupled with electronic jonometer (Orion 920A). Net ion fluxes were calculated from the change in volume and ion concentrations between the initial time and final samples.

RESULTS

Effects of deoxycholic acid, theophylline alone, in combining with DCA and EDTA on net water transport

The control solution of $NaCl/NaHCO_3$ introduced into the colonic loop provoked water absorption. Both DCA and theophylline (T) abolished this direction of water movement (Fig. 1). Consequently, as a result of their action the net water and Na^+, K^+ secretion was seen and it was more pronunced when these compounds were given separately (Tab. 1). Combining effects of DCA and T were less exaggerated. Under the influence of DCA final concentrations of both NA^+ and remaining cations in the lumen of colon were significantly augmented and higher than elicited water. Contrary to that, EDTA only decreased water absorption from colonic loops.

Effects of deoxycholic acid, theophylline alone, in combining with DCA and EDTA on colonic luminal composition

Introducing distillate water into the colonic loop (basal condition) evoked, except Cl^-, the secretion of all investigated cations (Na^+, K^+, Ca^{2+}).

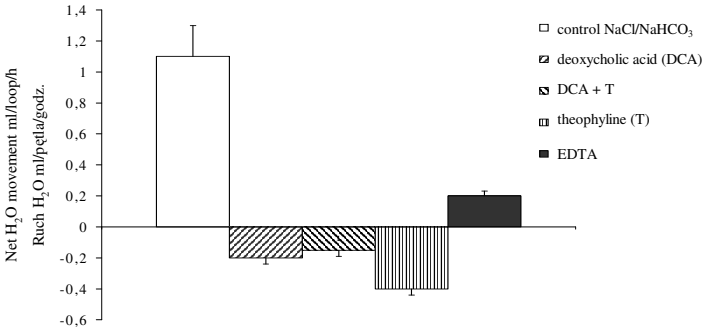

Fig. 1. Effect of experimental solutes on net water transport in colonic loops. Positive values designate net absorption; negative values, set secretion (±SEM, n = 4)
Rys. 1. Wpływ eksperymentalnych roztworów na transport wody netto w pętlach okrężnicy. Dodatnie wartości oznaczają wchłanianie netto; ujemne – wydzielanie netto

The range of changes of Na^+ under influence of experimental solutes was incomparable higher than changes in a concentration of Cl^- (Fig. 2 and 3). The response, induced by T, was depicted in a magnitude rise of Na^+ concentration and a drop in respect to Cl^- (Fig. 2 and 3). Under the influence of DCA and T, coincidently with augmentation of Na^+ and other analyzed cations in colonic luminal fluid, their decline in plasma was noted (Fig. 2 and 3). The only solute was able to induce significant ($p<0.05$) secretion of Na^+ and Cl^- to the loop of colon was DCA. The secretory effect evoked by this bile acid was suppressed by theophylline (Tab. 1). Electrolyte content in the final loop fluid under the influence of EDTA, was characterized by the greatest increase of Na^+, K^+, Ca^{2+} and a slight decline of Cl^-.

Table. 1. Net solute transport from rat colonic loops exposed to deoxycholic acid (DCA), theophylline (T), DCA+T, and ethylenediaminetetraacetate (EDTA). Positive values designate net absorption; negative values set secretion
Tabela 1. Transport roztworu netto z pętli okrężnicy u szczurów do DCA, T, DCA+T, EDTA. Dodatnie wartości oznaczają wchłanianie netto, ujemne – wydzielanie

Fluid component (per hour) Skład płynu (na godz.)	H_2O	DCA	DCA + T	T	EDTA
Na^+, mEq/loop	-2.99±0.25	-2.74±0.32*	-2.52 ± 0.29*	-3.53±0.19*	-1.53±0.43*
K^+, mEq/loop	-0.02±0.005	-0.07±0.01	-0.12 ± 0.04*	-0.16±0.02*	-0.14±0.03*
Cl^-, mEq/loop	-0.62±0.41	-0.34±0.12*	0.16 ± 0.05*	0.47±0.09*	0.99±0.19*
Ca^{2+}, mEq/loop	-0.02±0.004	-0.02±0.004	-0.01± 0.001	0.04±0.003*	-0.04±0.002

*Values are significantly ($p<0.05$) different from respective H_2O group, (n = 4).
*Wartości, które są istotnie ($p<0,05$) różne od odpowiednie grupy H_2O, (n = 4).

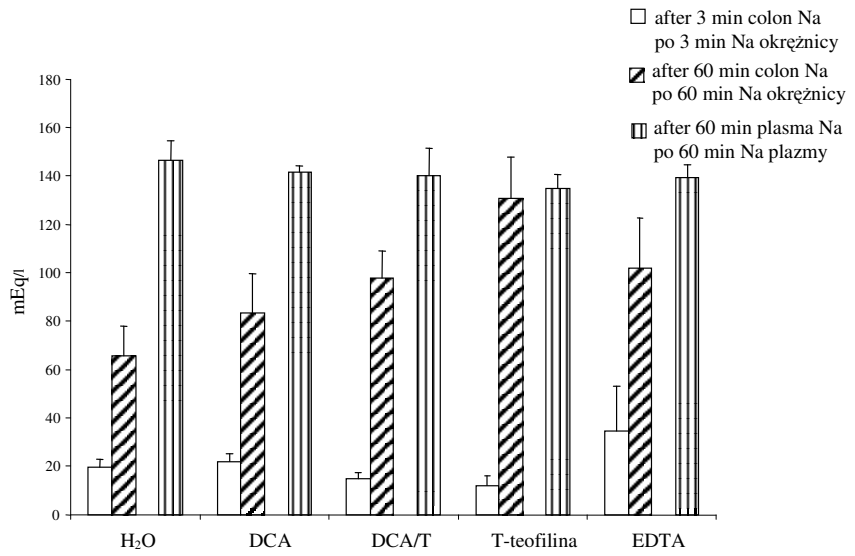

Fig. 2. Effect of DCA, DCA/T, T and EDTA on Na concentration in colon of rats (±SEM, n = 4)
Rys. 2. Wpływ DCA, DCA/T, T i EDTA na stężenie Na w okrężnicy szczurów

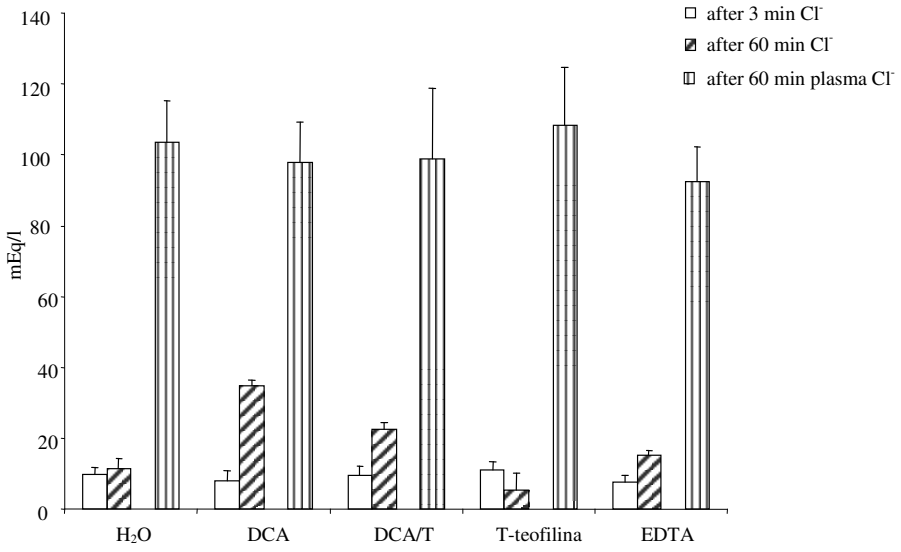

Fig. 3. Effect of DCA, DCA/T and EDTA on Cl concentration in colon (±SEM, n = 4)
Rys. 3. Wpływ DCA, DCA/T, T i EDTA na stężenie Cl w okrężnicy

DISCUSSION

In the normal colon water and electrolytes absorption dominates over secretion. About 50% of the extracellular fluid volume in the form of endogenous secretions is presented daily to the large intestine for absorption. Thus, colonic absorption alone appears to be critical in the maintenance of the extracellular fluid volume [Carpilli *et al*. 1995]. Under many diarrhogenic influences the excessive movement of these compounds is directed into the lumen of colon [Wanitschke *et al*. 1977]. Apart from colonization of colon by pathogenic microorganisms (*E. coli, Sallmonella typhimurium, Clostridium dificille),* an excessive amount of endogenic bile salts and many external solutes belong to the major cause of secretory diarrhea. These different kinds of reasons generate the same intracellular second messengers that lead to overstimulation of the secretory pathway [Edwards 1977, Cuthbert 1985]. The central role in secretory diarrhea is also played by the luminal cAMP-regulated Cl^- channel defined as a cystic fibrosis transmembrane conductance regulator (CFTR). Blocking luminal CFTR Cl^- channels would be the appropriate treatment for secretory diarrhea. Recently, specific water channels called aquaporins (AQP3, AQP4, AQP8) located in different parts of epithelial surface were implicated in colonic water permeability. However, although transepithelial water movement seems to be mediated by a special kind of aquaporins, their specific role is uncertain and have to be elucidated [Gelbmann *et al.* 1995]. AQP8 may play a dominant role, because it is expressed in absorptive columnar epithelial cells of colon.

The present findings showed that 3 employed solutes affect ions and water transport in a different way. Due to known chelating action of EDTA on Ca^{2+} ions, a small increase in secretion of Na^+, K^+ and Ca^{2+} but not Cl^- to the lumen of colon, was evoked. EDTA was shown [Argenzio and Whipp 1983] to alter mucosal permeability by chelation depletion of Ca^{2+} and Mg^{2+} and extensive mucosal damage with superficial erosion of the epithelium. Under such conditions a tissue hydrostatic pressure may be uncovered. This kind of pressure could attenuate net absorption and induced net water secretion to the loop of the colon with the most pronounced induction evoked by T. The highest increase in Na^+ secretion and lowest in Cl^- secretion to the lumen of colon were induced by T. Thus, the effects of theophylline on ion transport were strikingly different from those of EDTA or DCA.

The action of higher level of DCA in colon was different. It has been postulated that DCA when present in the colonic lumen at inappropriately high concentrations may induce secretory diarrhea in the colon [Hedgs *et al.* 1975, Mauricio *et al.* 2000]. DCA in low concentration (never higher than 1 mM), normally found within the colon, has a little effect on colonic structure or water absorption. On the other hand, abnormally high concentrations can alter colonic function [Hedgs *et al.* 1975, Wanitschke *et al.* 1977]. But the mechanism underlying the secretagogue activity of DCA has not been fully elucidated. Some authors postulated that the mechanism of DCA action on the colon may resemble the action of cholera toxin [Argenzio and Whipp 1983, Karbach and Wanitschke 1984]. While the secretory effect of dihydroxy bile acids, as has been shown in the presented results, was combined with chlorine secretion, and furthermore was inhibited by H1 histamine receptor antagonists and modified by the cyclooxygenase inhibitor indomethacin the responses to cholera toxin was quite opposite [Gelbmann *et al*. 1995]. Such contradictory responses led to the speculation that DCA causes fluid

secretion by filtration, whereas cholera toxin enhances the secretory activity of the epithelium. The obtained results also showed that higher than normal concentration of DCA (2 mM) turned the absorption of water, sodium, potassium and calcium to secretion. Other authors [Goerg *et al*. 1980] revealed that DCA increased reversibly the mucosal permeability, whereas cholera toxin decreased the colonic 51CrEDTA clearance. It is still possible that an elevated tissue pressure could drive a connective flow from blood to lumen. According to Karbach and Wanitschke [Karbach and Wanitschke 1984], transfer of water into colon is carried out by pressure-induced acting of DCA, predominantly on paracellular pathway. Furthermore, the secretory effect of bile salts is confined only to unconjugated bile salts [Breuer *et al*. 1983] which cause microscopic mucosal damage but their conjugated forms prevents the cathartic effect of α-dihydroxyl bile acid in the colon.

In conclusion, results of the presented study show that under the influence, of DCA, EDTA and T the secretion is more preponderant over absorption. Furthermore, the mechanism of their action is different. Inasmuch as T induces principally changes in electrical properties of mucosa, the response to EDTA depends on chelating effect but DCA provokes secretion by many different mechanisms. These results are not the consequence of an increase in mucosal permeability as determined by PEG 4000 clearances.

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STRESZCZENIE

Przeprowadzone doświadczenia (*in situ*) na izolowanej pętli okrężnicy szczurów zmierzały do wykazania zmian w wydzielaniu/wchłanianiu elektrolitów i wody pod wpływem takich czynników, jak kwas dezoksycholowy (DCA), teofilina (T) i kwas etylenodiaminotetraoctowy (EDTA), w różny sposób zmieniających mechanizmy transportowe w okrężnicy. Czasowe zmiany objętości wody w badanej pętli oraz ruch jonów wyliczono po oznaczeniu stężenia markerowego glikolu polietylenowego (PEG 4000). W przeciwieństwie do roztworu kontrolnego, zarówno DCA, jak i T powodowały wydzielanie wody i elektrolitów do okrężnicy. Połączenie obu stosowanych związków zmniejszało odpowiedź wydzielniczą okrężnicy. Po podaniu EDTA notowano, zamiast wydzielania, jedynie obniżenie wchłaniania wody. DCA powodował zwiększenie wydzielania Na^+ i największy, spośród stosowanych substancji, wzrost wydzielania Cl^-, podczas gdy T wzbudzała największe wydzielanie sodu, a zmniejszała wydzielanie chloru. Z kolei pod wpływem EDTA następowało znaczne zwiększenie wydzielania Na^+ i niewielkie Cl^-. Otrzymane odpowiedzi sekrecyjne wskazują, iż zastosowane substancje wpływają na odmienne procesy transportowe w okrężnicy. Działania EDTA wynika z jego własności chelatujących jony Ca^{2+} wewnątrz komórek. Należy przypuszczać, iż tylko niesprzężone kwasy żółciowe, do których zaliczany jest DCA, zwiększają filtrację do okrężnicy na drodze obokkomórkowej, wzbudzając mikroskopijne uszkodzenia śluzówki.

Słowa kluczowe: kwas dezoksycholowy, teofilina, EDTA, izolowana pętla okrężnicy, sód, potas, wapń, chlor