# Regulation of polyethylene glycol 400 intestinal permeability by endogenous and exogenous prostanoids. Influence of non-steroidal anti-inflammatory drugs

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## Abstract

Polyethylene glycol 400 (PEG 400) is a clinically useful intestinal permeability probe whose rate of intestinal permeation is influenced in part by solvent drag. As mucosal prostanoids are increased in inflammatory bowel disease and affect water transport we examined the possible relationship between prostaglandin $E_2$ (PGE$_2$) and the inhibitors of endogenous prostaglandins – the non-steroidal anti-inflammatory drugs (NSAIDS) – on PEG 400 absorption in vivo using segmental perfusion of rat small intestine. We found that the addition of exogenous PGE$_2$ in concentrations of 0·5, 1·0, and 1·5 µg/ml significantly (p<0·01) decreased PEG 400 and water absorption. Addition of 5 mmol/l of the cyclooxygenase inhibitors acetylsalicylic acid (ASA) or indomethacin in concentrations 2·5 or 5·0 mmol/l to the perfusate significantly (p<0·01) increased PEG 400 and water absorption. The simultaneous addition of 1·0 µg/ml of exogenous PGE$_2$ to the perfusate with 5 mmol/l of ASA or with 2·5 mmol/l of indomethacin reversed the increase of PEG 400 and water transport (p<0·01). There were no differences in PEG 400 and water absorption when PGE$_2$ was given alone or in combination with ASA or indomethacin. This study suggests that endogenous or exogenous prostanoids play an important role in the regulation of PEG 400 permeation. PGE$_2$ and NSAIDS modify PEG 400 permeation in parallel with changes in water transport indicating that their effect on permeability is through changes in solvent drag. These findings provide a mechanism which might explain the increase in PEG 400 intestinal permeability in Crohn's disease patients and the increase in intestinal permeability found in patients receiving NSAIDS.

The selective ability of the intestinal epithelium to provide a barrier to the absorption of potentially harmful compounds is often referred to as permeability. Abnormal permeability may be important in the pathogenesis and pathophysiology of various diseases such as coeliac disease,[1,2] rheumatoid arthritis,[3] indomethacin-associated enteritis,[4,5] and Crohn's disease.[6]

PEG 400 is a water soluble mixture of at least nine different polymers, ranging in molecular weight from 242 to 594 daltons and has an average cross sectional diameter of 0·53 nm. Polyethylene glycol 400 was introduced as an 'ideal' probe for measuring intestinal perme-

ability by Chadwick and his colleagues.[7]

We have recently described increased permeability of PEG 400 in patients with Crohn's disease and their clinically healthy relatives.[8] The mechanisms which are responsible for this increase in PEG 400 permeability, however, are not presently understood. In our previous work we found that passive absorption modulated by solvent drug is the main mechanism of PEG 400 permeation.[9]

The purpose of this study was to examine the possible relationship between prostaglandins and non-steroidal anti-inflammatory drugs on the small intestinal permeation of PEG 400. We questioned the possible relationship between prostaglandins and PEG 400 absorption because prostaglandins are known to affect sodium and water transport in the small intestine[10-12] Moreover, an increased concentration of prostaglandins in colonic mucosa is found in patients with ulcerative colitis[13] and Crohn's disease.[14]

## Methods

### ANIMALS
Non-fasted male fisher 344 rats (180-300 g) from Charles River Breeding Laboratories were used in all experiments.

### SOLUTIONS
The basic perfusate solution consisted of tracer $^3$H-PEG 400 (New England Nuclear) and 1 mmol/l PEG 400 (Sigma Chemical Company) which were solubilised in a standard Krebs-phosphate saline buffer at pH 6·5 with a final osmolarity of 300 mOsm. $^{14}$C-Inulin was used as a non-absorbable marker.[15] Acetylsalicylic acid and indomethacin were purchased from Sigma Chemical Company, ethyl alcohol was purchased from Gold Shield Chemical Company, and 16,16,-dimethyl prostaglandin E$_2$ was purchased from Calbiochem. Each of these was added to the basic perfusate solution as indicated.

### IN VIVO PERFUSION
Using well standardised methods of recycling perfusion previously described,[16] we cannulated 30 cm segments of proximal jejunum, starting approximately 5 cm distal to the ligament of Treitz. Perfusion lasted for three hours. The 30 ml perfusate was recirculated at 1 ml/min from a continuously stirred reservoir. Duplicate 100 µl samples were taken from the reservoir every 30

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minutes and radioactivity counted in a Beckman LS9000 scintillation counter. ^{14}C-Inulin was used as the non-absorbable marker to correct each sample for fluid shifts. The absorption rate of PEG 400 was calculated by measuring its disappearance from the perfusate. Absorption values were calculated per 100 cm length after standardised stretching and drying of the intestinal segments because this length correlates best with intestinal surface area.[17] Water absorption or secretion was calculated by the differences between initial and final volume of perfusate and by the differences between initial and final concentrations of ^{14}C-Inulin[15] and expressed as ml/100 cm of intestinal length per hour.

### STATISTICAL ANALYSIS

All values are reported as mean (SEM) and statistical comparisons of control and experimental conditions are made by using ANOVA and Student's $t$ test.

## Results

### EFFECT OF 16,16,-DIMETHYL PROSTAGLANDIN $E_2$ ON JEJUNAL PEG 400 ABSORPTION

Jejunal absorption of PEG 400 was studied after the addition of three different concentrations of 16,16-dimethyl prostaglandin $E_2$ (0·5, 1·0, and 1·5 µg/ml) to the standard Krebs-phosphate saline buffer. The PEG 400 and water absorption were compared with the rats that were perfused with the standard Krebs-phosphate saline buffer without 16,16,-dimethyl prostaglandin $E_2$. Experimental conditions were similar in both groups. We found that the treatment with 16,16,-dimethyl prostaglandin $E_2$ significantly (p<0·01) decreased PEG 400 and water absorption as compared to the control values of PEG 400 absorption of 13·1 (0·5) µmol/100 cm/h and water absorption of 8·9 (0·4) ml/100 cm/h. Absorption of PEG 400 of 8·3 (0·5), 8·8 (0·5), and 6·0 (1·9) µmol/100 cm/h and water absorption of 4·8 (0·2), 4·5 (0·6), and 1·2 (1·2) ml/100 cm/h were recorded at 16,16,-dimethyl prostaglandin $E_2$ concentrations of 0·5, 1·0, and 1·5 mg/ml respectively (Fig 1). PEG 400 absorption was linearly related to time (three hours) of perfusion in control group (r=0·97) and in group perfused with 1 µg/ml of 16,16,-dimethyl prostaglandin $E_2$ (r=0·98) (Fig 2).

### EFFECT OF ACETYSALICYLIC ACID ON JEJUNAL ABSORPTION OF PEG 400

Influence of acetylsalicylic acid on jejunal absorption of PEG 400 was determined by the addition of 2·5 and 5 mmol/l acetylsalicylic acid to the basic perfusate solution. Addition of 2·5 mmol/l acetylsalicylic acid did not change PEG 400 and water absorption, but addition of 5 mmol/l acetylsalicylic acid to the perfusate significantly (p<0·01) increased PEG 400 absorption to 18·1 (0·5) µmol/100 cm/h and water absorption to 12·1 (0·8) ml/100 cm/h, as compared with PEG 400 absorption of 13·1 (0·5) µmol/100 cm/h and water absorption of 8·9
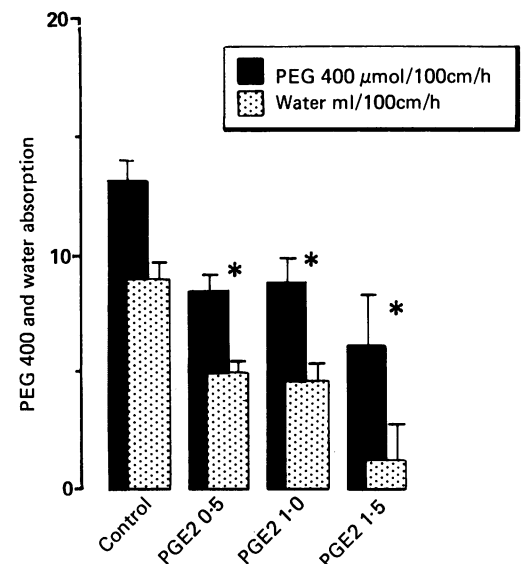


Figure 1: Effect of 16,16-dimethyl prostaglandin $E_2$ (0·5, 1·0, and 1·5 µg/ml) on jejunal absorption of PEG 400 and water. Three to 11 rats were studied in each group. Each rat provided six separate data points. Perfusate composed of 1 mmol/l PEG 400 and Krebs-phosphate saline buffer at pH 6·5 with flow rate of 1 ml/min. Height of the bars indicates mean (SEM). Solid bars represent PEG 400 absorption in µmol/100 cm/h. Open bars represent water absorption in ml/100 cm/h. * denotes a significant (p<0·01) difference from control.

(0·4) ml/100 cm/h in controls. The results are illustrated in Fig 3. PEG 400 absorption was linearly related to time (three hours) of perfusion with 5 mmol/l ASA (r=0·98) (Fig 2).

### EFFECT OF INDOMETHACIN ON JEJUNAL PEG 400 ABSORPTION

The influence of indomethacin (cyclooxygenase inhibitor) on PEG 400 absorption was tested after the addition of three different concentrations of indomethacin (1·0, 2·5, and 5·0 mmol/l) in the perfusate, which contained 10% ethyl alcohol in the Krebs-phosphate saline buffer in order to dissolve the indomethacin. The results were compared with the control rats (n=11) perfused with the basic perfusate solution alone, and to additional control rats (three) perfused with 10% ethyl alcohol solution in the standard Krebs-phosphate saline buffer.
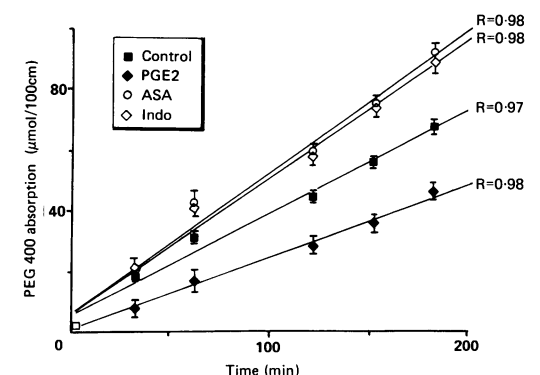


Figure 2: Relationship of perfusion time to PEG 400 absorption in control group of rats, in rats perfused with 1 µg/ml of PGE[2], 5 mmol/l acetylsalicylic acid (ASA) and 2·5 mmol/l indomethacin (INDO). The perfusate consisted of 1 mmol/l PEG 400 and Krebs-phosphate saline buffer at pH 6·5 with flow rate of 1 ml/min. Plotted values are mean (SEM) of three to 11 different experimental runs. Values were fitted to a linear plot using a linear regression analysis program.
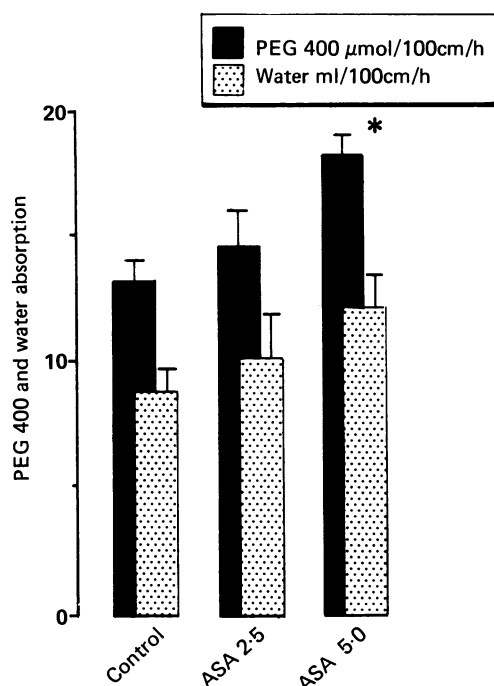
*Figure 3: Effect of 2·5 mmol/l and 5 mmol/l acetylsalicylic acid (ASA) on jejunal absorption of PEG 400 and water. Three to 11 rats were studied in each group. Each rat provided six separate data points. Perfusate was composed of 1 mmol/l PEG 400 and Krebs-phosphate saline buffer at pH 6·5 with flow rate of 1 ml/min. Height of bars indicates mean (SEM). Solid bars represent PEG 400 absorption in μmol/100 cm/h. Open bars represent water absorption in ml/100 cm/h. * denotes a significant (p<0·01) difference from control.*
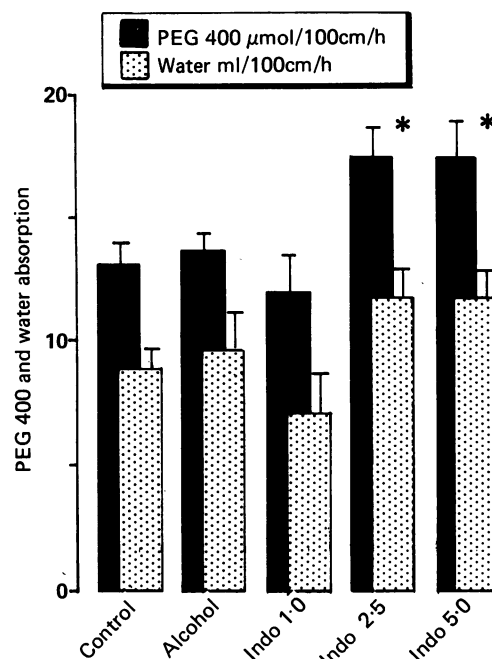


*Figure 4: Effect of 1·0, 2·5 and 5·0 mmol/l of indomethacin (INDO) and 10% alcohol (alcohol) on jejunal absorption of PEG 400 and water. Three to 11 rats were studied in each group. Each rat provided six separate data points. Perfusate composed of 1 mmol/l PEG 400 and Krebs-phosphate saline buffer at pH 6·5 with flow rate of 1 ml/min. Height of the bars indicates mean (SEM). Solid bars represent PEG 400 absorption in μmol/100 cm/h. Open bars represent water absorption in ml/100 cm/h. * denotes a significant (p<0·01) difference from control.*

Polyethylene glycol 400 absorption was similar between the control groups (13·1 (0·5) μmol/100 cm/h), the group perfused with 10% ethyl alcohol in the basic perfusate solution (13·6 (0·3) μmol/100 cm/h), and in group perfused with 1·0 mmol/l indomethacin (11·9 (1·1) μmol/100 cm/h), but was significantly (p<0·01) increased in the rats perfused with higher (2·5 mmol/l and 5·0 mmol/l) concentrations of indomethacin (17·4 (0·5) and 17·3 (1·1) μmol/100 cm/h respectively). Water absorption was similar in control group (8·9 (0·4) ml/100 cm/h), the group perfused with 10% ethyl alcohol in the standard Krebs-phosphate saline buffer (9·6 (1·2) ml/100 cm/h), and in group perfused with 1·0 mmol/l indomethacin (7·1 (0·8) ml/100 cm/h), but significantly (p<0·01) higher in the rats perfused with higher (2·5 mmol/l and 5·0 mmol/l) concentrations of indomethacin (11·9 (0·5) and 11·6 (0·8) ml/100 cm/h respectively). The results are illustrated in Figure 4. No differences were found in PEG 400 and water absorption between rats perfused with 2·5 and 5·0 mmol/l of indomethacin. PEG 400 absorption was linearly related to time (three hours) of perfusion with 2·5 mmol/l indomethacin (r=0·98) (Fig 2).

EFFECT OF ACETYSALICYLIC ACID WITH
16,16,-DIMETHYL PROSTAGLANDIN E₂ ON
JEJUNAL ABSORPTION OF PEG 400
Influence of acetysalicylic acid with the simultaneous addition of 16,16,-dimethyl prostaglandin E₂ on jejunal absorption of PEG 400 was determined by the addition of 5 mmol/l acetyl-

salicylic acid with 1 μg/ml 16,16,-dimethyl PGE₂ to the basic perfusate solution.

We found that addition of acetylsalicylic acid together with 16,16,-dimethyl prostaglandin E₂ significantly (p<0·01) decreased PEG 400 and water absorption compared with the control (Table). There were no significant differences in PEG 400 and water absorption between rats perfused with both acetylsalicylic acid and 16,16,-dimethyl prostaglandin E₂ and rats perfused with 16,16,-dimethyl prostaglandin E₂ alone.

EFFECT OF INDOMETHACIN TOGETHER
WITH 16,16,-DIMETHYL PROSTAGLANDIN
E₂ ON JEJUNAL PEG 400 ABSORPTION
We tested jejunal absorption of PEG 400 after the simultaneous addition of 2·5 mmol/l indomethacin with 1 μg/ml of 16,16,-dimethyl

TABLE    *Effect of PGE₂ and cyclooxygenase inhibitors on PEG 400 and water absorption*

| Addition | Rats (n) | PEG400 absorption μmol/100 cm/h | Water absorption ml/100 cm/h |
|---|---|---|---|
| Control | 11 | 13·1 (0·5) | 8·9 (0·4 |
| PGE₂ 1 μg/ml | 3 | 8·8 (0·5)* | 4·5 (0·6)* |
| Aspirin 5 mmol/l (ASA) | 4 | 18·1 (0·5)* | 12·1 (0·8)* |
| Indomethacin 2·5 mmol/l (INDO) | 4 | 17·4 (0·5)* | 11·9 (0·6)* |
| ASA 5 mmol/l and PGE₂ 1 μg/ml | 3 | 8·8 (0·3)* | 5·1 (0·5)* |
| INDO 2·5 mmol/l and PGE₂ 1 μg/ml | 4 | 8·3 (0·3)* | 3·5 (0·3)* |

* Denotes a significant (p<0·01) difference from control absorption. Values are mean (SEM) of the six data points per rat.

prostaglandin $E_2$ in 10% ethyl alcohol solution in the standard Krebs-phosphate saline buffer.

Addition of both indomethacin and 16,16,-dimethyl prostaglandin $E_2$ significantly ($p<0.01$) decreased PEG 400 and water absorption compared to the control values (Table). There were no significant differences in PEG 400 and water absorption between rats perfused with both indomethacin with 16,16,-dimethyl prostaglandin $E_2$ and rats perfused by 16,16,-dimethyl prostaglandin $E_2$ alone.

## Discussion

We studied the influence of exogenous 16,16,-dimethyl prostaglandin $E_2$ (PGE$_2$) and cyclo-oxygenase inhibitors on the intestinal absorption of PEG 400 because of the known influence of prostaglandins on water transport and because of the documented increase in prostaglandins in the lumen and in the mucosa of patients with inflammatory bowel disease.[13] We have previously established that the rate and the direction of water transport are major factors which regulate and modulate the intestinal permeation of PEG 400 and PEG 900.[9 18] The present data show that endogenous and exogenous prostaglandins are also potent regulators of PEG 400 permeation through regulation of net water flow.

In the present study we found that the addition of PGE$_2$ in concentrations of 0.5, 1.0, and 1.5 µg/ml to the perfusate resulted in parallel decrease in both PEG 400 and water absorption (Fig 1). When we added the cyclooxygenase inhibitor ASA in concentration of 5 mmol/l or indomethacin in concentrations of 2.5 and 5.0 mmol/l the absorption of PEG 400 and water increased (Figs 3, 4). The increase in transport was reversed by the simultaneous addition to the perfusate of exogenous PGE$_2$ (Table). These experiments clearly show that PGE$_2$, ASA and indomethacin change PEG 400 permeation in parallel with water absorption. We concluded that ASA and indomethacin influenced PEG 400 and water absorption by blocking the cyclooxygenase pathway and thereby decreasing endogenous synthesis of prostanoids.

Our observations are in concert with the findings of Bjarnson *et al*, who showed that NSAID increased permeability of $^{51}$Cr-EDTA.[4] Our findings provide an explanation and a mechanism which could account for Bjarnson's results. By giving NSAID to the patients with rheumatologic disorders or to normal volunteers, the endogenous synthesis of prostaglandins was decreased by blocking the cyclooxygenase enzyme system and intestinal permeability of $^{51}$Cr-EDTA and water increased.

At this point, we can only speculate as to the mechanisms by which endogenous or exogenous prostaglandins inhibit water absorption and PEG 400 permeation. Our previous data clearly indicate that PEG 400 and PEG 900 absorption is modulated by solvent drag. As net water absorption increases, net PEG 400 and PEG 900 permeation follow.[9 18] PEG 400 penetration of the intestinal barrier is mediated by two separate forces – simple passive diffusion along its concentration gradient and solvent drag or convection. In the small intestine we found that 43% of

the total transport was the result of diffusion while 57% of the transport was mediated by convection.[9] In the colon, only 14.3% of total PEG 400 transport is caused by passive diffusion while 85.7% was due to convection (submitted data). Thus permeability changes after NSAID or ASA administration are probably secondary to changes in water flux which in turn are regulated by the concentration of prostaglandins in the mucosa.

There are data which suggest that the inhibitory effect of prostaglandins on net water absorption may be mediated, at least in part, by prostaglandin effect on sodium and chloride transport. Sernka and coworkers showed that PGE$_2$ inhibited sodium absorption and stimulated chloride secretion *in vitro*.[19] These changes would result in decreased net water absorption.

It has been shown that PGE$_2$ stimulates mucosal adenylate cyclase through cyclic 3' 5'-AMP as a mediator, similar to cholera exotoxin[20] and cyclic 3' 5'-AMP stimulates active secretion of chloride and inhibits the active absorption of sodium.[21] Therefore, a possible mechanism by which prostaglandins may inhibit water and PEG 400 absorption is by their effect of stimulating mucosal cyclic 3' 5'-AMP. In addition, according to Brunsson *et al*[22] PGE$_2$ acts on intestinal fluid transport by both neural and non-neural mechanisms. Neural response to PGE$_2$ is not mediated through cAMP, but may decrease water absorption *via* hormonally independent mechanisms.

Cyclic AMP may also have a specific role in the orientation of tight junctional intramembranous strands,[23] thereby perhaps affecting paracellular transport of larger molecules such as PEG 400.

Water transport occurs secondary to osmotic forces, and is influenced by active sodium, glucose and amino acid absorption. According to recent reports some hydrophilic electrically neutral molecules are absorbed by osmotic forces, mostly through the paracellular pathway.[24] It has been shown that sodium coupled active transport increases fluid absorption through intercellular junctions.[25] These physiological changes correlate well with the morphological changes seen on light microscopy, transmission electron microscopy, and freeze fracture techniques which showed dilatation of tight junction after perfusion with glucose, alanine or leucine.[26] In addition it has been shown that junctional dilatation is an active process involving contractile proteins of the terminal web and can be affected by cAMP.[27] Therefore prostaglandins can be modulators of junctional permeability by acting through cAMP. Decrease in PEG 400 and water absorption by PGE$_2$ in the present work correlate well with the results obtained by Duffey *et al*.[23] They showed in *Necturus* gall bladder epithelium that cyclic AMP increased the resistance to passive ion flow through the paracellular pathway by aggregation of microfilaments in regions adjacent to the tight junctions.

In summary our studies show that 16,16,-dimethyl prostaglandin $E_2$ decreased PEG 400 permeability in parallel with decreasing water absorption. Non-steroidal anti-inflammatory drugs increased PEG 400 and water absorption probably by decreasing endogenous mucosal

prostanoid synthesis. We conclude that prostaglandins are potent regulators of PEG 400 intestinal permeability through their direct and indirect effects on water transport. As permeability changes are potentially related to the pathogenesis of Crohn's disease[28] these observations are important in clarifying our understanding of inflammatory bowel disease.

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