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ORIGINAL CONTRIBUTION

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Modulation by fish oil diet of eicosanoid-induced anion secretion in the rat distal colon

Modulation Eicosanoid-induzierter Sekretionsvorgänge am Kolon der Ratte durch eine Fischöldiät

Summary Eicosanoids are involved in the mediation of inflammatory and allergic processes in the gut. In order to evaluate a potential beneficial effect of the diet, the effect of mediators of inflammation and of a sensitization against egg albumin on anion secretion across the colon was tested using rats fed on a diet containing 15 % fish oil as compared to 15 % olive oil as donor animals.

Feeding on a fish oil diet significantly reduced the response to bradykinin or phospholipase C, known agonists of prostaglandin-induced secretion, by about 50 %. The increase in short-circuit current (Isc) induced by the phospholipase A_2 stimulator, melittin, or by distension of the gut wall were only insignificantly inhibited by 15–30 %. Administration of egg albumin to the mucosas from animals sensitized against egg albumin induced an indomethacin- and tetrodotoxin-sensitive increase in Isc. This response was, however, only insignificantly (30 %) reduced by the fish oil diet.

In conclusion, the effect of fish oil diet depends on the stimulus used for activation of prostaglandin release. This suggested that different pools of arachidonic acid are differentially affected by the diet or that certain stimuli for phospholipases are strong enough to overcome the effect of a reduced substrate availability. Consequently, a diet rich in polyunsaturated n-3 fatty acids may only play an adjuvant role for the therapy of inflammatory or allergic intestinal diseases.

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Zusammenfassung Eicosanoide sind an der Entstehung entzündlicher und allergischer Darmerkrankungen beteiligt. Um die Möglichkeit einer diätetischen Beeinflussung dieser Erkrankungen zu untersuchen, wurde die sekretorische Wirkung verschiedener Entzündungsmediatoren und einer Sensibilisierung gegen Hühnereialbumin am Kolon von Ratten in vitro getestet, die eine Diät mit 15 % Fischöl erhielten. Als Kontrolle dienten Ratten, die eine Diät mit 15 % Olivenöl erhielten.

Die Fütterung mit der Fischöldiät hemmte signifikant die prostaglandinvermittelte Sekretion, die durch Bradykinin oder Phospholipase C hervorgerufen wurde, um circa 50 %. Der Anstieg des Kurzschlußstroms (Isc), der durch Melittin, einem Stimulator der Phospholipase A_2, oder Dehnung der Darmwand induziert wurde, war dagegen nur geringfügig (um 15–30 %; nicht signifikant) vermindert. Zugabe von Hühnereialbumin zu den Schleimhautpräparaten von Tieren, die gegen dieses Protein sensibilisiert worden waren, rief einen Indomethacin- und Tetrodotoxin-sensitiven Anstieg des Isc hervor. Auch diese Antwort wurde nur in nicht signifikantem Ausmaß (30 %) durch die Fischöldiät vermindert.

Diese Ergebnisse legen den Schluß nahe, daß die Wirkung einer Fischöldiät von der Art des Stimulus abhängig ist, der die Prostaglandinsynthese anregt. Dies weist möglicherweise auf die Existenz verschiedener Arachidonsäurepools hin, die von verschiedenen Stimuli aktiviert und unterschiedlich durch die Diät beeinflußt werden. Dementsprechend kommt einer Diät mit n-3-mehrfach ungesättigten Fettsäuren höchstens eine Rolle als Adjuvans bei der Therapie entzündlicher oder allergischer Darmerkrankungen zu.

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Schlüsselwörter Kolon – Ratte – Fischöl – n-3-mehrfach ungesättigte Fettsäuren – Prostaglandine

Abbreviation index *Isc* = Short circuit current · *Gt* = tissue conductance · *PLC* = phospholipase C *SEM* = standard error of the mean

Introduction

Arachidonic acid, released by phospholipases from phospholipids in cellular membranes, is the precursor of eicosanoids like prostaglandins, thromboxanes or leukotrienes, which are produced by the key enzymes cyclooxygenase and 5-lipoxygenase. Replacement of arachidonic acid by eicosapentaenoic acid leads to the production of e.g. prostaglandins of the 3-series, which have a low biological activity (5). Fish oil is rich in eicosapentaenoic acid (36). Fish oil diets or diets enriched with eicosapentaenoic acid have been shown to exert antiinflammatory effects (18, 25) and to reduce cardiovascular risks (32). An antiproliferative effect suggesting a protective role against colon cancer has been observed in humans (3) as well as in certain tumour cell lines in vitro (29).

In the colon, metabolites of arachidonic acid play a role as mediators of inflammation (see e.g. 24, 33), of intestinal allergy (for review see 7), and of physiological responses, e.g. secretion induced by distension of the gut wall (16, 28). Consequently, the aim of the present study was to investigate, whether a diet rich in n-3 polyunsaturated fatty acids has an effect on physiological and pathophysiological responses mediated by prostaglandins. Therefore, the effect of mediators of inflammation, distension or allergically induced secretion was tested in colon preparations from rats fed on a fish oil diet as compared to those from rats fed on an olive oil diet, which is poor in n-3 polyunsaturated fatty acids.

Material and methods

Animals and diet

Female SIVZ-50 rats (Institut für Labortierkunde, Universität Zürich, Switzerland) with a weight of 180–220 g were used. The animals had free access to water and food until the day of the experiment. If not indicated differently, animals were fed on a standard chow diet (Nafag 890; Nafag Ecosan, Gossau, Switzerland). In the experiments, in which the effects of a fish oil diet were to be tested, the animals were fed for 2–3 weeks with a fish oil diet (for composition see Tables 1 and 2). They were compared with rats fed on an olive diet, in which the fish oil was replaced by olive oil while leaving the other components of the diet unchanged (Table 1). Butylhydroxytoluol (Fluka, Buchs, Switzerland) was added to both diets as an antioxidant. The increase in body weights was measured over 2 weeks with 6 rats in each of both feeding groups. Linear regression analysis of the data ($r \geq 0.93$) revealed an increase in body weight of 2.42 ± 0.17 $g \cdot d^{-1}$ in the olive oil group and of 2.50 ± 0.25 gid^{-1} in the fish oil group (difference not significant; analysis of co-variances). The fatty acid content (Table 2) of the diets was commercially analyzed by UFAG Laboratorien, Sursee, Switzerland.

Table 1 Composition of diets

	Fish oil diet	Olive oil diet
	% (w/w)	
Casein	12.87	12.87
Corn starch	46.00	46.00
Methionine	0.13	0.13
Fish oil	15.00	–
Olive oil	–	15.00
Butylhydroxytoluol	0.10	0.10
Mineral mixture	4.00	4.00
Vitamin mixture	3.00	3.00
Polyethylene	16.00	16.00

The sources for the main components of the diets were: Zentralschweizerischer Milchverband Luzern, Dagmarsellen, Switzerland (Ca caseinate); Blattmann, Wädenswil, Switzerland (corn starch); Nef, Zürich, Switzerland (olive oil); Fuga, Luzern, Switzerland (fish oil). 1 kg mineral mixture contained 162.14 g Ca, 80.75 g P, 66.31 g Na, 90.88 g K, 38.99 g Mg, 102.00 g Cl, 2.92 g Fe, 665 mg Mn, 174 mg Cu, 411 mg Zn, 27 mg J, 63 mg F, 13 mg Co, and 9 mg Se. 1 kg vitamin mixture contained 700 000 IU A, 70 000 IU D_3, 4.91 g E, 1.80 g C, 1.00 g B_1, 0.60 g B_2, 0.45 g B_6, 1.20 mg B_{12}, 1.80 g nicotinic acid, 1.50 g pantothenate, 100 mg folic acid, 30 mg biotin, and 18.75 g choline. Polyethylene (Lupolen 1800 SP 15) was obtained from BASF, Wädenswil, Switzerland.

Sensitization

Animals were sensitized to chicken egg albumin (grade V; Sigma, Buchs, Switzerland) by subcutaneous injections of 10 µg egg albumin in 50 µl 0.9 % NaCl (w/v) together with 100 µl complete Freund's adjuvans. The rats were injected at day-14 and day-1 before the in vitro experiments. The experiments were approved by the Administration Bureau for Veterinary Medicine of the Kanton Zürich.

Table 2 Fatty acid content of the diets

Fatty acid	Fish oil diet	Olive oil diet
	% of total fatty acids (w/w)	
C12	0.1	not detected
C14	8.0	not detected
C16	19.5	11.2
C16:1	7.6	0.6
C17	0.6	not detected
C18	4.0	3.0
C18:1	9.8	70.7
C18:2	1.7	9.8
C18:3	0.8	0.5
C18:4	2.2	0.2
C20	0.2	0.5
C20:1	0.9	0.3
C20:2	0.2	not detected
C20:3	not detected	not detected
C20:4	0.8	not detected
C20:5	13.7	not detected
C22	not detected	0.1
C22:1	0.2	not detected

Tissue preparation

Animals were stunned by a blow on the head and killed by exsanguination. The serosa and muscularis propria were stripped away by hand to obtain the mucosa-submucosa preparation of the distal part of the colon. Two (in some experiments three) pieces of the mucosa of the distal colon, which was defined by the absence of palm leaf-like striae (27), were mounted in an Ussing chamber.

Solutions

All experiments in vitro were carried out in a bathing solution containing ($mmol \cdot l^{-1}$): NaCl 107, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, $NaH2PO_4$ 0.2, $CaCl_2$ 1.25, $MgSO_4$ 1 and glucose 12. The solution was gassed with 95 % O_2 + 5 % CO_2 and was kept at a temperature of 37 °C and a pH of 7.4.

Short-circuit current measurement

The tissue was fixed in a modified Ussing chamber (1), bathed with a volume of 4 ml on each side of the mucosa and short-circuited by a voltage clamp (Aachen Microclamp, AC Copy Datentechnik, Aachen, Germany) with correction for fluid resistance. The exposed surface of the tissue was 1 cm^2. Short-circuit current (Isc) was continuously recorded on a chart-recorder. In addition, Isc and tissue conductance (Gt) were printed every min by a computer printer.

In the experiments with sensitized rats, at least one piece of mucosa from the rat was left untreated in order to evaluate the individual response to antigen under control conditions. If the increase in Isc in this control tissue was less than 0.5 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$, sensitization was considered insufficient and the data from this animal were excluded. The success rate of sensitization against egg albumin defined by this criterion was 81 % (30 out of 37 rats).

Data evaluation

Experiments were started 60–90 min after mounting the tissue in the chamber, when the initial high Isc had stabilized to a plateau. The effect of secretagogues on Isc and Gt were measured, when the Isc had increased to a maximal value. Data in the tables are given as differences to the former baseline, i.e. the mean over 3 min just prior to the addition of the respective secretagogue (Δ Isc).

Drugs

Bradykinin, melittin and phospholipase C (PLC; type XIV from *clostridium perfringens*; specific activity 210 $U \cdot mg^{-1}$) were dissolved in aqueous stock solution containing 0.1 % (w/v) bovine serum albumin. Indomethacin was dissolved in ethanol (final concentration 0.1 %, v/v). Tetrodotoxin was dissolved in citrate buffer (5 mg per mg tetrodotoxin; pH 4.3). Egg albumin and mepyramine were dissolved in the bathing solution (composition see above) just before use. If not indicated differently, drugs were from Sigma, Buchs, Switzerland).

Statistics

Results are given as means ± one standard error of the mean (SEM). If more than one group had to be compared, the significance of differences was tested by analysis of variances and, if indicated, by paired or unpaired two-tailed Student's *t*-test or an U-test, respectively. An F-test was applied to decide which test method was to be used. The quality of linear regressions was checked by the linear regression coefficient (r), that of nonlinear regression by the squared nonlinear regression coefficient (r^2). Linear regression lines were compared by analysis of co-variances (23).

Results

Effect of fish oil diet on the response to secretagogues

Basal electrical parameters in the distal colon from rats fed on the olive oil diet, which served as control, amounted to: Isc 2.1 ± 0.2 $\mu Eq \cdot h^{-1} \cdot cm^2$ and Gt 12.3 ±

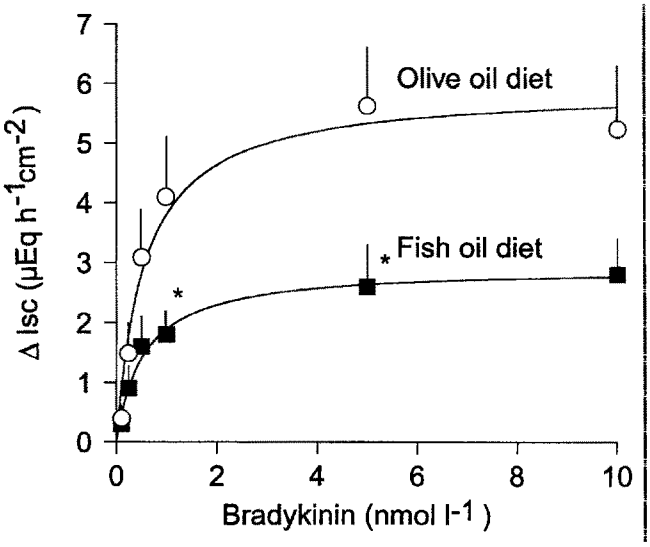

Fig. 1 Effect of bradykinin (10^{-10} – 10^{-8} mol·l^{-1} administered at the serosal side) on Isc across rat distal colon from animals fed on a fish oil diet (filled squares) or an olive oil diet (open circles). Bradykinin was administered in increasing concentrations to the tissue with an intermediate washing step (washing the serosal compartment three times within 15 min with 5 x the chamber volume). The effect of bradykinin was significantly different from zero ($p < 0.05$) for concentrations $\geq 10^{-10}$ mol·l^{-1} in both groups. The lines were obtained by nonlinear fitting to the Michaelis-Menten equation assuming affinities of 0.54 nmol·l^{-1} or 0.55 nmol·l^{-1} and maximal responses of 2.9 μEq·h^{-1}·cm^{-2} or 5.9 μEq·h^{-1}·cm^{-2} in the fish oil or the olive oil group, respectively ($r^2 \geq 0.96$ for both). Values are given as difference to the baseline Isc just prior administration of bradykinin and are means ± SEM, n = 8, * $p < 0.05$ versus olive oil group.

0.4 mS·cm^{-2} (n = 65). There was a tendency for a reduction of the baseline parameters in the tissues from rats fed on the fish oil diet, in which basal Isc amounted to 1.7 ± 0.1 μEq·h^{-1}·cm^{-2} at a Gt of 11.2 ± 0.4 mS·cm^{-2} (n = 65). None of these differences were statistically significant.

Administration of bradykinin induced a concentration-dependent increase in Isc in both dietary groups (Fig. 1). A half-maximal increase was reached in both groups at a concentration of about $5 \cdot 10^{-10}$ mol·l^{-1}, i.e., fish oil diet did not alter the potency of bradykinin. The response to bradykinin saturated at concentrations $\geq 5 \cdot 10^{-9}$ mol·l^{-1}. However, the maximal increase in Isc induced by the kinin was reduced by more than 50 % in rats fed on the fish oil diet, i.e. the diet reduced the efficacy of bradykinin. In parallel, bradykinin evoked an increase in Gt, which was slightly (but insignificantly) reduced in the fish oil group (Table 3).

Melittin, a component of bee venom, is another stimulator of phospholipase A_2 inducing Cl^- secretion in the rat colon. In the olive oil group melittin (2 μg·ml^{-1} at the mucosal and the serosal side) induced an increase in Isc of 2.1 ± 0.5 μEq·h^{-1}·cm^{-2} above baseline (n = 15, $p < 0.05$; Table 3), which was paralleled by an increase in Gt (Table 3). Fish oil diet appeared to attenuate the increase in Isc (1.5 ± 0.3 μEq·h^{-1}·cm^{-2}, n = 15), although this effect did not reach statistical significance due to the large variability (Table 3).

Phospholipase C (PLC) from clostridium perfringens is known to induce a biphasic increase in Isc; the first phase of this response is suppressed by indomethacin. In the olive oil diet group, PLC (100 U·l^{-1} at the serosal side) induced a first peak in Isc of 1.5 ± 0.3 μEq·h^{-1}·cm^{-2} above baseline (n = 14, $p < 0.05$) after 9.9 ± 0.8 min

Table 3 Response to secretagogues, antigen or distension

	Δ Isc (μEq·h^{-1}·cm^{-2})		Δ Gt (mS·cm^{-2})	
Diet:	Olive oil	Fish oil	Olive oil	Fish oil
Bradykinin	5.6 ± 1.0*	2.6 ± 0.7*#	3.4 ± 0.7*	2.0 ± 1.3
PLC 1. peak	1.5 ± 0.3*	0.7 ± 0.1*#	1.0 ± 0.3*	0.6 ± 0.2*
PLC 2. peak	4.3 ± 0.3*	4.5 ± 0.6*	4.6 ± 0.7*	4.0 ± 0.6*
Melittin	2.1 ± 0.5*	1.5 ± 0.3*	2.2 ± 0.6*	1.3 ± 0.3*
Distension	5.0 ± 0.6*	4.3 ± 0.4*	10.3 ± 2.8*	6.4 ± 1.3*
Egg albumin	4.3 ± 0.7*	3.1 ± 0.5*	3.5 ± 1.1*	2.4 ± 0.6*

Effect of secretagogues and antigen on Isc and Gt across rat distal colon from animals fed on a fish oil or an olive oil diet. Concentrations of agonists were: bradykinin $5 \cdot 10^{-9}$ mol·l^{-1} (administered at the serosal side), melittin (2 μg·ml^{-1} administered at the mucosal and the serosal side), phospholipase C (PLC; 100 U·l^{-1} administered at the serosal side). Egg albumin (100 μg·ml^{-1} at the mucosal and the serosal side) was administered to tissues from sensitized animals. Values are given as difference to the baseline just prior secretagogue administration and are means ± SEM, n = 8–15. * $p < 0.05$ versus baseline, # $p < 0.05$ versus olive oil group.

(Fig. 2A). Then Isc decreased transiently, until it reached a second peak of 4.3 ± 0.3 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ above baseline (n = 14, $p < 0.05$) after 52.9 ± 6.2 min. In the tissues from the animals fed on fish oil diet, the size of the first peak, which was reached after 12.5 ± 2.7 min, was significantly diminished (0.7 ± 0.1 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ above baseline, n = 15, $p < 0.05$ vs. olive oil group; Fig. 2B). The second peak in Isc, which was reached after 55.6 ± 4.4 min, was unaffected (4.5 ± 0.6 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ above baseline, n = 15; not significantly different from that of the olive oil group). The increase in Gt induced by PLC was not significantly reduced in the fish oil group (Table 3).

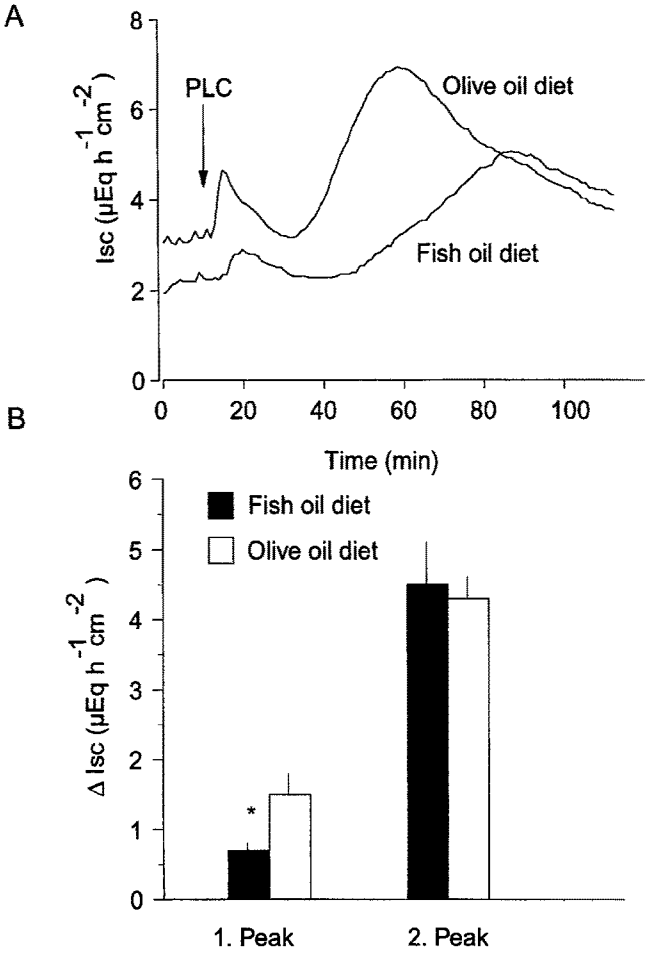

Fig. 2A Effect of phospholipase C (PLC; 100 U·l^{-1} administered at the serosal side) on Isc across rat distal colon from animals fed on an olive oil diet (upper tracing) or a fish oil diet (lower tracing). The tracings are representative for 14–15 experiments in each group. **B** Mean increase in Isc induced by PLC in the fish oil (filled bars) and the olive oil group (open bars). Values are given as difference to the baseline Isc just prior to administration of PLC and are means ± SEM, n = 14–15, * $p < 0.05$ versus olive oil group.

Prostaglandins are involved in Cl^- secretion evoked by distension of the gut wall. Therefore, the effect of the diet on distension-induced Isc was tested. Distension was performed by an occlusion for 5 s of the opening at the top of the serosal half-chamber, by which the carbogen used to gas the tissue normally escaped. In the olive oil group, distension induced an increase in Isc of 5.0 ± 0.6 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ above baseline (n = 11, $p < 0.05$; Table 3), which was accompanied by an increase in Gt (Table 3). The increase in Isc decreased with a half-time of 4.3 ± 0.6 min. In the fish oil group, the increase in Isc induced by distension appeared to be slightly reduced (4.3 ± 0.4 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ above baseline; n = 10) and shortened (half-time 3.1 ± 0.4 min). None of the changes were statistically significant.

Effect of fish oil diet on antigen-induced secretion

In a first series of experiments, the response to antigen was tested in rats fed on a standard chow diet and sensitized to (chick) egg albumin. Basal Isc in these tissues amounted to 2.1 ± 0.2 $\mu Eq \cdot -^{-1} \cdot cm^{-2}$ at a Gt of 13.2 ± 0.6 $mS \cdot cm^{-2}$ (n = 42). Orientating experiments revealed that the secretory effect of antigen was more pronounced after serosal compared to mucosal administration. Therefore, the egg albumin was applied at both the mucosal and the serosal side. Administration of egg albumin to the colon of sensitized animals induced a concentration-dependent increase in Isc (Fig. 3). The threshold, at which the antigen induced a first significant increase in Isc was 10 $\mu g \cdot ml^{-1}$. Sensitization was successful in 30 out of 37 rats (see Methods for exclusion criterion). Egg albumin, in concentrations up to 100 $\mu g \cdot ml^{-1}$, had no effect on Isc across the colon from non-sensitized animals (n = 10). For all further experiments, a concentration of 100 μg–ml^{-1} was chosen. In parallel with the increase in Isc, an increase in Gt of 3.4 ± 0.7 $mS \cdot cm^{-2}$ above baseline (n = 13, $p < 0.05$) was observed during antigen exposure (Table 4). The secretory effect of antigen declined rapidly with a half-time of 2.3 ± 0.2 min. The effect of egg albumin showed a rapid and complete desensitization, i.e. subsequent administration of fresh antigen after termination of the response induced by a first antigen administration had no more effect on Isc, even after severe washing of the chamber with fresh buffer solution.

The response to egg albumin was inhibited by tetrodotoxin, a neuronal toxin. Tetrodotoxin itself (10^{-6} mol·l^{-1} at the serosal side) induced a decrease in basal Isc from 2.4 ± 0.4 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ to 1.1 ± 0.3 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ (n = 6, $p < 0.05$). In the presence of the neurotoxin, the response to egg albumin (100 $\mu g \cdot ml^{-1}$ at the mucosal and the serosal side) amounted to only 0.9 ± 0.3 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ above baseline (n = 6), which was significantly smaller than that of an untreated control group, in which egg albumin induced an increase in Isc of 5.3 ± 1.4 $\mu Eq \cdot h^-$

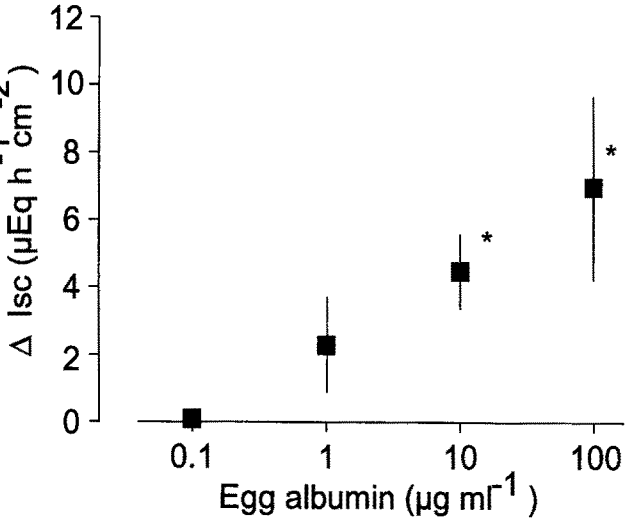

Fig. 3 Concentration-dependent effect of egg albumin (administered at the mucosal and the serosal side) on Isc across rat distal colon from sensitized animals. Values are given as difference to the baseline Isc just prior to administration of the antigen and are means ± SEM, n = 4–6, * $p < 0.05$ versus baseline.

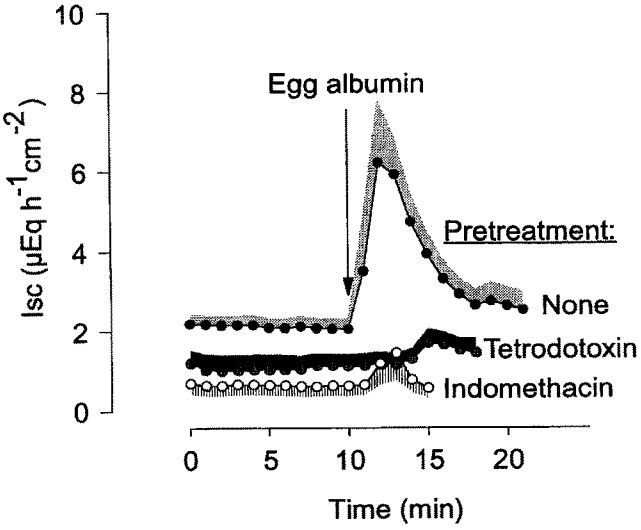

Fig. 4 Effect of egg albumin (100 μg·ml^{-1} administered at the mucosal and the serosal side) on Isc across rat distal colon from sensitized animals under control conditions (closed circles), in the presence of indomethacin (10^{-6} mol·l^{-1} at the serosal and the mucosal side; open circles), and in the presence of tetrodotoxin (10^{-6} mol·l^{-1} at the serosal side; crossed circles). Values are means (symbols) ± SEM (shaded area), n = 6–13.

Table 4 Effect of inhibitors on the antigen response

Inhibitor	Δ Isc (μEq·h^{-1}·cm^{-2})	Δ Gt (mS·cm^{-2})
No inhibitor	5.3 ± 1.4*	4.5 ± 1.1*
Indomethacin	0.9 ± 0.5#	0.5 ± 0.2#
Tetrodotoxin	0.9 ± 0.3*#	1.3 ± 0.6
Mepyramine	2.0 ± 0.2*	1.7 ± 0.3*

Effect of egg albumin (100 μg·ml^{-1} at the mucosal and the serosal side) on the colon of sensitized rats in the absence of inhibitors, and in the presence of indomethacin (10^{-6} mol·l^{-1} at the mucosal and the serosal side), tetrodotoxin (10^{-6} mol·l^{-1} at the serosal side), or mepyramine (10^{-4} mol·l^{-1} at the serosal side). Values are given as difference to the baseline just prior antigen administration and are means ± SEM. n = 6–16. * $p < 0.05$ versus baseline, # $p < 0.05$ versus antigen response in the control group (no inhibitor).

$^{-1}$·cm^{-2} (n = 13, $p < 0.05$; Fig. 4; Table 4). A similar inhibition was observed with the cyclooxygenase inhibitor, indomethacin. Indomethacin (10^{-6} mol·l^{-1} at the mucosal and the serosal side) caused a decrease in basal Isc from 2.1 ± 0.5 μEq·h^{-1}·cm^{-2} to 0.6 ± 0.3 μEq·h^{-1}·cm^{-2} (n=7, $p<0.05$). In the presence of indomethacin, egg albumin (100 μg·ml^{-1} at the mucosal and the serosal side) induced only an increase in Isc of 0.9 ± 0.5 μEq·h^{-1}·cm^{-2} above baseline (n = 7, $p < 0.05$ versus response under control conditions) suggesting the involvement of prostaglandins and enteric neurons in the mediation of the antigen-induced secretion. In contrast, the partial inhibition of the antigen-induced Isc by mepyramine, a histamine H_1-receptor blocker, did not reach statistical significance (Table 4). Mepyramine alone (10^{-4} mol·l^{-1} at the serosal side) caused a decrease in basal Isc from 1.9 ± 0.3 μEq·h^{-1}·cm^{-2} to 0.8 ± 0.2 μEq·h^{-1}·cm^{-2} (n = 16, $p < 0.05$).

When the response to egg albumin was tested in sensitized rats fed on olive oil or fish oil diet, the secretory Isc induced by the antigen appeared to be slightly reduced from 4.3 ± 0.7 μEq·h^{-1}·cm^{-2} in the olive oil group (n = 14) to 3.1 ± 0.5 μEq·h^{-1}·cm^{-2} in the fish oil group (n = 15); an effect, which was, however, not statistically significant due to the large variability.

Discussion

Feeding rats on a fish oil diet, i.e. a diet rich in n-3 polyunsaturated fatty acids (36), has a modest effect on basal electrical parameters of the rat distal colon. It causes a marginal (and insignificant) decrease in Isc and Gt suggesting a reduction of basal anion secretion. Baseline Isc in the rat colon is known to be stimulated by the spontaneous activity of submucosal enteric neurons (1), which are basically activated by prostaglandins, e.g. prostaglandin I_2 (14). This is confirmed in the present study by the decrease in basal Isc induced by the neurotoxin, tetrodotoxin, blocking the propagation of action potentials (9), and by the cyclooxygenase inhibitor, indo-

methacin, preventing the production of prostaglandins. These observations are in contrast to results obtained with the rat jejunum. In this part of the intestine, an increase in basal Isc has been reported both for rats fed on a diet with a high content (18 %) fo fish oil as well as a high content of olive oil (6). The reasons for this discrepancy, e.g. segment heterogeneity, are not clear.

Fish oil diet has a variable effect on the secretion induced by activation of phospholipase(s) A_2. Both bradykinin, melittin and distension have been shown to induce secretion in the rat colon sensitive to the cyclooxygenase inhibitor, indomethacin, and to the phospholipase A_2 inhibitor, quinacrine (12, 13, 16). They differ, however, by their mechanism of activation of the enzyme. Bradykinin induces a receptor-mediated activation of phospholipase A_2 (20), whereas the hydrophobic protein melittin is thought to improve the presentation of the substrate of the enzyme, i.e. membrane phospholipids, by complex formation (30). The mode of activation of phospholipase A_2 by distension is not clear, it might be caused by an increase in the substrate availability for the enzyme due to a distension-induced change in membrane phospholipid packing (26). These processes were affected differentially by the fish oil diet. The fish oil diet significantly reduced the maximal increase in Isc induced by bradykinin by about 50 % (Table 3), while leaving the potency of bradykinin (i.e. the affinity of the peptide for its receptor) constant (Fig. 1). This is consistent with the hypothesis that the fish oil diet has led to a decrease in the content of arachidonic acid in the cellular membrane, which serves as precursor for the biologically active prostaglandins of the 2-series. The response to melittin, however, was only insignificantly reduced by about 30 %. A similar, insignificant reduction was observed with the secretory response to distension: the increase in Isc was reduced by about 15 % and the half-time was shortened by about 30 % when compared with the olive oil group. The reason for this discrepancy is not known. One might speculate that physical activation of phospholipase A_2 by distension or changing the substrate availability by melittin causes a stronger activation of the enzyme, which finally is able to counteract – at least in part – the reduction of the concentration of the correct substrate, arachidonic acid. Alternatively, there may be different pools of arachidonic acid in the submucosa, i.e. the part of the intestine, where prostaglandin synthesis takes place quantitatively (10), which are modified differentially by the diet.

In addition to the phospholipase A_2 pathway, also phipholipase(s) C is able to stimulate the production of prostaglandins, because the product of the phospholipase C reaction, diacylglycerol, can be further cleaved by a diacylglycerol lipase to form arachidonic acid (34). Administration of a phospholipase C from *clostridium perfringens* has been shown to induce a biphasic Cl^- secretion (17). The first phase of this secretion is due to the release of prostaglandins as indicated by the complete sensitivity to indomethacin, whereas the second phase of its action seems to be mediated by an activation of the protein kinase C pathway (17). The first phase of the PLC-effect is significantly reduced by about 50 % in the fish oil group compared to the olive oil group (Fig. 2), suggesting an effective reduction in the level of arachidonic acid in the PLC-sensitive pool.

Prostaglandins and other arachidonic acid metabolites are not only involved in inflammatory processes, but play also a role in intestinal anaphylactic reactions (for review see 8). In order to investigate whether the fish oil diet has a potential beneficial effect on allergic intestinal responses, rats were sensitized to egg albumin. Administration of egg albumin in vitro to the colon from sensitized (but not from non-sensitized) animals induces an increase in Isc (Fig. 4), suggesting an increase in anion secretion similar as it has been observed with other antigens or other intestinal segments (2, 7, 11, 35). The response to antigen showed a quite large variability (Table 4) and even 7 from 37 sensitized rats had to be excluded due to insufficient sensitization (see Methods for exclusion criterion). This increase in Isc was inhibited by tetrodotoxin or indomethacin, suggesting – in accordance with the results obtained at other intestinal segments (7, 11, 22) – the involvement of enteric secretomotor neurons and of prostaglandins in the mediation of this response. Indeed, a hyperexcitability of submucosal neurons in sensitized animals has been observed by Frieling et al. (19). A partial (60 %) inhibition by the histamine H_1-receptor blocker mepyramine just failed to reach statistical significance and thus did not allow to definitively answer the question whether histamine is involved in this secretion or not. Despite of the clear participation of prostaglandins indicated by the sensitivity to indomethacin, the fish oil diet had only a small and insignificant effect on the antigen-induced increase in Isc, which was reduced by about 30 % when compared to the olive oil group (Table 3). This suggests that either the pool of arachidonic acid released during the antigen reaction is only moderately affected by the diet or that the stimulus for the phospholipases responsible for the release of arachidonic acid is strong enough to partially counteract the reduced concentration of the enzyme substrate.

Which conclusion can be drawn from these in vitro data concerning a possible beneficial effect of a diet rich in n-3 polyunsaturated fatty acids for the treatment of inflammatory or allergic intestinal diseases? The results of the present study indicate that the response to some of the tested secretagogues, which depend on the eicosanoid pathway, but not to all of them, can be significantly reduced by a fish oil diet. The effect of the diet itself, e.g. on baseline parameters, exhibits a quite large variability, excluding statistically significant effects of the diet on such responses which themselves show a strong variability like, for example, the response induced by anti-

gen. Overall, the effect of the diet, despite of its high content (15 %) of fish oil, is much weaker than that of indomethacin, which suppresses, for example, the secretion induced by bradykinin or the first phase of the PLC response, whereas the fish oil diet causes only an inhibition of about 50 %. Consequently, a diet rich in n-3 polyunsaturated fatty acids may only play an adjuvant role for the treatment of inflammatory or allergic intestinal diseases.

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