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Nitric Oxide Involved in the IL-1-Induced Inhibition of Fructose Intestinal Transport

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ABSTRACT

Interleukin-1 (IL-1) is a pleiotropic cytokine produced by cells of the immune system and a large variety of other cell types including endothelial cells. It is released during inflammatory and infectious diseases, and possesses a wide spectrum of autocrine, paracrine and endocrine activities. The aim of this work was to examine the IL-1 effect on D-fructose transport across rabbit jejunum and try to identify the mediators implicated in this process. A sepsis condition was induced for 90 min after intravenous (iv) administration of IL-1 and body temperature was recorded. Studies on cellular intestinal integrity have not shown modifications of the epithelium and the basement membrane. D-fructose intestinal transport was studied in rabbit jejunum from control and treated animals and it was reduced in the latter ones. This cytokine decreased both the mucosal to serosal transepithelial flux and the transport across brush-border membrane vesicles of D-fructose. The inhibition was reversed by L-NAME (nitric oxide [NO] synthase inhibitor), but not by indomethacin (cyclooxygenase 1 and 2 inhibitor). Both inhibitors were administered iv 15 min before the IL-1. The protein levels of GLUT5 were not changed in all animal groups and those of mRNA were even increased. In summary, these findings indicate that IL-1, at the time assayed, induced a significant reduction in the relative intrinsic activity of GLUT5 and in this decrease are involved NO signalling pathways. In this way, blockage of D-fructose intestinal uptake by IL-1 may be playing an essential role in the pathophysiology of septic shock. J. Cell. Biochem. 111: 1321–1329, 2010.

KEY WORDS: GLUT5; INTESTINAL TRANSPORT; IL-1β; RABBIT; FRUCTOSE; NO; PROSTAGLANDINS

The intestinal absorption epithelium serves as a dynamic barrier which, under normal conditions, maintains the regulated uptake of nutrients and water while excluding potential pathogens [McKay and Baird, 1999].

A variety of bacteria and their excreted/secreted products have direct effects on epithelial ion transport and permeability and release of cytokines during bacterial infection may impact directly on epithelial function [Lewis et al., 1995; Sears and Kaper, 1996].

There is evidence that immune-derived cytokines can mediate metabolic alterations during the course of infective, inflammatory, autoimmune and neoplastic processes [Powanda and Beisel, 2003; Riedemann et al., 2004], either by acting locally or by interacting with different endocrine mechanisms [Bachmann and Kopf, 2002; Powanda and Beisel, 2003]. Studies at subcellular, cellular and tissues levels allow to investigate how cytokines interact with each other and with non-cytokine neuroimmune mediators to regulate homeostasis in the intestine [McKay and Baird, 1999].

The interleukins (IL) are pleiotropic cytokines that affect a wide variety of cells and they are small soluble mediators that participate in a variety of physiological and pathological events [Church et al., 2007]. IL-1 consists of three members: IL-1α, IL-1β and IL-1 receptor antagonist (IL-1ra). IL-β is secreted into surrounding interstitial fluid and blood circulation during inflammation and mediates wide-ranging proinflammatory actions [Dinarello, 2005,

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2009]. IL-1β was originally identified as the endogenous pyrogen and exogenous it causes fever in experimental animals [Dinarello, 1988]. Studies in vivo show that the administration of Il-1β may also induce hypoglycaemia in mice [Metzger et al., 2004] and leucopenia and hypotension in rabbits [Charles and Dinarello, 1988; Weinberg et al., 1988].

During inflammatory states, interleukins regulate the intensity of the intestinal immune response and mediate altered physiology in response to the inflammatory process either directly or via production of additional effector molecules that plays a central role in the intestinal inflammation amplification cascade [Al-Sadi and Ma, 2007]. In this way, Il-1β activates NO synthase and increases NO secretion by endothelial cells [Xu et al., 1999] and induces prostaglandins E production [Dinarello, 2005; Yoon and Dinarello, 2007].

Aside from their role in the regulation of the immune cascade, interleukins have also been shown to modulate intestinal transport function. In vitro studies have shown that Il-1β causes an increase in intestinal epithelial tight junction permeability in Caco-2 cells [Al-Sadi and Ma, 2007]. This cytokine stimulates anion secretion in chicken intestine [Chang et al., 1990] while the IL-α has been reported to inhibit Na^+ and Cl^- absorption [Chiossone et al., 1990] and increased 3-*O*-methyl glucose jejunal absorption in rabbit in vitro [Hardin et al., 2000]. Another results show that treatment of rats (injected i.p.) with this cytokine inhibits the intestinal transport of 3-*O*-methylglucose [Kreydiyyeh et al., 1998].

The aim of this work was to examine the effect of the proinflammatory Il-1β on jejunal D-fructose transport and GLUT-5 transporter expression and to determine the mode of action of this cytokine.

In a more general context, the regulation of IL-1β action, could be an effective therapeutic target for improving nutrient malabsorption during the course of sepsis.

MATERIALS AND METHODS

MATERIALS

D-fructose, *N*-nitroarginine methyl ester (L-NAME), 1-(*p*-chloro-benzoyl) 5-methoxy-2-methylindole-3-acetic acid (indomethacin), D-mannitol, HEPES, Tris (hydroxymethyl) amino-methane, sucrase, albumin bovine, cytochalasin B, ATP and anti-actin were obtained from Sigma Chemical (Madrid, Spain). IL-1β recombinant human was obtained from PeproTech GmbH (Hamburg, Germany). D-[U-14C] fructose, anti-rabbit IgG peroxidase and Biodegradable Counting Liquid Scintillation were obtained from GE Healthcare Life Sciences (Madrid, Spain). The membrane filters were obtained from Millipore (Barcelona, Spain).

ANIMALS, PREPARATION OF INTESTINAL TISSUE

The experimental animals were housed, handled and euthanised according to European Union Legislation 86/609/EEC. All experimental protocols were approved by the Ethical Committee of the University of Zaragoza (Spain). Male New Zealand rabbits weighing 1.8–2.0 kg were maintained at constant room temperature (24°C) with free access to water and standard rabbit fodder (25.1% proteins, 3.8% fat, 18.05% cellulose). The experiment was performed after a 24 h fast and all procedures were carried out while the rabbits were in an opaque restraining box. The animals were assigned to three main groups. One group of rabbits received an intravenous solution of 200 μl saline solution (control animals), another received 200 μl IL-1β at 0.05; 0.2 or 0.5 μg/kg body weight (bw) (treated animals). In the third group, the inhibitors (L-NAME and indomethacin) were injected 15 min before IL-1β. The inhibitors were made in saline solution and the doses were calculated from published EC50 in relation to body weight (40 μg/kg bw). It was administered in a final volume of 200 μl to avoid any changes in blood volume. The saline solution, IL-1β and inhibitors tested were administered intravenously (iv) through the lateral ear vein.

Rabbits were killed 90 min after injection, following previously published protocols including ours [Amador et al., 2007a,b; García-Herrera et al., 2007] and intestinal samples were taken. The proximal jejunum was removed and rinsed with ice-cold Ringer's solution which contained (in mM): 140 NaCl, 10 $KHCO_3$, 0.4 KH_2PO_4, 2.4 K_2HPO_4, 1.2 $CaCl_2$ and 1.2 $MgCl_2$, pH 7.4.

SUGAR UPTAKE MEASUREMENTS

Tissue uptake. Rings of everted jejunum weighing about 100 mg were continuously bubbled with 95% O_2–5% CO_2. Tissue rings were incubated for 3 min in Ringer's solution at 37°C containing 0.01 μCi/ml D-[U-14C] fructose plus 5 mM unlabeled substrate. After incubation, tissue pieces were washed with three gentle shakes in ice-cold Ringer's solution and blotted carefully on both sides to remove excess of solution, weighed, and incubated overnight in 0.5 ml of 0.1 M HNO_3 at 4°C to extract the labelled fructose from tissue. Aliquots of 200 μl from extract and bathing solutions were counted in 2 ml of scintillation fluid biodegradable (NBCS 104, Amersham Biosciences). The measurements were expressed as μmol D-fructose per gram of tissue.

Transepithelial flux measurements. Jejunal tissue was stripped of its serosal and external muscle layers and mounted as a flat sheet in Ussing-type chambers. The bathing solutions containing 5 mM D-fructose at mucosal and serosal surfaces of the tissue were maintained for a 40 min preincubation at 37°C using a circulating water bath. Mucosal-to-serosal sugar fluxes (Jm-s) and serosal to mucosal fluxes (Js-m) were then measured by adding 0.04 μCi/ml D-[U-14C] fructose to the mucosal or serosal chamber, respectively. Samples were removed from the non-radioactively labelled side at 20 min intervals for 60 min. Starting radioactivity at time zero and timely samples were counted with a liquid scintillation counter. The results are expressed as μmol D-fructose per square centimetre per hour.

BBMV uptake assays. Brush-border membrane (BBM) vesicles (BBMVs) were prepared using the Mg^{2+} EGTA precipitation method [Brot-Laroche et al., 1986]. Prepared BBMVs containing 300 mM mannitol and 10 mM HEPES–Tris pH 7.4 buffer were used for transport studies. Protein content was measured with the Bradford method using bovine serum albumin as standard. BBMV purity was determined by measuring sucrase activity enrichments and levels of basolateral plasma membranes were assayed by measuring Na^+/K^+ ATPase activity.

Fructose uptake was measured at different times: 5, 40 s; 2, 10 and 30 min. Incubations were performed at 37°C and started by adding

5 μl (200 μg) of BBMV to 45 μl of incubation medium containing 10 mM HEPES–Tris, 100 mM NaCl, 0.01 μCi/ml D-[U-14C] fructose plus 5 mM unlabelled substrate, and D-mannitol to compensate for osmolarity (300 mOsmol/L). The results are expressed as D-fructose uptake in picomoles per milligram protein.

WESTERN BLOTTING ANALYSIS

Similar amounts of BBMVs protein (10 μg) from control and treated animals were loaded and resolved on a 12% SDS–polyacrylamide gel. Proteins were transferred electrophoretically to PVDF membranes (GE Healthcare Life Sciences) using a semidry transblot transfer apparatus (Bio-Rad). The protein transfer efficiency was visualised with Ponceau S. GLUT5 was detected by using a rabbit polyclonal anti-GLUT5 antibody Millipore AB1348 (LOT LV1371782) corresponding to the C-terminus of rat GLUT5 (aa 490–502). Equal loading was confirmed by using an anti-actin antibody (Sigma Chemical). Detection was carried out using an anti-rabbit IgG conjugated to horseradish peroxidase (GE Healthcare Life Sciences) and ECL chemiluminescence (GE Healthcare Life Sciences). Membranes were exposed to ECL films (GE Healthcare Life Sciences) for several time periods to achieve signal intensity within the dynamic range of quantitative detection, and films were scanned at a 600 dpi resolution. Intensity of bands for each condition, taken as volume of pixels per square millimetre, was calculated with Quantity One Software to that corresponding to the actin signal.

IMMUNOFLUORESCENCE AND CONFOCAL MICROSCOPY

Immunofluorescence confocal microscopy analysis of GLUT5 was performed in cryosections of jejunum from the different experimental groups. Sections after permeabilisation with 0.2% Triton X-100 PBS were incubated at 4°C overnight with a Glut5 affinity purified goat antibody sc-14841 from Santa Cruz Biotechnology (Santa Cruz, CA) diluted at 1:250. After washing with PBS, sections were incubated with Alexa fluor 488 donkey anti goat IgG (Invitrogen, Madrid, Spain) at dilution 1/2,000 for 1 h and stained with propidium iodide. The stained sections were visualised with a Leica SP2 AOBS confocal scanning microscope. Images were collected using a 20× lens (lens specification, HC PL FLUOTAR NA 0.50; Leica). The confocal pinhole was 1 AU.

RNA PREPARATION AND ANALYSIS

At the end of the experimental period jejunum of all rabbits were immediately removed and frozen in liquid nitrogen. RNA from each jejunum was isolated using Trigent reagent (MRC, Cincinnati, OH). DNA contaminants were removed by TURBO DNase treatment using the DNA removal kit from AMBION (Austin, TX). RNA was quantified by absorbance at A260/280 (the A260/280 ratio was greater than 1.75). The integrity of the 28S and 18S ribosomal RNAs was verified by agarose formaldehyde gel electrophoresis followed by ethidium bromide staining and the 28S/18S ratio was greater than 2.

Equal amounts of RNA from each rabbit were used in quantitative real-time reverse transcriptase–polymerase chain reaction (RT-PCR) analyses. First-strand cDNA synthesis and the PCR reactions were performed using the SuperScript II Platinum Two-Step quantitative RT (qRT)-PCR Kit with SYBR Green (Invitrogen), according to the manufacturer's instructions and as previously described [Arbonés-Mainar et al., 2006]. The following primers were used in real-time PCR: for glut5-sense, 5′-CGGTTCGTCTTTCCAGTATGCT-3′, glut5 antisense, 5′-GTGTCGTTGTAGAACTCCGTCATG-3′ and for cyclophilin B-sense, 5′-TCCCAGTTCTTCATCACCACAG-3′, cyclophilin B antisense, 5′-TCCATGCCCTCCAGAACTTT-3′. Real-time PCR reactions were performed in an ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA). The relative amount of all mRNAs was calculated using the comparative $2^{-\Delta\Delta C_t}$ method. Cyclophilin B mRNA was used as the invariant control.

HISTOLOGICAL STUDY

A histological study was carried out to test the tissue viability in the different experimental conditions (control and IL-1β treated animals). Jejunal tissue samples were fixed in 10% buffered formaldehyde and embedded in paraffin. Sections of 4 μm were stained with haematoxylin–eosin. The Electron Microscopy Core Facility of the University of Zaragoza analysed the ultrastructure of the intestines. Images were captured and digitised by use of a Nikon microscope equipped with Cannon digital camera.

STATISTICAL ANALYSIS

All results are expressed as means ± SE. Means were compared using an one-way analysis of variance (ANOVA). Significant differences at $P < 0.05$ were compared using a Bonferroni's multiple comparison test. The statistical analysis and the graphics were performed using the GraphPad Prism Version 5.02 program on a PC computer.

RESULTS

DOSE-DEPENDENT IL-1β EFFECT ON D-FRUCTOSE INTESTINAL UPTAKE

In relation to previous IL-1β assays [Yamashiro et al., 1993], we studied the effect of i.v. administration of IL-1β (0.05, 0.2 and 0.5 μg/kg bw) on the intestinal absorption of D-fructose 5 mM. Following 90 min of cytokine treatment, the animals were sacrificed and the sugar intestinal uptake was measured (3 min incubation). As shown in Table I, IL-1β at 0.2 and 0.5 μg/kg bw inhibited (about 20%) D-fructose uptake. For the following experimental studies we chose the significantly smaller concentration (0.2 μg/kg bw).

Histological examination of the jejunum showed that the epithelial structure and the basement membrane were similar in

TABLE I. Dose-Dependent IL-1β Effect on D-fructose Intestinal Absorption

Animal conditions	D-fructose uptake
Control	2.29 ± 0.04
0.05 μg/kg IL-1-β	1.87 ± 0.08
0.2 μg/kg IL-1β	1.85 ± 0.09*
0.5 μg/kg IL-1β	1.78 ± 0.13*

D-fructose 5 mM uptake (3 min) was measured in everted jejunal rings taken from control and IL-1β-treated rabbits. Animals were injected intravenously (iv) with either saline solution (control) or 0.05, 0.2 and 0.5 μg/kg bw IL-1β for 90 min. Results were expressed as μmol D-fructose/g tissue and represent the means ± SE of six determinations per animal, with six animals.

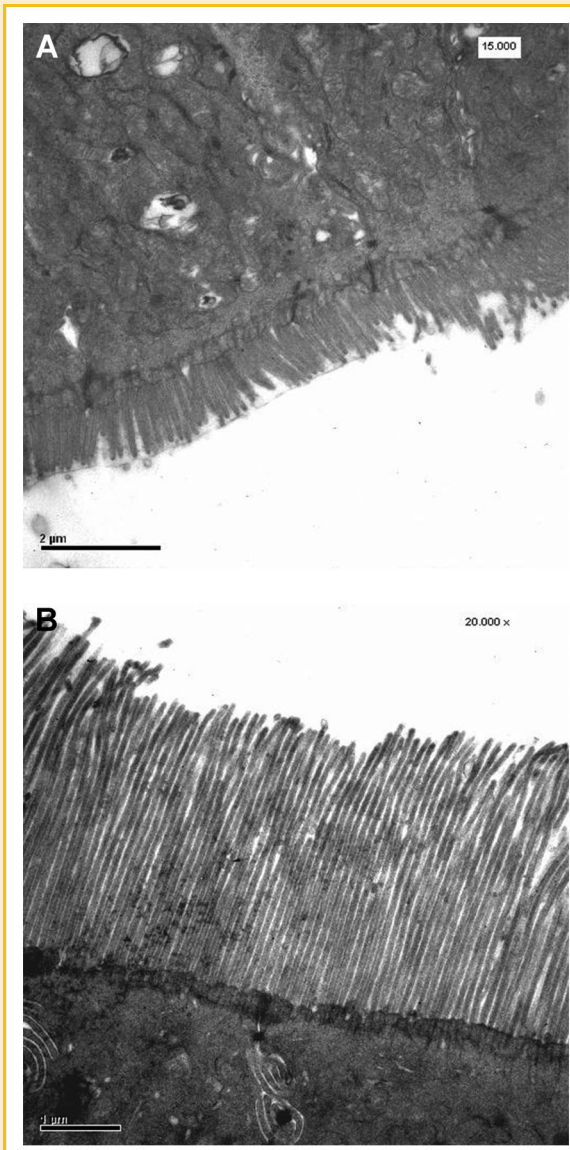

Fig. 1. Electron microscopy image of small intestine (microvilli) from control and treated rabbits with 0.2 μg/kg IL-1β bw for 90 min. A: corresponds to control jejunum at 15,000× magnification. Bar corresponds to 2 μm. B: Corresponds to treated jejunum at 20,000× magnification. Bar corresponds to 1 μm.

control and 0.2 μg/kg bw IL-1β-treated animals (data not shown). Electron microscopy analysis of the BBMs integrity was shown unaltered after cytokine treatment (Fig. 1A,B). In addition, this cytokine concentration increases the rectal temperature rabbit around 1.05°C (Table II), therefore a septic state was evoked.

TABLE III. Action of IL-1ra on Il-1β Effect in D-Fructose Intestinal Transport

Animal conditions	D-fructose uptake
Control	2.28 ± 0.09
(0.2 μg/kg) IL-1-β	1.85 ± 0.09*
(1 μg/kg) ILRa + (0.2 μg/kg) IL-1-β	1.91 ± 0.04*
(2 μg/kg) ILRa + (0.2 μg/kg) IL-1-β	2.31 ± 0.05
(2 μg/kg) ILRa	2.08 ± 0.05

D-fructose 5 mM uptake (3 min) was measured in everted jejunal rings taken from several groups of rabbits. Animals were injected intravenously (iv) with either saline solution (control), 0.2 μg/kg bw IL-1β or different IL-1β receptor antagonist concentrations (1, 2 μg/kg bw IL-1ra) for 90 min. The antagonist was administered 15 min before IL-1β administration. Results were expressed as μmol D-fructose/g tissue and represent the means ± SE of six determinations per animal, with six animals.
*$P < 0.05$ compares results with control.

TABLE IV. Effect of 0.2 μg/kg bw IL-1β on 5 mM D-fructose Mucosal-to-Serosal (Jm-s) and Serosal-to-Mucosal (Js-m) Fluxes

	Jm-s	Js-m
μmol D-fructose/cm^2/h		
Control	0.75 ± 0.06	0.28 ± 0.02
IL-1β	0.42 ± 0.04*	0.22 ± 0.02

Results are expressed as μmol D-fructose per square centimetre per hour and represent the means ± SE of eight determinations per animal, with six animals (control and IL).
*$P < 0.05$ compares results obtained in IL with control.

IL-1β EFFECT ON D-FRUCTOSE INTESTINAL TRANSPORT

The effect of IL-1β (0.2 μg/kg bw) on D-fructose transport was abolished by the specific receptor antagonist IL-1ra (2 μg/kg bw) (Table III). IL-1ra alone at this concentration had no effect significative on fructose transport.

Since, IL-1β inhibits D-fructose absorption across rabbit jejunum, this effect could indicate either a decrease in D-fructose uptake across the mucosal border or an increase in D-fructose transport from the cell to the medium across the serosal border. In order to clarify this point, the fluxes mucosal-to-serosal (Jm-s) and serosal-to-mucosal (Js-m) of 5 mM D-fructose was measured in control and treated animals (i.v. administration of 0.2 μg/kg bw IL-1β) by Ussing chambers. Mucosal-to-serosal D-fructose flux decreased in treated animals (about 44% inhibition) but no change was reported in relation to serosal-to-mucosal (Js-m) D-fructose flux (Table IV).

TABLE II. Effect of 0.2 μg/kg bw IL-1β on Body Temperature

Animal conditions	Initial temperature	Final temperature	Increase temperature
Control	39°C ± 0.24	38.9°C ± 0.18	–
Interleukin 1-β	38.8°C ± 0.08	39.8°C ± 0.12	1.05°C
L-NAME + IL-1β	39.0°C ± 0.10	39.9°C ± 0.12	0.90°C
Indomethacin + IL-1β	39.1°C ± 0.06	39.5°C ± 0.08	0.40°C

The rectal temperature was measured every 30 min for 90 min. L-NAME or indomethacin (40 μg/kg bw) was administered 15 min before the cytokine. The results are from six animals at each condition.

TABLE V. Effect of 0.2 μg/kg bw IL-1β on Jejunal Brush-Border Membrane Vesicles (BBMV)

Time	Control	IL-1β	L-NAME + IL-1β	Indomethacin + IL-1β
pmol D-fructose/mg protein				
5 s	5.97 ± 0.70	5.42 ± 1.02	5.64 ± 0.92	6.11 ± 0.66
40 s	14.09 ± 1.18	15.88 ± 0.54	15.13 ± 2.45	16.21 ± 1.50
2 min	44.00 ± 4.28	30.64 ± 1.51*	40.30 ± 1.54	25.50 ± 1.07*
10 min	71.82 ± 6.41	45.07 ± 2.09*	68.30 ± 4.78	53.65 ± 3.09*
30 min	80.89 ± 3.30	54.98 ± 2.87*	79.61 ± 4.49	57.73 ± 5.45*

Time course of 5 mM D-fructose uptake in BBMV from control and IL-1β (± L-NAME or indomethacin 40 μg/kg bw) treated animals after 90 min. Uptakes, in pmol per mg of membrane protein ± SE, represent the mean value of three determinations per point for six animals.
*$P < 0.05$ compared to control.

IL-1β EFFECT ON BRUSH-BORDER MEMBRANE VESICLES

Intestinal fructose transport can occur via GLUT2 and GLUT5 facilitative transporters and depends on the sugar concentration gradient across the membrane. Changes in fructose uptake might therefore result from changes in transporter abundance in the plasma membrane and/or from metabolic alterations by increasing the slope of sugar gradients [Mesonero et al., 1995; Helliwell et al., 2000; Le Gall et al., 2007; Leturque et al., 2009]. We performed fructose uptakes in purified BBMVs at different incubation times: 5, 40 s; 2, 10 and 30 min. Fructose uptake was decreased up to 40% at 30 min of incubation time in purified BBMVs prepared from IL-1β compared with control animals (Table V).

To decipher the respective contribution of GLUT2 and GLUT5 to fructose transport, BBMVs were incubated for 10 min with 0.1 mM cytochalasin B, a competitive inhibitor of GLUT2 but not GLUT5 [Burant et al., 1992]. Fructose uptake was not changed in control and IL-1β BBMVs by cytochalasin B, indicating the absence of GLUT2 contribution (Fig. 2A). Moreover, the cytokine did not inhibit D-

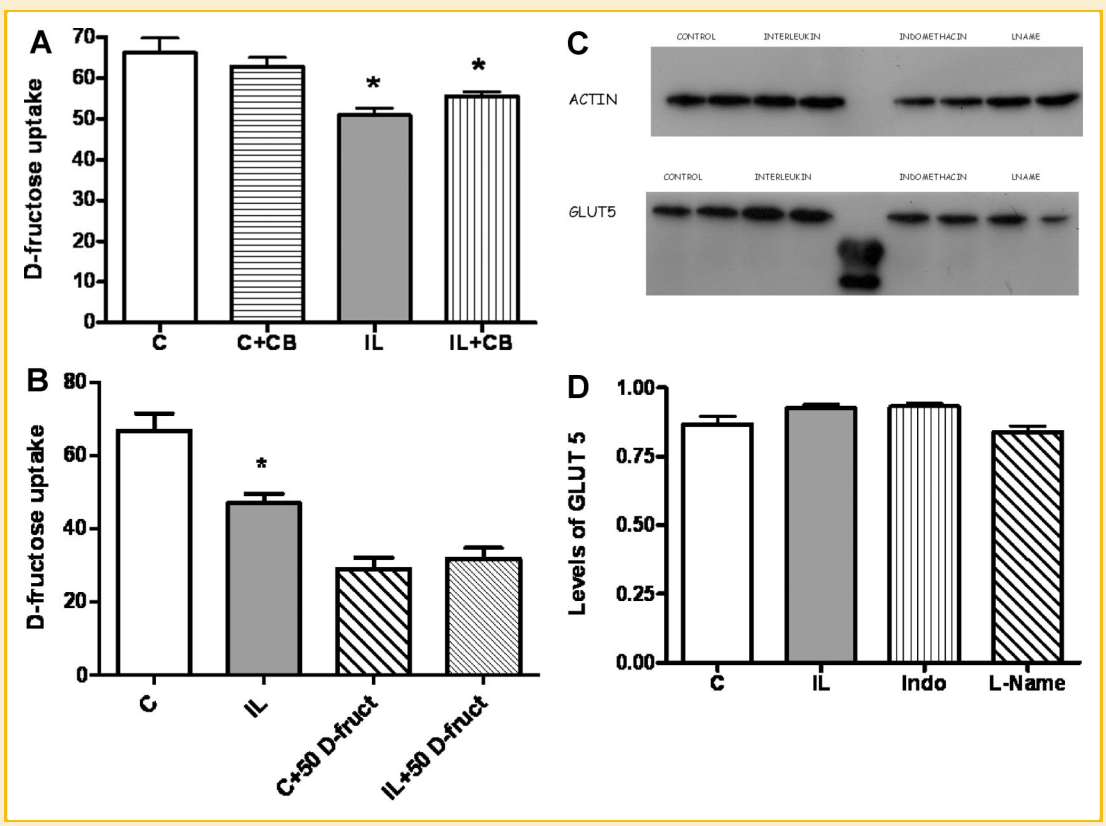

Fig. 2. GULT5 transporter in jejunal brush-border membrane vesicles (BBMVs) from control and 0.2 μg/kg bw IL-1β treated animals after 90 min. A: Effect of 0.1 mM cytochalasin B (CB) on 5 mM D-fructose uptake for 10 min in BBMVs from control and IL-1β-treated animals. B: Effect of 50 mM D-fructose on 5 mM D-fructose uptake for 10 min in BBMVs from control and IL-1β treated animals. Uptakes, in pmol per mg of membrane protein ± SE, represent the mean value of three determinations per point for six animals for each condition. C: Effect of IL-1β and several inhibitors on GLUT5 protein expression in BBMVs. Representative Western blot analysis of GLUT5 BBMVs prepared from control and IL-1β (± inhibitors)-treated animals. The results represent data obtained by densitometric analysis of immunoblotted signals for proteins normalised to those of β-actin on the same gels. D: Representative blots and data expressed as percent of control values (means ± SE) are given. The preparations of intestinal vesicles per animal of each group ($n = 6$) were prepared and analysed in duplicate.

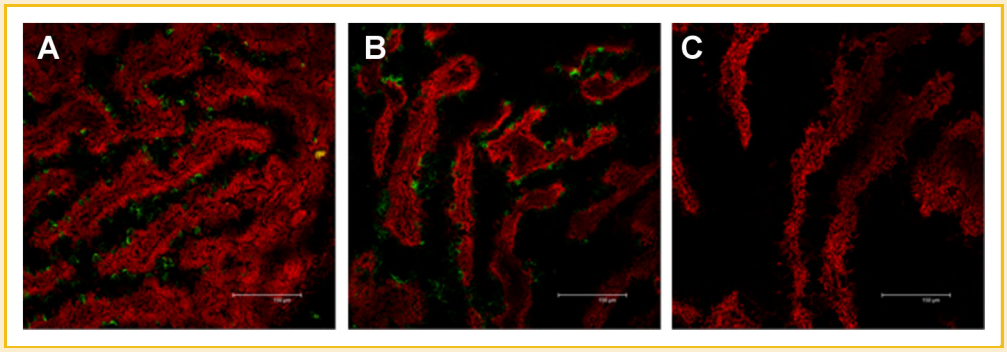

Fig. 3. Representative figures of immunofluorescence confocal microscopy of GLUT5 (green) and nucleus (red, propidium iodide) in jejunum cryosections from rabbit control group (A), rabbit IL group (B) and secondary antibody control (no primary antibody used) (C).

fructose BBMVs uptake when 50 mM D-fructose (acting as an inhibitor of mediated transport) was added to media for 10 min (Fig. 2B). This result indicates that the endotoxin does not seem to modify sugar simple diffusion. All together these results indicate that IL-1β-sensitive fructose transport is by GLUT5 at the level of the BBM excluding any effect of metabolism.

To measure GLUT5 levels in BBM from control and IL-1β (0.2 μg/kg bw) treated animals, a Western blot analysis was performed. The GLUT5 antibody recognised a single band in control and IL BBMVs (Fig. 2C). The densitometric analysis for GLUT5 normalised to actin levels was performed in six separate experiments, indicating no significant changes of GLUT5 protein in any of the conditions (Fig. 2D).

IMMUNOFLUORESENCE ASSAYS

The above results suggest that the protein level of GLUT5 was similar to different animal groups. However, the sugar uptake was inhibited by IL-1β. In order to further evaluate the characteristics of the cytokine inhibitory effect on GLUT5 transporter, the immunofluoresence with its own antibody was performed. The treatment of rabbits with IL 0.2 μg/kg bw does not lead to a change in the GLUT5 transporter expression in the BBM as shown in Figure 3.

IL-1β AND FRUCTOSE TRANSPORTER GLUT5 mRNA EXPRESSION

With the object of studying the IL-1β effect on GLUT5 transporter in-depth, we assayed by RT-PCR, the RNA expression in different conditions. In jejunum of IL-1β-treated rabbit, a no significant increase was observed in the expression of GLUT5 mRNA in relation to jejunum of control animals (Fig. 4).

MEDIATORS IMPLICATED IN IL-1β ACTION ON D-FRUCTOSE INTESTINAL ABSORPTION

Some studies have shown that nitric oxide (NO) [Jourd'Hevil et al., 1997; Jergens, 1999] and prostaglandin E2 (PGE2) [Takeuchi et al., 2003; Wallace and Devchand, 2005] play a very important role in modulating gastrointestinal mucosa in a variety of contexts like inflammatory bowel disease. To establish whether these mediators are implicated in the IL-1 inhibitory effect on D-fructose intestinal uptake, we assayed L-NAME (an inhibitor of NO synthases) and Indomethacin (cyclooxygenase 1 and 2 inhibitor). These inhibitors were administered i.v. 15 min before IL-1β treatment. L-NAME at concentration 40 μg/kg bw completely abolished the cytokine effect on 5 mM D-fructose uptake in rings of everted jejunum and in BBMVs but indomethacin at 40 μg/kg not changed the IL-1β effect (Tables V and VI). The inhibitor concentrations were assayed in previous studies [García-Herrera et al., 2004].

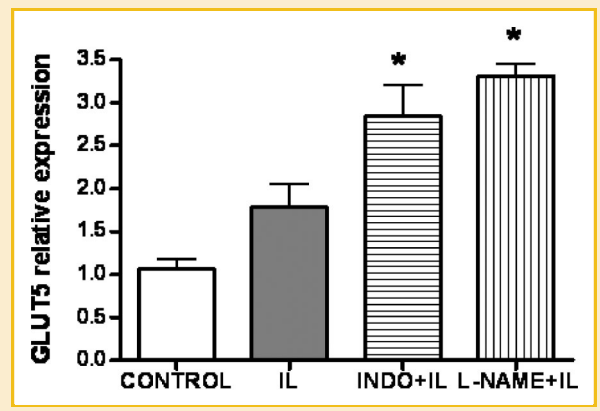

Fig. 4. Effect of 0.2 μg/kg IL-β on GLUT5 mRNA expression. The expression was measured in jejunum of control and IL-1β treated animals ± L-NAME or indomethacin 40 μg/kg bw. Values are expressed as means ± SE of six animals per group. The quantitative fold changes in mRNA expression were determined as relative to cyclophilin mRNA levels in each corresponding group and calculated using the $2^{-\Delta\Delta C_t}$. $^*P < 0.05$ compared with control group.

TABLE VI. Mediators Implicated in 0.2 μg/kg bw IL-1β Action on D-fructose Intestinal Absorption

Animal conditions	D-fructose uptake
Control	2.28 ± 0.06
Interleukin 1-β	1.82 ± 0.04*
L-NAME + IL-1β	2.23 ± 0.06
Indomethacin + IL-1β	1.85 ± 0.09*

D-fructose 5 mM uptake (3 min) was measured in everted jejunal rings taken from several groups of rabbits. Animals were injected intravenously (iv) with either saline solution (control) or 0.2 μg/kg bw IL-1β for 90 min. The inhibitors (L-NAME or indomethacin) were administered at 40 μg/kg bw 15 min before IL-1β administration. Results were expressed as D-fructose/g tissue and represent the means ± SE of six determinations per animal, with six animals.
*$P < 0.05$ compared with control animals.

On the other hand, in presence of these inhibitors, the GLUT5 protein levels were not modified (Fig. 2C,D). However, GLUT5 mRNA underwent a significant increase in these groups of animals (Fig. 4).

DISCUSSION

LPS endotoxin stimulates monocytes and macrophages to release cytokines such as IL-1, IL-6 and TNF-α which induce a multitude of host responses like alter nutrient intestinal absorption. Previous studies in our laboratory have shown that adding LPS to intestinal tissue inhibits the transport of L-leucine [Abad et al., 2001a, 2002a], D-fructose [García-Herrera et al., 2003] and D-galactose [Amador et al., 2008]. Moreover, after intravenous administration of the endotoxin, the intestinal absorption of these nutrients was inhibited. This LPS effect could be produced by the secretagogue action of TNF-α on the gut, also implying protein kinases and other mediators such as NO and prostaglandins [Abad et al., 2001b, 2002b; García-Herrera et al., 2004, 2007; Amador et al., 2007a,b].

In this study, we showed that in the jejunum of IL-1β-treated rabbits, the intestinal absorption of fructose was reduced. The levels of GLUT5 mRNA were increased and protein did not change suggesting a post-transcriptional down-regulation of GLUT5 in the intestine of these rabbits. Besides, NO could be a mediator involved in the inhibition of D-fructose intestinal uptake.

Fever is a systemic inflammatory response, and several endogenous pyrogens have been identified including the proinflammatory cytokine IL-1 [Dinarello, 1988; Opp and Krueger, 1991].

In our work, following the injection of IL-1β (0.2 mg/kg bw) in normal rabbits there was a significant temperature increase at 90 min (∼1.05°C) and the fever was ameliorated by cyclooxygenase inhibitor indomethacin (Table II).

Morphologically, when a cell undergoes apoptosis, the cell shows a lot of changes in cell volume, cytoplasmic organelles, microvilli, etc. According to these cellular integrity analysis, the IL-1β (0.2 μg/kg bw) did not affect the intestinal mucosa (Fig. 1B) but there were biological effects on gut.

D-fructose intestinal transport was significantly inhibited in rabbits after IL-1β injection (0.2 μg/kg bw) (Table I) and this effect was abolished by the specific receptor antagonist IL-1ra at concentration 2 μg/kg bw (Table III). The cytokine inhibited Jm-s and sugar uptake across purified BBMVs indicating that mucosal side of the cell is affected (Tables IV and V).

The fructose and glucose transporter GLUT2 is involved in the exit of sugars at the basolateral membrane of the cells, and GLUT2 was reported to traffic into the BBM of enterocytes in response to several physiological signals, including sugar consumption, fasting, and feeding, and to AMPK as well as PKCβ2 and P38 MAPK activation [Kellet and Brot-Laroche, 2005; Kellet et al., 2008]. In the present experiments, the BBM-associated transport was insensitive to cytochalasin B, an inhibitor of GLUT2 but not GLUT5 (Fig. 2A). Explanation for the lack of GLUT2 related uptake can also come from the composition of fodder that delivers very low amounts of simple sugars in the lumen of the intestine and limits to a minimum level the priming of the enterocytes for GLUT2 insertion. Thus, in the absence of any GLUT2 in the apical membrane, the results presented in this article show that the cytokine induces changes in GLUT5 transporter. This event was confirmed when the D-fructose mediated transport was inhibited by 50 mM D-fructose (Fig. 2B). GLUT5 mRNA expression was slightly increased in IL-1β treated animals (Fig. 4) but this increase did not modify the protein expression (Figs. 2C,D and 3). Therefore, at the time assayed in this work, the inhibition in D-fructose intestinal uptake is provoked by a reduction in the relative intrinsic activity of GLUT5 protein.

Cox-1 and Cox-2 synthesise prostaglandins from arachidonic acid [Backlund et al., 2005]. While intestinal epithelial cell express Cox-1 constitutively and is critical for housekeeping action in the gastrointestinal mucosa, Cox-2 is responsible for inflammation [O'Neill and Ford-Hutchinson, 1993; Ikari et al., 1999]. The administration of indomethacin, a prostaglandin synthesis inhibitor (Cox-1 and Cox-2 inhibitor) [Takeuchi et al., 2003] before IL-1β administration, abolished the hyperthermia in rabbits (Table II) but did not modified the inhibitory effect of cytokine on D-fructose intestinal transport (Tables V and VI). In addition, indomethacin did not alter the number of GLUT5 transporters at the BBM (Fig. 2C,D) despite the significant increase observed in GLUT5 mRNA levels (Fig. 4). Several mechanisms could be involved in this discrepancy, first it can not be ruled out a different time course of mRNA and protein levels, second a post-transcriptional regulation of mRNA and third a regulatory effect at the GLUT5 cargo to be directed towards BBM.

On the other hand, NO is a potent cell-signalling effector that plays important roles in diverse biological effects in many tissues [Kone et al., 2003] and alters the function of many metalloenzymes, carrier proteins and structural proteins [Opal, 2007]. NO is a highly diffusible gas that plays an essential role in the pathophysiology of septic shock. The short half-life (1–3 s) of NO limits its activity to the tissues in which it is generated by one of three isoforms of NO synthase. Full expression of inducible NO synthase is complex requiring TNF-α, IL-1, LPS and probably other regulatory elements [Opal, 2007]. In this way, the intravenous administration of L-NAME (inhibitor of NO synthases) before IL-1β treatment, blocked completely the cytokine inhibitory effect on fructose intestinal absorption (Tables V and VI). This fact suggests that NO is involved in the IL-1β action. However, not only did not this inhibitor modify the GLUT5 level proteins (Fig. 2C,D) but it also increased mRNA gene expression significantly (Fig. 4). These facts are indicating that the reduction in the relative intrinsic activity of GLUT5 protein by IL-1β is abolished when L-NAME was administered.

In conclusion, our results show that 90 min after the intravenous administration of IL-1β (0.2 μg/kg bw), the transport of fructose across brush border of rabbit jejunum is inhibited by a reduction in the relative intrinsic activity of GLUT5 protein. Furthermore, L-NAME abolishes the cytokine effect on sugar transport suggesting that NO could play a role in mediating this action mainly exerted at the activity level.

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