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Modulation of amylase release and intracellular Ca^{2+} mobilization by dietary fat in isolated rat pancreatic acinar cells

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A number of *in vivo* studies indicate that alteration in dietary fat intake results in adaptation of exocrine pancreatic secretion. At the cellular level, the mechanism of this adaptation remains largely unknown. Dietary fats influence the lipid composition of plasma membranes in several tissues, including pancreas (Martinez *et al.* 2002), and this may, in turn, induce functional changes. This study was designed to clarify the effects of feeding different oils on cholecystokinin octapeptide (CCK-8)-evoked amylase release and Ca^{2+} mobilization in isolated rat pancreatic acinar cells.

Male weaning Wistar rats were fed *ad libitum* with one of two semi-purified diets differing only in the type of fat added (10 % wt): virgin olive oil (group O) and sunflower oil (group S). After 8 weeks, rats were humanely killed and the pancreas removed. A fraction was used for plasma membrane isolation and analysis of fatty acid composition by gas-liquid chromatography. The rest of the gland served to provide acini by collagenase digestion. Amylase release was determined by incubating the cells with CCK-8. The activity released into the medium was quantified by the Phadebas blue starch method (Ceska *et al.* 1969) and expressed as a percentage of the cell total activity at the beginning. $[Ca^{2+}]_i$ was measured fluorimetrically in a suspension of fura-2 AM-loaded cells by established methods.

The lipid composition of acinar cell membranes reflected the type of dietary fat given. Membranes of olive oil-fed animals displayed a level of total monounsaturated fatty acids of 47.36 ± 2.37 (percentage of total fatty acids, mean ± S.E.M., $n = 11$), significantly higher ($P < 0.05$, Student's unpaired t test) than did the sunflower oil-fed group (29.72 ± 1.41, $n = 15$). Accordingly, a significantly higher ($P < 0.05$, Student's t test) percentage of total polyunsaturated fatty acids was found in membranes of group S rats (29.94 ± 2.25, $n = 15$) than in those of group O (11.26 ± 1.73, $n = 11$). No differences in the saturated fatty acid level were observed among the groups. Adaptation to different fats resulted in significant differences ($P < 0.05$, Student's t test) in basal amylase release (group O: 5.96 ± 0.60 %, $n = 9$); group S: 12.20 ± 0.48 %, $n = 13$). The increase above basal evoked by CCK-8 10^{-10}, 10^{-9} and 10^{-8} M in group O was 15.32 ± 1.39 % ($n = 23$), 14.22 ± 1.20 % ($n = 23$) and 12.50 ± 0.32 % ($n = 15$), respectively. These responses were significantly higher ($P < 0.05$, Student's t test) than those observed in group S (9.61 ± 0.51 %, $n = 35$; 8.02 ± 0.44 %, $n = 37$ and 6.90 ± 0.52 %, $n = 22$ for CCK-8 10^{-10}, 10^{-9} and 10^{-8} M, respectively). Dietary fat had no influence on basal $[Ca^{2+}]_i$. However, the rise in $[Ca^{2+}]_i$ evoked by all three concentrations of CCK-8 was significantly enhanced ($P < 0.05$, Student's t test) in group O compared with group S. CCK-8 10^{-10}, 10^{-9} and 10^{-8} M resulted in group O in a peak response of 556.49 ± 30.24 nM ($n = 4$), 677.44 ± 40.28 nM ($n = 5$) and 601.18 ± 48.79 nM ($n = 4$), respectively, whereas values of 306.07 ± 12.07 nM ($n = 7$), 404.70 ± 12.20 nM ($n = 5$) and 455.29 ± 10.45 nM ($n = 6$) were reached in group S in response to the same concentrations of the secretagogue.

The results suggest that dietary fat can modulate not only the composition of membrane lipids of rat pancreatic acinar cells but also the secretory activity and signal transduction evoked by CCK-8. How these facts are linked and whether more complex mechanisms are involved deserve further investigation.

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All procedures accord with current National and local guidelines.

P184

Role of somatostatin receptor subtypes in the control of gastric acid secretion in mice

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Somatostatin, acting in a paracrine fashion, is the main inhibitor of gastric acid secretion (GAS). Somatostatin actions are mediated through five different receptors subtypes, termed sst_1 to sst_5, all of which are found in the stomach.

The aim of this study was to investigate the role of the different somatostatin receptor subtypes in the control of GAS in mice, using receptor-selective agonists and antagonists and sst_2 knockout mice.

In urethane (1.25 g kg^{-1}, I.P.) anaesthetized sst_2 knockout, and wild-type mice (Merck Research Laboratories), GAS was monitored at 10 min intervals by continuous intragastric perfusion with saline (pH 7.0, 36 °C) and backtitration to pH 7.0 with NaOH (0.001 N). The ileal veins were cannulated for I.V. infusion of compounds. The following receptor-selective somatostatin analogues were tested: L-797-591 (sst_1), DC 32-87 (sst_2), BIM-23056 (sst_3), L-803-087 (sst_4) and BIM-23052 (sst_5) (20–200 μg kg^{-1}, I.V.) (Robrer *et al.* 1998; Martínez *et al.* 2000). The effects on GAS of the sst_2 selective antagonist PRL 2903 (1.56 mg kg^{-1}; Rossowiski *et al.* 1998) and the *in vivo* immunoneutralization of somatostatin (somatostatin monoclonal antibody CURE.S6, 150 μg per mouse; CURE:DDRC, UCLA, Los Angeles) were also investigated. Data are means ± S.E.M. and were analysed using Student's t test or one-way ANOVA, paired or unpaired as appropriate. At the end of the experiments animals were humanely killed accordingly to currently accepted procedures.

In wild-type animals the selective sst_2 agonist DC 32–87 inhibited pentagastrin-stimulated GAS in a dose-dependent manner (27.1 ± 6.3, 37.1 ± 7.1, 53.7 ± 8.8, 57.9 ± 14.3 and 63.7 ± 8.7 % for 1, 5, 10, 15 and 20 μg kg^{-1}, respectively, $n = 5$) Somatostatin-14 and DC 32-87 (20 μg kg^{-1}) inhibited pentagastrin-stimulated GAS with similar efficacy (somatostatin-14: 0.62 ± 0.07 μmol h^{-1}; DC 32-87: 0.69 ± 0.07 μmol h^{-1}; both $P < 0.05$ *vs.* vehicle: 1.27 ± 0.05 μmol h^{-1}; $n = 4$–8). None of the other receptor selective agonists, tested at doses up to 200 μg kg^{-1}, modified GAS (L-797-591: 1.29 ± 0.05 μmol h^{-1}; BIM-23056: 1.31 ± 0.12 μmol h^{-1}; L-803-087: 1.23 ± 0.17 μmol h^{-1}; BIM-23052: 1.27 ± 0.13 μmol h^{-1}; vehicle: 1.28 ± 0.16 μmol h^{-1}, $n = 3$–6). In sst_2 knockout mice basal GAS was approximately 15-fold higher than in wild-type animals (10.8 ± 1.7 *vs.* 0.7 ± 0.02 μmol h^{-1}, respectively; $n = 4$–5; $P < 0.05$). Neither somatostatin-14 nor DC 32-87 affected the high GAS observed in knockout mice. In wild-type mice, *in vivo* immunoneutralization of somatostatin or infusion of the selective sst_2 antagonist PRL 2903 increased basal GAS to 2.01 ± 0.14 μmol h^{-1} ($n = 5$) and 6.1 ± 1.2 μmol h^{-1} ($n = 4$), respectively ($P < 0.05$ *vs.* vehicle: 0.54 ± 0.09 μmol h^{-1}; $n = 5$). Neither somatostatin immunoneutralizacion nor PRL 2903 affected GAS in the knockout animals.

These results indicate that, in mice, somatostatin inhibits GAS through activation of sst_2 receptors, while other somatostatin receptors subtypes do not seem to play a significant role. Selective agonists and antagonists of sst_2 receptors, as well as sst_2 knockout mice, might be useful tools to characterize the mechanisms of control of gastric acid secretion.

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All procedures accord with current National guidelines.

P185

Superoxide dismutase in offspring: effects of ethanol and folic acid supplementation

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In this work we show the protective effect of folic acid on oxidative stress in offspring caused by chronic maternal ethanol consumption during pregnancy and the lactation period. Animals were randomized into three groups: control group (CG) received only water and rat basal diet (2 p.p.m. folic acid) during pregnancy and the lactation period; ethanol-treated rats (EG) were administered ethanol (20% v/v) during the pregnancy and the lactation period; the ethanol–folic acid group (EFG) received the folic acid supplement (8 p.p.m.) concomitantly with ethanol administration. After the lactation period, the offspring were anaesthetized and the livers were removed and weighed. The animals were killed humanely.

Superoxide dismutase (SOD) specific activity was assayed in the offspring liver. The determination of SOD was performed as described by Fredovich (1985). We used the Tamhane statistical test. The results indicated that in the EG the SOD activity increased in the liver of the offspring (EG: 14.21 ± 1.3 *vs.* 8.86 ± 0.77 U (mg protein)$^{-1}$, $P < 0.01$). The specific activity of this enzyme in the offspring supplemented with folic acid remained unchanged with respect to CG (EFG: 10.3 ± 0.4 U (mg protein)$^{-1}$). However, there was a significant reduction in the enzymatic activity in the EFG with respect to EG ($P < 0.05$).

The results suggested that folic acid may be useful in the prevention of damage and promotion of health of ethanol-fed rat progeny.

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All procedures accord with current National and local guidelines.

P186

Effect of insulin on pancreatic juice secretion in normal and diabetic rats *in vivo*

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Diabetes mellitus (DM) is a metabolic disease characterised by hyperglycaemia which is associated with absolute and relative deficiencies in insulin secretion or action (Saltiel & Kahn, 2001). People with DM suffer from a number of medical complications including exocrine pancreatic insufficiency. In several *in vitro* studies it has been demonstrated that insulin has little or no effect on exocrine pancreatic secretion but the islet hormone can markedly potentiate the secretory effects of acetylcholine and cholecystokinin-octapeptide (Singh *et al.* 1998). In this study we investigate the secretagogue effect of insulin in normal and diabetic animals employing the *in vivo* anaesthetised rat preparation.

Animals were rendered diabetic by a single injection of streptozotocin (STZ, 60 mg kg^{-1} I.P.; Sharma *et al.* 1985). Age-matched controls were injected with an equal volume of citrate buffer. The rats were tested for hyperglycaemia 4 days after STZ injection and 7 weeks later when they were used for the experiments. Following general anaesthesia (1 g kg^{-1} urethane I.P.), laparotomy was performed and the pancreatic duct cannulated for the collection of juice using established methods (Singh *et al.* 1992). After the experiments, rats were humanely killed by urethane overdose.

At 7 weeks after STZ treatment diabetic rats gained significantly ($P < 0.001$; Student's unpaired *t* test) less weight (219.8 ± 6.8 g, mean ± S.E.M., $n = 10$) compared to control (373.6 ± 6.7 g, $n = 10$). Other characteristics of diabetic animals include elevated blood glucose (380.0 ± 25.9 mg dl^{-1} at fasting, $n = 10$) and reduced pancreatic weight (1.012 ± 0.054 g, $n = 20$) compared with age-matched controls (73.3 ± 3.4 mg dl^{-1}, $n = 12$ and 1.289 ± 0.069 g, $n = 15$, respectively). Basal pancreatic juice flow rate, total protein output and amylase secretion in control and diabetic rats were 0.65 ± 0.07 μl min^{-1}; 0.75 ± 0.08 μg min^{-1} and 101.4 ± 9.5 mU min^{-1} ($n = 12$ for all parameters) compared with 0.71 ± 0.08 μl min^{-1}, 0.24 ± 0.05 μg min^{-1} and 0.28 ± 0.11 mU min^{-1} ($n = 14$ for all parameters), respectively. These results show that diabetes is associated with a significant ($P < 0.001$, Student's *t* test) reduction in both protein output and amylase secretion compared with control. Administration of insulin (1 IU, I.P.) resulted in time dependent and significant ($P < 0.05$, ANOVA plus DMS *post-hoc* test) increases in pancreatic flow rate, protein output and amylase secretion in control animals compared to basal secretory parameters. Maximal effects occurred after 40 min of insulin administration. The action of insulin was also associated with a time dependent decrease in blood glucose levels (152.7 ± 16.9 mg dl^{-1} ($n = 6$) prior to insulin and 42.0 ± 8.4 mg dl^{-1} ($n = 4$) 100 min later). In diabetic rats insulin (4 IU, I.P.) evoked delayed increases in flow, protein output and amylase secretion with maximal responses occurring after 120 min of insulin administration. Blood glucose level was 467.6 ± 14.0 mg dl^{-1} ($n = 10$) prior to insulin and this value decreased slowly to 386.6 ± 43.6 mg dl^{-1} ($n = 7$) at 120 min post-insulin. The results indicate that insulin can evoke marked pancreatic secretagogue effects and stimulate glucose metabolism in the healthy rats. However, during diabetes these effects of insulin were reduced compared with age-matched control animals.

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Singh J *et al.* (1992). *Exp Physiol* **77**, 191–204.
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All procedures accord with current National and local guidelines.

P187

Effect of tumour necrosis factor-α on D-fructose intestinal transport in rabbit

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Tumour necrosis factor-α (TNF-α) is a central immuno-regulatory cytokine involved in septic responses during bacterial infection (Damas *et al.* 1997, Van Dullemen *et al.* 1997). Previous studies in our laboratory have shown that this cytokine is able to inhibit L-leucine intestinal absorption (Abad *et al.* 2002). In this work, D-fructose transport was studied in rabbit jejunum (obtained from a rabbit killed by a blow to the head) from control and treated (intravenously with TNF-α 2 μg (kg body weight)$^{-1}$, 1 h 30 min) animals.

We studied the sugar transport using three experimental methods: (a) tissue accumulation (incubations were for 3 min, at 37 °C, under 95 % O_2–5 % CO_2 bubbling, the measurements were expressed as micromoles of D-fructose per millilitre of cell water), (b) transepithelial flux in Ussing-type chambers (the bathing solutions on the mucosal and serosal surfaces of the tissue were maintained at 37 °C, under 95 % O_2–5 % CO_2 bubbling, the results are expressed as micromoles of D-fructose per cm^2 per hour) and (c) brush-border membrane vesicles (BBMV) were prepared using the Mg^{2+}, EGTA precipitation method (Hauser *et al.* 1980) with minor modifications (Brot-Laroche *et al.* 1986). All results are expressed as means ± S.E.M. Means were compared using a one-way analisis of variance (ANOVA). Significant differences ($P < 0.05$) were compared using a Student's unpaired two-tailed *t* test. Handling, equipment and killing of animals was in accordance with the European Council Legislation 86/609/EEC concerning experimental animal protection.

The results have shown that TNF-α decreases significantly (about 30 %) the accumulation (control 2.50 ± 0.05, TNF-α 2.12 ± 0.01), the mucosal-to-serosal transepithelial flux (control 0.41 ± 0.01, TNF-α 0.28 ± 0.02) and the uptake across brush-border membrane vesicles of D-fructose at different times. This inhibition could be related to the TNF-α secretagogue effect on the gut because the intracellular tissue water was affected. Likewise, the absence of chloride ion in the incubation medium removed, in part (about 90 %), the cytokine inhibition. To explain this inhibition, the density of the D-fructose transporter GLUT5 was analysed by Western blot. GLUT5 levels were lower in TNF-α-treated animals, indicating a significant reduction in the expression of GLUT5 protein and, therefore, in the transport capacity.

To sum up, the inhibition of D-fructose intestinal absorption by intravenous administration of TNF-α could be mainly produced by a reduction in the number of GLUT5 transporters and, at least in part, by the secretagogue action of this endotoxin on the gut.

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The rabbit polyclonal antibody GLUT5 was kindly provided by Dr E. Brot-Laroche (INSERM U505, Paris, France). This work was supported by grants from CICYT (SGPN Y+D) AGL 2000-1228 and University of Zaragoza, Spain.

All procedures accord with current National and local guidelines.

P188

Dietary fatty acids modify pancreatic membrane lipid composition in rabbits with experimental atherosclerosis. Is this fact involved in the enzymatic secretion pattern?

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Cell membrane fluidity is modified by their lipid structure and saturated fatty acids (SFA)/unsaturated fatty acids (UFA) proportions and alterations in this coefficient (particularly, SFA/monounsaturated fatty acids (MUFA)) are related with numerous pathologies (Ntambi, 1999). However, the fatty acid incorporation pattern is different for each tissue (Soriguer *et al.* 2000). On the other hand, there is controversy about the influence of the quality of fat (fatty acid chain length and unsaturation index) on the pancreatic enzyme content and secretion. Studies with conscious dogs show that long-term adaptation to diets high in fat provide higher levels of lipase secretion after a sunflower-seed oil dietary intake (Yago *et al.* 2000).

We find that literature is scarce that relates both changes in membranes lipid composition and pancreatic adaptation, by dietary fatty acids (Begin *et al.* 1990).

Thirty-six male New Zealand rabbits were divided into two groups and fed for 50 days with either a standard chow (Panlab, Barcelona; control group, C, $n = 6$) or a atherogenic diet (95 % standard chow, 3.5 % lard and 1.5 % cholesterol, w/w; atherogenic groups, A, $n = 30$). Prior to analysis the animals were anaesthetized with sodium pentothal (30 mg kg^{-1}, I.V.) and humanely killed according to international guidelines. The pancreas was removed in order to measure enzymatic activities by an enzymatic–colorimetric (amylase) and by titrimetric (lipase, colipase, trypsin and chymotrypsin) methods and fatty acid composition of plasma membrane by gas–liquid chromatography.

In the atherogenic rabbits, the intake of differents diets (98.25 % standard chow, 1.75 %: virgin olive (V), olive (O), sunflower seed (S) and fish (F) oils, w/w), during the next 30 days provide modifications in the pancreatic enzymes content, when compared with control group. The lipase activity (U (g pancreas)$^{-1}$), significantly higher ($P < 0.05$, one-way ANOVA) in atherogenic than control group (38.54 ± 4.577, $n = 6$ *vs.* 10.14 ± 2.859, $n = 6$, means ± S.E.M.), continues increasing in the sunflowerseed group (87.17 ± 25.941, $n = 6$) when compared

with the other experimental groups (25.18 ± 5.781, $n = 6$), 57.15 ± 12.837, $n = 6$, 31.40 ± 3.783, $n = 6$, V, O and P, respectively). These results seem to indicate a role of fat quality in lipolytic enzyme adaptation, specifically, for fats with a high content in polyunsaturated fatty acids (PUFA) (n–6). The acinar cell membrane lipid composition analysis (percentage of total fatty acids, means ± S.E.M.) indicates statistical significance in the adaptation ($P < 0.05$, one-way ANOVA) to dietary fatty acids profile (Table 1).

	Control Group	*Atherogenic Group*	*Virgin Olive Group*
SFA (%)	47.54±1.774	44.04±0.808 +	40.41±0.980 +*a
MUFA (%)	34.47±0.679	37.51±0.269 +	40.12±0.824 +*a
SFA/MUFA (%)	1.38±0.056	1.17±0.023	1.01±0.046 a
PUFA (%)	17.99±1.963	18.46±0.855	19.47±0.339 a
PUFA ($_{n-6}$) (%)	16.24±1.774	17.12±0.857	17.79±0.347 a
PUFA ($_{n-3}$)>18C (%)	0.22±0.026	0.21±0.023	0.13±0.016 +*a
UI (%)	1.56±0.134	1.72±0.066	2.02±0.074

	Olive Group	*Sunflower Group*	*Fish Group*
SFA (%)	42.37±0.657 +a	41.85±1.223 +a	42.84±0.677 +a
MUFA (%)	38.33±0.704 +b	35.49±1.240 a	37.96±1.046 +ab
SFA/MUFA (%)	1.11±0.028 +ab	1.19±0.073 +b	1.13±0.037 +ab
PUFA (%)	19.30±0.922 a	22.65±0.926 +*b	19.20±1.180 a
PUFA ($_{n-6}$) (%)	17.44±0.679 a	20.82±0.815 a	16.60±1.187 +*b
PUFA ($_{n-3}$)>18C (%)	0.16±0.027 +a	0.17±0.033 +*a	1.11±0.209 +a
UI (%)	1.90±0.089	1.98±0.104	1.89±0.061

Table 1. Fatty acid profile (%) of pancreatic acinar cell membranes in different experimental groups: control (C), atherogenic (A), virgin olive (V), olive (O), sunflower (S) and fish (F). Values are expressed as means ± S.E.M. ($n = 6$) for all groups. Means with different superscripts indicate significant differences ($P < 0.05$, ANOVA plus *post hoc* DMS and Bonferroni test): (+) *vs.* C, (*) *vs.* A, (different letters *vs.* V, O, S and F groups).

As previous studies show that the quality of fat affects different gastrointestinal peptide levels implicated in the pancreas secretion activity, the results obtained for enzymatic content and those observed for membrane lipid composition suggest that the type of fat preferentially affects the enzymatic secretion (Hedemann *et al.* 2001). This fact may to be relevant in the investigation and treatment of pancreatic diseases such as pancreatitis or pancreatic cancer.

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All procedures accord with current National guidelines.

P189

Lipopolysaccharide, from *E. coli*, inhibits D-fructose uptake across rabbit jejunum

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Lipopolysaccharide (LPS) is an endotoxin causative agent of sepsis (Morrison *et al.* 1987). Recent studies in our laboratory have shown that this endotoxin inhibits L-leucine absorption across rabbit jejunum (Abad *et al.* 2001). The aim of this work was to study the LPS effect on D-fructose intestinal absorption.

The experimental methods used in the intestinal transport were: (a) tissue accumulation (incubation was for 3 min, at 37 °C, under 95 % O_2–5 % CO_2 bubbling, and the measurements were expressed as micromoles of D-fructose per millilitre of cell water), (b) transepithelial flux in Ussing-type chambers (the bathing solutions on the mucosal and serosal surfaces of the tissue were maintained at 37 °C, under 95 % O_2–5 % CO_2 bubbling, and the results are expressed as micromoles of D-fructose per cm^2 per hour), and (c) brush-border membrane vesicles (BBMV) were prepared using the Mg^{2+}, EGTA precipitation method (Hauser *et al.* 1980) with minor modifications (Brot-Laroche *et al.* 1986). D-Fructose transport was studied in rabbit jejunum from control and treated animals (2 μg (kg body weight)$^{-1}$ LPS by intravenous route, 1 h 30 min previously). All results are expressed as means ± S.E.M. Means were compared using a one-way analysis of variance (ANOVA). Significant differences ($P < 0.05$) were compared using a Student's unpaired two-tailed t test. Handling, equipment and killing of animals was in accordance with the European Council Legislation 86/609/EEC concerning experimental animal protection.

The results show that the sugar absorption in LPS-treated rabbits was reduced significantly compared with control animals (control 2.71 ± 0.07, LPS 2.20 ± 0.04). The endotoxin decreases both the mucosal-to-serosal transepithelial flux (control 0.41 ± 0.02, LPS 0.28 ± 0.02) and the transport across brush-border membrane vesicles of D-fructose at different times. To explain this inhibition of sugar transport, the density of the D-fructose transporter, GLUT5, was analysed by Western blot. The levels of GLUT5 were lower in LPS-treated animals (about 28 %), indicating a significant reduction in the expresión of GLUT5 protein and, therefore, in transport capacity. These results suggest that the inhibition in D-fructose intestinal absorption by intravenous administration of LPS could be produced by a reduction in the number of GLUT5 transporters. We also found that the LPS intestinal effect induced by intravenous administration was blocked by a TNF-α antagonist, indicating that TNF-α is a mediator in the LPS effect on D-fructose intestinal uptake inhibition (control 2.47 ± 0.06, LPS 1.90 ± 0.05, antagonist TNF-α/LPS 2.46 ± 0.05).

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All procedures accord with current National and local guidelines.

P190

Modulatory effects of PAMP and AM on intestinal function

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Adrenomedullin (AM) and proadrenomedullin N-terminal 20 peptide (PAMP) are two peptides derived from preproadrenomedullin, a common polypeptide precursor encoded by the AM-gene. Both AM and PAMP appear to play important roles in the regulation of vascular tone, hormone secretion from the anterior pituitary gland and adrenal function. Recent data suggest that both peptides may have some effects on blood glucose levels, food intake and gastric emptying after exogenous administration, as well as an antimicrobial effect in human colonic epithelia. Since the expression of these peptides has been found in a variety of mammalian tissues, including neuroendocrine cells in the stomach and small intestine, the aim of the present study was to investigate possible effects of PAMP and AM on intestinal functions such as motility and absorption of sugars and amino acids.

Intestinal absorption of sugars and amino acids was measured in rat jejunum with *in vitro* (everted rings) and *in vivo* techniques, as well as in monolayers of Caco-2 cells. Intestinal motility was measured by using strips of rat jejunum and guinea-pig ileum in an organ bath. Animals were anaesthetized by I.M. administration of 0.5 mg medetomidine and 10 mg ketamine per kg body weight and humanely killed at the end of the experiment. All procedures for the animal handling were performed according to the ethics committee of the University of Navarra.

The uptake of 1 mM α-methylglucoside by everted jejunal rings was significantly increased by the presence of 10^{-8} M AM or PAMP. The effect was already observed after 5 min incubation and enhanced after 30 min incubation. At this exposure time, 10^{-12} to 10^{-8} M PAMP increased sugar absorption, 10^{-10} M being the most effective concentration. Interestingly, PAMP did not show any effect at 10^{-7} or 10^{-6} M. AM seems to be less potent than PAMP, since no effect was found at concentrations below 10^{-8} M, 10^{-6} M being the most effective concentration. In contrast, the uptake of 1 mM alanine or proline was not modified by PAMP. In *in vivo* experiments, intraluminal infusion of 10^{-7} or 10^{-8} M PAMP for 15 min prior to the infusion of 1 mM α-methylglucoside for 5 min significatively reduced the absorption of this sugar. In Caco-2 cells, the presence of PAMP (10^{-9}–10^{-7} M) for 30 or 60 min in the apical side of the monolayer significantly increased the uptake of 0.1 mM α-methylglucoside. The results of the experiments in which intestinal motility was studied showed that PAMP induced a contractile response at concentrations higher than 10^{-6} M.

On the whole, our results suggest that the novel cardiovascular-active peptides PAMP and AM may have a physiological role in the modulation of intestinal motility and sugar absorption.

All procedures accord with current local guidelines.

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Driving potentials of interstitial cells of Cajal in a mathematical model

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Rhythmical contractions of the gastrointestinal tract are associated with the pacemaking electrical activity generated in the muscle layers: it occurs at low frequency in the absence of an extrinsic nervous stimulation. Interstitial cells of Cajal (ICC), distributed in the myenteric region of the gastric wall, have been suggested to initiate the pacemaking activity (Tomita, 1981). This pacemaking activity then propagates to the smooth muscle cells through gap junctions to generate the contraction of the whole gastrointestinal tract. This pacemaking activity is recorded as the driving potential of ICC.

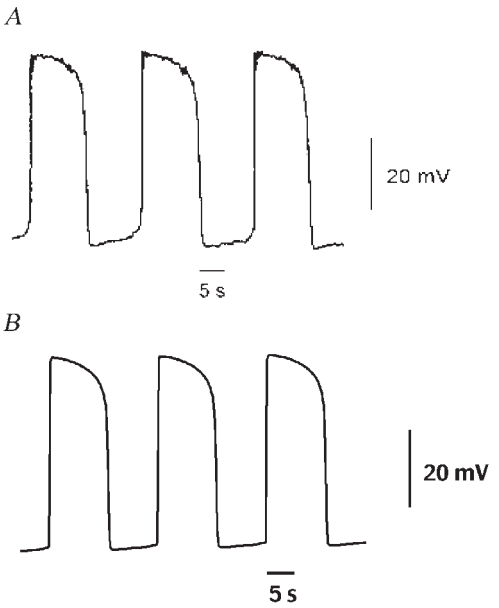

Figure 1. Driving potential of ICC. *A*, recording of driving potentials from interstitial cells in the myenteric region (ICC_{MY}) of ginea-pig antrum (Dickens *et al.* 2000). *B*, recording of driving potentials by simulation.

In this study, driving potentials of ICC from experimental records were simulated with a mathematical model to explain the regenerative nature of the slow potentials and the underlying $[Ca^{2+}]_i$ changes. Included in the model are the apparatus for $[Ca^{2+}]_i$ regulation and pacemaker current. Ca^{2+} entry through a dihydropyridine-resistant conductance triggers IP_3-mediated Ca^{2+} release (Ward & Sanders, 1992). Subsequent mitochondrial Ca^{2+} uptake transiently reduces $[Ca^{2+}]_i$ in the space close to the non-selective cation channels. Activation of these channels generates the pacemaker current and it makes the initial component of the driving potential (Sanders *et al.* 2000). The depolarization by the pacemaker current facilitates the production of IP_3 and the plateau phase of the driving potential continues (Ganitkevitch & Isenberg, 1993). Gradual decrease of IP_3 and $[Ca^{2+}]_i$ terminates the plateau phase. In the model, the membrane potentials between successive driving potentials are in the range from −66 to −72 mV (Fig. 1). Peaks of driving potentials are between −30 and −25 mV. The duration of driving potentials ranges from 8 to 12 s. Analysis of behaviours of the

pacemaker current and the $[Ca^{2+}]_i$ regulation in the model reveals the complexity of the underlying process in the generation of the driving potential.

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P192

Involvement of prostaglandins in the LPS effects on ACh-induced contractions in rabbit small intestine

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The aim of this study was to investigate the role of PGE_2 in LPS effects on ACh-induced contractions of rabbits intestinal segments *in vitro* with two different protocols.

In the first protocol, rabbits were treated with saline or LPS (0.2, 2 or 20 μg kg^{-1}) I.V. injected as a bolus. Rabbits were humanely killed by a blow on the head. Intestinal segments were removed 90 min after LPS administration. Pieces of duodenum, jejunum and ileum (10 mm × 5 mm) were vertically suspended in the direction of longitudinal muscle fibres, in a thermostatically controlled organ bath (10 ml capacity). The bath contained Tyrode solution at 37 °C to reach pH 7.4 and was gassed with 95 % O_2 and 5 % CO_2. Segments were stretched passively to an initial tension of 20 mN. Each segment was connected to an isometric transducer and mechanical activities were recorded, stored and analysed with a computer program. ACh non-cumulative concentration–response curves were constructed with and without LPS. In the second protocol, intestinal segments isolated from LPS non-treated rabbits were incubated with Ringer or LPS (0.03, 0.3, 3, and 30 μg ml^{-1}). ACh 10^{-4} M contractions were recorded without LPS as the control. After, LPS was added to the bath and ACh contractions were compared with the control.

DE_{50} values of ACh were 3.6×10^{-7}, 4.9×10^{-7} and 5.1×10^{-7} M in duodenum, jejunum and ileum, respectively. After LPS treatment (0.2 μg kg^{-1}), DE_{50} was 2.6×10^{-6}, 1.0×10^{-6} and 4.5×10^{-7} M in duodenum, jejunum and ileum, respectively. When added to the bath, LPS also significantly reduced ACh-induced contractions (43 % reduction in duodenum, 35 % in jejunum and 17 % in ileum). Indomethacin (1 mg kg^{-1}) antagonised LPS effects when it was administered before LPS. PGE_2 (8 μg kg^{-1}, I.V.) reduced ACh-induced contractions and mimicked LPS actions.

The results show that LPS decreases ACh-induced contractions and that prostaglandins are involved in this action.

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All procedures accord with current National and local guidelines.

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Prostaglandin E_2 receptors in rabbit small intestine

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PGE_2 may interact with at least four cell surface receptors (EP1–EP4). Pharmacological studies suggest that PGE_2 contricts the smooth muscle through EP1 and EP3. In contrast, PGE_2 relaxes vascular and intestinal smooth muscle through EP2 and EP4. EP1 receptor is presumed to stimulate intracellular Ca^{2+} mobilisation. The EP3 receptor inhibits adenylate cyclase and reduces levels of cAMP (Narumiya *et al.* 1999).

The aim of this study was to examine the PGE_2 receptors in intestinal longitudinal segments from rabbits *in vitro*.

Rabbits were humanely killed by a blow on the head. Pieces of duodenum, jejunum and ileum (10 mm × 5 mm) were cut into longitudinal smooth muscle segments. The preparations were vertically suspended in a thermostatically controlled organ bath (10 ml capacity). The bath contained Krebs solution at 37 °C to reach pH 7.4 and was gassed with 95 % O_2 and 5 % CO_2. Segments were stretched passively to an initial tension of 20 mN. Each segment was connected to an isometric transducer and mechanical activities were recorded, stored and analysed with a computer program. Non-cumulative concentration–response curves to PGE_2 and PGE_2 agonists (10^{-9}–10^{-5} M) were constructed in duodenum, jejunum and ileum. Also, non-cumulative concentration–response curves to PGE_2 were made in the presence of atropine (10^{-6} M), guanethidine (10^{-6} M), tetrodotoxin (10^{-6} M), and hexamethonium (10^{-5} M).

PGE_2-induced contractions were dose dependent in duodenum, jejunum and ileum. Atropine, guanethidine, tetrodotoxin and hexamethonium did not modify the effect of PGE_2. These results suggest that the PGE_2 action was myogenic. Misoprostol, sulprostone, 17-phenyl PGE_2 and 16,16-dimethyl PGE_2 caused contractions in duodenum, jejunum and ileum. However, butaprost did not alter spontaneous contractions. The PGE_2-induced contractions were blocked in Ca^{2+}-free medium plus EGTA 0.5 mM. Verapamil 10^{-7} M, a Ca^{2+} channel antagonist, and staurosporine 5×10^{-7} M, a protein kinase C inhibitor, diminished the contractions of PGE_2 in small intestine.

PGE_2-induced contractions in rabbit small intestine appear to act directly on smooth muscle cells and EP1 receptors probably are implicated. Furthermore, voltage-dependent Ca^{2+} channels and protein kinase C mediate the responses.

Narumiya S *et al.* 1999). *Physiol Rev* **79**, 1193-1226.

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P194

Effect of ageing on proinflammatory molecule production and membrane lipids in rat hepatocytes

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It has been described that ageing induces changes in liver function and structure, such as a decrease in hepatic size, blood flow, metabolism of certain drugs and urea and cholesterol synthesis. The hepatocytes' capacity for regeneration is also reduced. Proinflamatory molecule production could play an important role during ageing. The present study has investigated whether nitric oxide (NO) and carbon monoxide (CO) release is modified by age in isolated rat hepatocytes, and if these changes are accompanied by modifications in cGMP and lipid hydroperoxides (LPO) levels.

Forty male ($n = 8$ each group) and fifty-four female young (2 months old) and old (14, 18, 22 and 24 months old) Wistar rats were used. Half of the old female rats were ovariectomized after Equithesin anaesthesia (0.3 ml (100 g)$^{-1}$ of weight; intraperitoneal) at 12 months of age ($n = 6$ for each group). After decapitation, hepatocytes were isolated and cultured in RPMI 1640 medium with serum, glutamine, antibiotics and insulin. After 24 h of culture, cells and medium were separately collected for measuring CO and NO release to the medium, and cGMP, phosphatidylcholine (PC) and LPO content of the cells. The results are presented as the mean ± standard error of the mean. Mean comparison was done by Friedman's analysis of variance followed by a two-tailed Wilcoxon's test for paired data; a confidence level of 95 % ($P < 0.05$) was considered significant. The experimental procedures employed in this study are in accordance with the principles and practices of the 1986 Animals Act, published in Spain (RD 223/1988).

Hepatocytes isolated from 14-month-old female rats released significantly higher amounts of NO (nmol (μg protein)$^{-1}$) than young ones (1.54 ± 0.02 *vs.* 1.23 ± 0.02; $P < 0.001$), an increase that was more apparent in males (1.81 ± 0.03 *vs.* 1.24 ± 0.005; $P < 0.001$) and ovariectomized females. With age, NO production was further increased, and the increase was even higher in males and ovariectomized females (1.71 ± 0.05, 2.11 ± 0.03 and 2.01 ± 0.03; $P < 0.001$; females, males and ovariectomized females respectively, at 24 months). CO (nmol ml^{-1}) release was significantly augmented with age (5.334 ± 0.23 *vs.* 1.92 ± 0.09; 24-month-old male *vs.* 2-month-old male; $P < 0.001$), although this increase occurred later in females compared to males and ovariectomized females (2.21 ± 0.05 *vs.* 5.334 ± 0.23 *vs.* 5.5 ± 0.5; 24-month-old female *vs.* 24-month-old male *vs.* 24-month-old ovariectomized female). As expected, the increase in NO and CO release was accompanied by an increase in intracellular cGMP (fmol (μg protein)$^{-1}$) content (248 ± 2.1 *vs.* 46.6 ± 4.1; 24-month-old male *vs.* 2-month-old male; $P < 0.001$). Age also increased cellular LPO levels (5.6 ± 0.3 *vs.* 0.9 ± 0.05; 24-month-old male *vs.* 2-month-old male; $P < 0.001$), whereas PC (pmol (mg protein)$^{-1}$ in 24 h) levels were decreased (5.82 ± 0.8 *vs.* 317 ± 7.1; 24-month-old male *vs.* 2-month-old male; $P < 0.001$).

These data suggest that the harmful effect of age on hepatic function could be partially due to an increase in proinflamatory molecule production, which could induce alterations in membrane lipids.

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All procedures accord with current National and local guidelines.

P195

Female sex-specific antisecretory effects of oestrogen in isolated rat distal colon

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The rapid non-genomic effects of 17-β-oestradiol (E2) on transepithelial bumetanide-sensitive Cl^- secretion(I_{Cl}) and cell signalling were compared between male and female rat isolated distal colon mounted in Ussing chambers. The rats were humanely killed by cervical dislocation. The data are presented as means ± S.E.M. and were compared using Student's paired *t* test.

E2 (100 nM) inhibited basal I_{Cl} by 60 ± 4 % ($n = 8$, $P < 0.01$) and prevented chloride secretion in response to heat-stable enterotoxin, cholera toxin, carbachol or forskolin. The rapid time course of the anti-secretory effect of E2 (< 5 min) and the insensitivity to cycloheximide, actinomycin or tamoxifen suggest the participation of a novel receptor. The anti-secretory effect of E2 was associated with a stimulation of PKCδ activity of 50 ± 4 % ($n = 18$, $P < 0.01$). E2 also produced an increase in adenyl cyclase and cyclic AMP activity and a transient rise in intracellular Ca^{2+} from 280 ± 10 to 415 ± 12 nM, ($n = 8$, $P < 0.01$), followed by an enhanced Na^+/H^+ exchange activity (measured as amiloride-sensitive pH_i recovery rate in BCECF-loaded crypts). These latter signalling effects were dependent on the prior activation of PKCδ. The effects of E2 on chloride secretion and cell signalling were observed only in tissues of female origin and were absent from the male. Inhibition of the PKCδ isoform by rottlerin or chelation of intracellular Ca^{2+} with BAPTAM prevented the anti-secretory effects of E2. In nystatin-perforated luminal membrane preparations, E2 inhibited a chromanol 293B-sensitive basolateral K^+ current which was insensitive to charybdotoxin, apamin, tolbutamide or verapamil.

In conclusion, E2 inhibits chloride secretion in female distal colon via a PKA signalling pathway and targets a basolateral membrane K^+ channel of type KCNQ1/KCNE3.

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All procedures accord with current National and local guidelines.