Intrauterine low protein diet increases fetal β-cell sensitivity to NO and IL-1β: the protective role of taurine

S Merezak, A A Hardikar[1], C S Yajnik[2], C Remacle and B Reusens

Laboratory of Cell Biology, World Health Organization Collaborating Center for the Biology of Development of the Endocrine Pancreas, Université Catholique de Louvain, B-1348 Louvain-La-Neuve, Belgium

[1]Tissue Engineering and Banking Laboratory, National Center for Cell Science, Ganeshkhind, Pune 411007, India

[2]Diabetes Research Center, KEM Hospital, Rasta peth, Pune 411001, India

(Requests for offprints should be addressed to B Reusens, Laboratoire de Biologie Cellulaire, Université Catholique de Louvain, Place croix du sud 5, 1348 Louvain-La-Neuve, Belgium; Email: reusens@bani.ucl.ac.be)

Abstract

We have demonstrated earlier that a low-protein (8% protein) diet during gestation alters fetal β-cell development. Here, we investigated the effect of a low-protein diet as compared with a control (20% protein) diet, during gestation, on the sensitivity of fetal β-cells against nitric oxide (NO) or interleukin-1β (IL-1β), and assessed the protective effect of taurine *in vitro* and *in vivo*. Neoformed islets from control fetuses or fetuses of dams fed a low-protein diet (LP group) were incubated with taurine, methionine or β-alanine and then exposed to sodium nitropruside (SNP), a NO donor, or to IL-1β. To understand the effect of taurine *in vivo*, LP or control pregnant rats received 2·5% of taurine in the drinking water. Mortality and rate of apoptosis were quantified by confocal microscopy. Without treatment, rate of apoptosis was greater in LP group islets than in control islets (1·38±0·18% compared with 0·66±0·21% respectively, $P<0·05$). Addition of SNP 100 μM showed an augmentation in cell death, which was greater in the LP than in the control group (17·88±0·69% compared with 11·89±0·44% respectively, $P<0·01$). LP islets were more sensitive than control islets to IL-1β. Taurine was protective against SNP and IL-1β in both the groups, methionine provided a less protective effect than taurine, and pretreatment with β-alanine had no protective effect. Taurine supplementation of the maternal diet reduced the rate of apoptosis induced by IL-1β in control islets and suppressed that induced by IL-1β in LP islets. Our findings indicate that a low-protein diet during gestation augments the sensitivity of fetal islet cells to NO and IL-1β. However, through *in vitro* and *in vivo* experiments our studies indicate that such effects can be rescued using amino acids such as taurine.

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Introduction

Recent epidemiological findings suggest that several adult diseases, including diabetes, may originate in the womb (Barker *et al.* 1993, Barker 1998). Intrauterine malnutrition has been known to induce alterations in the development of several tissues, among which are the endocrine pancreas. Indeed, a low-protein diet given to the dams throughout gestation has been shown to decrease islet cell proliferation, islet size and pancreatic insulin content in the progeny at birth (Snoeck *et al.* 1990). It also leads to a reduced insulin secretion from fetal islet *in vitro* (Dahri *et al.* 1991). Moreover, apoptosis is increased in these fetal and neonatal β-cells (Petrik *et al.* 1999). Likewise, in the rat, general food restriction during gestation leading to intrauterine growth retardation, decreases the β-cell mass and the pancreatic insulin content at birth (Garofano *et al.* 1997). In addition, apoptosis of the β-cell is enhanced at 3 months even after an adequate quantity of food is given after weaning (Garofano *et al.* 1998).

The β-cells are exceedingly sensitive to different molecules such as streptozotocin (Bonner-Weir *et al.* 1981) or nitric oxide (NO) and cytokines involved in β-cell destruction as seen in insulin-dependent diabetes mellitus (Mandrup-Poulsen 1996). Interleukin IL-1β, alone or in combination with tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), demonstrates a cytotoxic activity on β-cells cultured *in vitro* that is partly mediated through the induction of free radicals such as NO (Cetkovic & Eizirik 1994). The production of NO is catalyzed by inducible nitric oxide synthase (iNOS) (Corbett *et al.* 1991). The release of NO in β-cells leads to apoptosis and, subsequently, to characteristic DNA fragmentation and cell

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death (Delaney *et al.* 1993, 1997, Fehsel *et al.* 1993). Consequently, the first aim of this study was to determine if a low-protein diet given to the dams during gestation (inducing altered fetal β-cell mass) increased the susceptibility of fetal β-cells to cytotoxic damage such as that induced by IL-1β and by a NO donor.

We have also shown previously that the level of taurine (2-amino ethanesulphonic acid) is markedly reduced in the plasma of fetuses of dams fed a low-protein diet (Reusens *et al.* 1995). Taurine supplementation of the low-protein diet of the mother restored a normal taurine concentration in fetal plasma, and a normal insulin secretion by fetal islets (Cherif *et al.* 1998). Taurine also restored a normal proliferation rate in β-cells of fetuses of dams fed a low-protein diet (Boujendar *et al.* 1999, 2000). Taurine, a β-amino acid derived from methionine and cysteine metabolism, is the most abundant intracellular free amino acid present in mammalian cells (Jacobsen & Smith 1968). This sulphonated β amino acid is also present at high concentration in the endocrine pancreas (Bustamante *et al.* 1998). Among the several properties of taurine, this semi-essential amino acid is also known to serve as an antioxidant agent (Huxtable 1992). The role of taurine against oxidative damage has been described in variety of cell types. This amino acid, possibly through its antioxidant activity and regulation of intracellular calcium flux can prevent the death of endothelial cells (Wang *et al.* 1996). Taurine has also been shown to attenuate apoptosis and necrosis of hepatocytes (Redmong *et al.* 1996) and inhibit the production of nitric oxide by glioma cells (Liu *et al.* 1998). Thus the second aim of this study was to determine if taurine, added *in vivo* to the drinking water of these dams or *in vitro* in the culture medium, could provide prevention of and protection against cytotoxic damage in fetal β-cells from control dams and dams fed a low-protein diet treated with IL-1β or NO.

Materials and Methods

Animals

Adult virgin female Wistar rats were caged overnight with males and copulation was verified the next morning. The animals were maintained at 25 °C with a 10 h darkness:14 h light cycle. One group of pregnant rats was fed a control diet (20% protein) and a second group received an isocaloric low-protein diet (8% protein; LP group). The compositions of these diets have been described previously (Snoeck *et al.* 1990); these diets are devoid of taurine but the 20% protein diet contained 0·2% methionine whereas the low-protein diet contained only 0·08%. The diets were purchased from Hope Farms (Woerden, Holland). Animals in both groups had free access to water. On day 21·5 of gestation, females were killed by decapitation and fetuses removed.

To investigate the effect of taurine *in vivo*, the pregnant rats were fed a control or low-protein diet supplied with 2·5% (w/v) of taurine in the drinking water throughout gestation.

Islet culture and treatment

Fetal pancreata were dissected and digested with collagenase as described previously (Mourmeaux *et al.* 1985). The cell suspension was cultured in 35 mm Petri dishes (Falcon plastics, Los Angeles, CA, USA) in RPMI 1640 medium (Gibco, Grand Island, NY, USA) with fetal bovine serum (10%) and antibiotics (penicillin 200 U/ml, streptomycin 0·2 mg/ml). Petri dishes were incubated at 37 °C with 5% CO_2 in air. The culture medium was changed daily after the second day. These cultures provided islets that aggregated progressively on the layer of non-endocrine cells.

Islet treatment

On the 5th day of culture, the dishes were rinsed twice with serum-free DMEM/F12 medium (1:1 v/v, Gibco, Paisley, Strathclyde, UK) and then incubated for next 42 h in the same medium without or with taurine (0·3 or 3 mM), methionine (0·1 or 1 mM) or β-alanine (0·3 or 3 mM) (Sigma Chemical Co., St Louis, MO, USA). During the last 18 h of treatment, the islets were exposed to 10 or 100 μM sodium nitropruside (SNP; Sigma Chemical Co.).

In other experiments, the islets were treated with 0·3 or 3 mM taurine for 48 h and exposed to IL-1β (Endogen, Woburn, MA, USA) at 50 U/ml for the last 24 h.

Islet labelling

Apoptosis was visualized as DNA strand breaks by TUNEL methodology (Li *et al.* 1995), using BODIPY-FL-X-14-dUTP (Molecular Probes, Eugene, OR, USA) to label the apoptotic nuclei. Propidium iodide (Molecular Probes) was used to label the total islet nuclei.

Global cell death was analysed using a non-specific probe staining the dead cells, the plasma membranes of which became permeabilized. For this purpose, the culture medium was removed and the dishes washed with phosphate buffered saline (PBS). These were then incubated for 20 min with 1 ml culture medium containing 20 μg/ml ethidium bromide to stain the permeabilized dead cells. The cultures were then fixed with 4% paraformaldehyde in PBS for 10 min, treated with 30% methanol for permeabilization of the remainder of the cells and then mounted in mowiol containing 20 μg/ml of Hoechst 33342, to stain the nuclei of all the cells.

Confocal microscopy

Staining probes were visualized through a confocal laser scanning microscopy system (MRC-1024 UV; Bio-Rad,

Hemel Hempstead, Herts, UK) equipped with argon ion and krypton/argon ion lasers. BODIPY-FL was excited at 503 nm and emitted at 522±32 nm, ethidium bromide was excited at 510 nm and emitted at 605±32 nm, propidium iodide was excited at 536 nm and emitted at 605±32 nm and Hoechst 33342 was excited at 346 nm and emitted at 455±30 nm. Four to six optical sections were collected at every 15 μm through the entire length of the islet. The number of BODIPY-FL- or ethidium bromide-positive nuclei were expressed as percentage of the total number of nuclei.

Nitrite assay

We quantified the concentration of NO in an acellular system in the presence of SNP alone or with taurine. Nitrite, a stable end product of NO oxidation, was measured by a fluorimetric procedure, based upon the reaction of nitrite with the 2,3-diaminonaphthalene (DAN) (Molecular Probes) to form the fluorescent product, 1-(H)-naphthotriazole (Misko *et al.* 1993). This method allows measurement of nitrite in concentrations of as little as 10 nM. In order to measure total NO production in the culture media, nitrate was converted to nitrite by the action of nitrate reductase from *Aspergillus* species (Sigma). The sample (100 μl) was incubated with 100 μl 20 mM Tris buffer, pH 7·6 containing, in final concentration, 80 μM NADPH (to initiate the reaction) and 56 mU enzyme. The reaction was stopped after 5 min of incubation at room temperature by dilution with 1800 μl ultrapure water, followed by the addition of DAN reagent (200 μl of a 0·05 mg/ml solution in 0·62 M HCl). Finally, 100 μl 2·8 M NaOH was added to each sample. Nitrite concentration was determined by using sodium nitrite (Sigma) as a standard. The fluorescence was measured in a Kontron fluorimeter. The excitation and emission wavelengths were 365 nm and 450 nm respectively.

Chemiluminescence measurements

Luminol (5-amino-2,3-dihydro-1–4-phthalazinedione, Sigma; 400 μM), taurine (0·3 or 3 mM), methionine (0·1 or 1 mM) and β-alanine (0·3 or 3 mM) stock solutions were prepared in PBS. 3-Morpholinosydnonimine (SIN-1; Sigma) at 100 μM was prepared in NaOH 1 M. The reaction was initiated by simultaneous injection of luminol and SIN-1 in the wells containing or not containing the amino acids in PBS. Chemiluminescence emitted by luminol in the presence of peroxynitrite was measured every 30 s during 20 min in the chemifluorophotometer (MicroLumat LB96P, EG & G Berthold).

HPLC measurements

Islets were incubated overnight at +4°C in 35% 5-sulphosalicylic acid in order to extract the amino acids. Their separation and quantification were performed with a standard, reverse phase HPLC method after derivatization with *o*-phthalaldehyde.

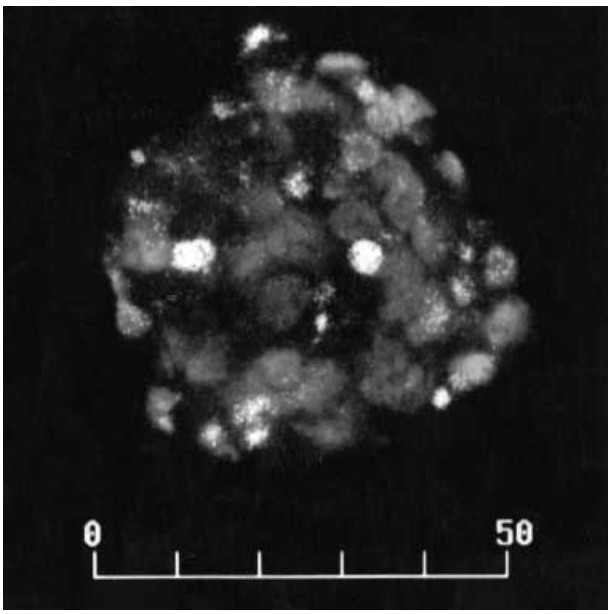

Figure 1 Confocal micrograph showing apoptotic nuclei labelled with BODIPY-FL-dUTP (in white) and total nuclei labelled with propidium iodide (in grey). Scale bar represents 50 μm.

Statistical analysis

Experimental results are reported as means±S.E.M. Significance of the differences between groups was analysed by Scheffé's test after one- or two-way ANOVA.

Results

Culture of control and LP group islets: apoptosis and taurine content

The culture of fetal pancreas provides islets that are exclusively composed of endocrine cells, of which >95% are β-cells (Mourmeaux *et al.* 1985). Apoptosis was quantified in the fetal islets by counting the nuclei labeled by a TUNEL method, on confocal sections (Fig. 1). Islet cell apoptosis was significantly greater in the LP group (1·38±0·2%, $P<0·001$) than in the controls (0·66±0·2%, $P<0·001$).

The concentration of taurine in islets after 7 days of culture was measured by HPLC. LP group islets showed a significantly ($P<0·01$) lower concentration of taurine (22·9±1·5 mM/μg protein) as compared with control islets (36·9±5·2 mM/μg protein).

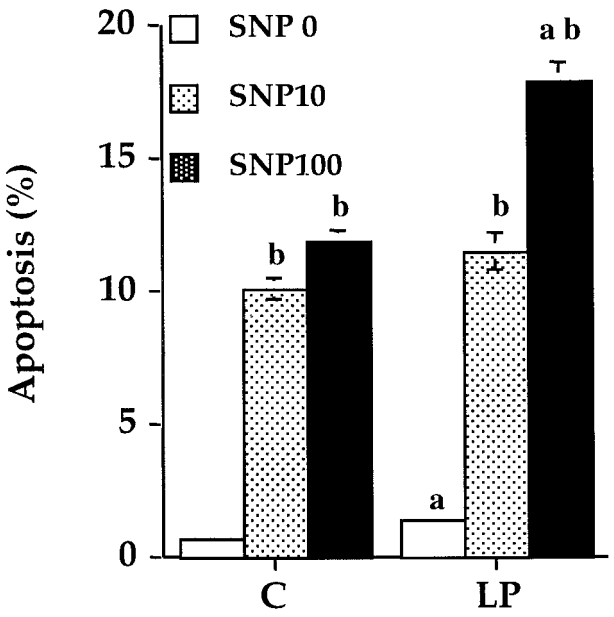

Figure 2 Effect of a low-protein diet on the sensitivity of control (C) and LP group islets towards SNP, a chemical donor of NO. Islets were cultured for 6 days in RPMI 1640 medium and then treated for 18 h without SNP (SNP 0) or with SNP 10 or 100 μM (SNP10, SNP100). Apoptosis was quantified by confocal microscopy using a TUNEL method. Values are the means of 11–14 islets pooled from three separate experiments with at least 2600 cells/group. [a]$P<0{\cdot}01$ compared with control; [b]$P<0{\cdot}01$ compared with SNP 0.

SNP-induced apoptosis in fetal β-cells of control and LP groups

When the islets were incubated with 10 or 100 μM of the NO donor SNP, the rate of apoptosis increased acutely in a dose-dependent manner (Fig. 2). With 100 μM SNP, apoptosis was significantly greater in LP islet cells than in controls. To confirm these results, we measured the percentage mortality in response to 100 μM SNP, using a test for cell permeability to ethidium bromide. LP group fetal islets showed 10·4±0·7% mortality, whereas in control islets the mortality was only 2·9±0·8%, which corroborated the results obtained using the TUNEL method.

Effect of taurine, methionine and β-alanine on SNP-induced apoptosis

In the second series of experiments, control fetal islets treated for 42 h with 0·3 mM or 3 mM taurine showed rates of apoptosis of 1·5±0·2% and 1·4±0·3% respectively, which are not significantly different from those of control islets without taurine (1·3±0·2%). In LP group islets, the percentage of apoptotic cells was approximately twofold greater (2·2±0·3%) as in the previous series and taurine did not affect this basal rate (2·1±0·3% and 2·1±0·4% respectively with 0·3 and 3 mM of taurine).

The protective effect of taurine against SNP cytotoxicity is presented in Fig. 3a. At physiological or supraphysiological concentrations, taurine significantly decreased the percentage of islet cells positive for apoptosis in both groups. However the protective effect of physiological concentrations of taurine was more marked in the LP islets (60% reduction of apoptosis compared with 30% in controls).

To determine if the activity of taurine was specific to this amino acid, we compared its effect with that of methionine, another sulphur-containing amino acid. In both groups (control and LP) the percentage of islet cells positive for apoptosis was significantly lower when methionine was used at physiological concentration (Fig. 3b). At supraphysiological concentration, methionine did not provide additional protection.

To determine the role of the sulphur moiety in the protective effect of taurine, β-alanine, an analogue of taurine lacking the sulphur moiety, was added in the same test conditions. The rate of apoptosis was similar with or without β-alanine, indicating that it afforded no protective effect against the toxicity of NO (Fig. 3c).

The protective activity of taurine was also verified by quantification of cell death using the cell-permeant probe. Figure 4 shows that the percentage of cell death was significantly diminished when islet cells were pretreated with taurine at physiological and supraphysiological concentrations. This was true for both groups, and this effect was dose dependent.

Interaction of taurine and NO

Nitric oxide is a reactive free radical that leads to peroxynitrite formation, another particularly reactive free radical, by interaction with superoxide ($NO+OO^{\bullet} \rightarrow ONOO^{\bullet}$). In order to determine if taurine had to act on islet cells in order to exert its protective effect or, alternatively, if the amino acid acted extracellularly, we investigated the possible direct molecular interaction between taurine and the NO donor, in addition to the formation of peroxynitrite. We then quantified the concentration of NO in an acellular system in the presence of SNP alone or with taurine. The concentration of NO released by SNP reached 26·7 nmol/ml and was not significantly different when taurine was added in a concentration of 0·3 mM (26·8 nmol/ml) or 3 mM (26·7 nmol/ml). To investigate the possibility that taurine decreased the formation of peroxynitrite from NO, we used luminol-derived chemiluminescence induced by peroxynitrite produced by the decomposition of sydnonimine (SIN-1). The effects of the addition of taurine (0·3 or 3 mM), methionine (0·1 or 1 mM) or β-alanine (0·3 or 3 mM) to this system are shown in Fig. 5. With 3 mM of taurine, luminol chemiluminescence was markedly decreased. No change was observed when the other amino acids were added.

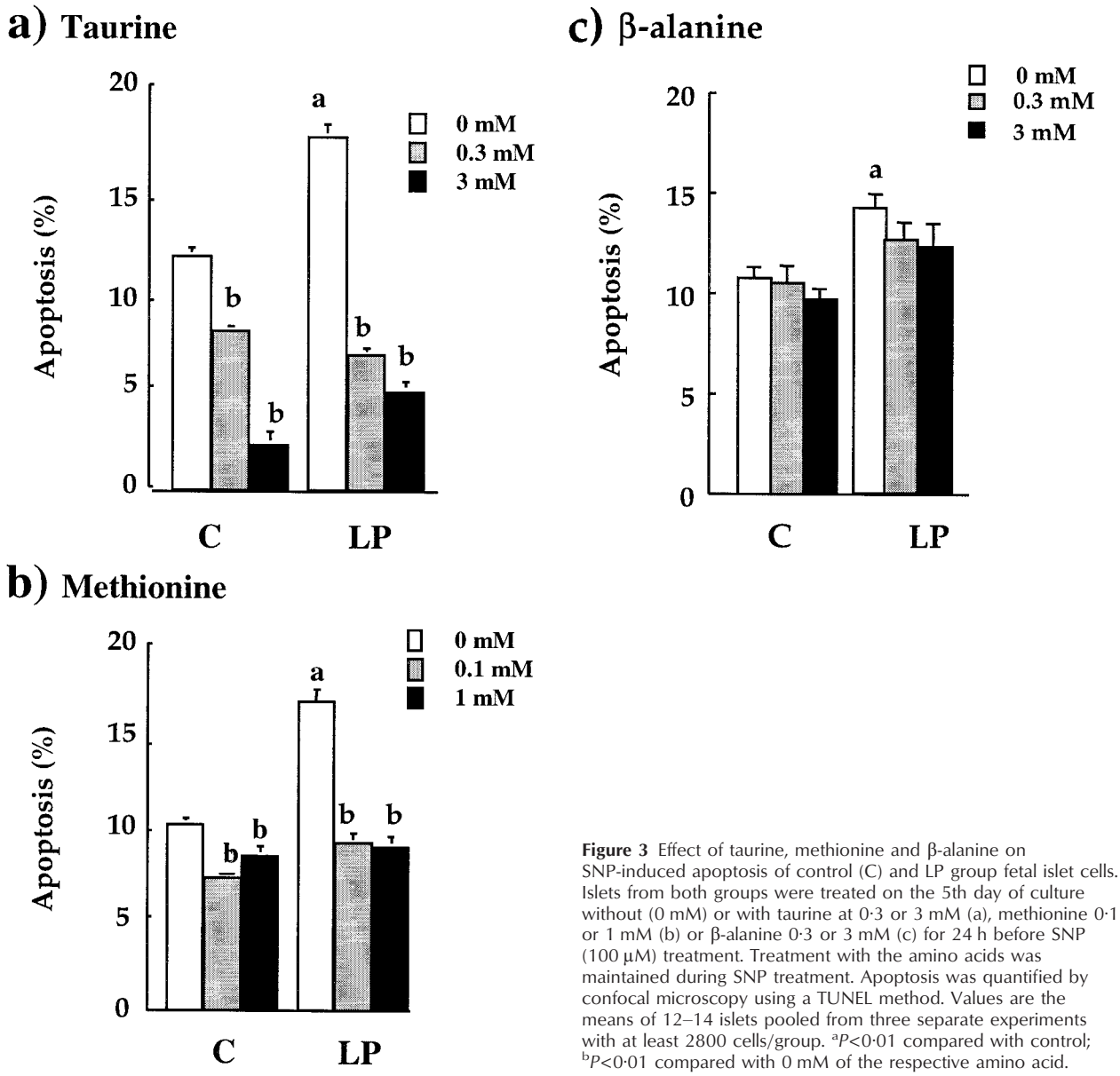

Figure 3 Effect of taurine, methionine and β-alanine on SNP-induced apoptosis of control (C) and LP group fetal islet cells. Islets from both groups were treated on the 5th day of culture without (0 mM) or with taurine at 0·3 or 3 mM (a), methionine 0·1 or 1 mM (b) or β-alanine 0·3 or 3 mM (c) for 24 h before SNP (100 μM) treatment. Treatment with the amino acids was maintained during SNP treatment. Apoptosis was quantified by confocal microscopy using a TUNEL method. Values are the means of 12–14 islets pooled from three separate experiments with at least 2800 cells/group. [a]$P<0·01$ compared with control; [b]$P<0·01$ compared with 0 mM of the respective amino acid.

Effect of taurine on IL-β-induced apoptosis

In this series of experiments, the basal rate of apoptosis in both groups was somewhat greater than that in the previous experiment (Fig. 6). Incubation of fetal islets with 50 U/ml IL-1β for 24 h increased the level of apoptosis in the control group and even more in the LP group.

To evaluate the protective effect of taurine against IL-1β-induced apoptosis, islets were pretreated with taurine for 24 h and then exposed to IL-1β for 24 h. In control islets, only the high dose of taurine significantly decreased the rate of apoptosis, but in LP group islets, taurine in both physiological and supraphysiological concentrations significantly decreased the number of islet cells positive for apoptosis.

Effect of taurine supplementation of the diet of the mother on SNP or IL-1β-induced apoptosis

To investigate the effect of a dietary supplementation of taurine, the amino acid was added to the drinking water of the dams during gestation and fetal islets were pretreated or not with taurine for 24 h and exposed to IL-1β for 24 h

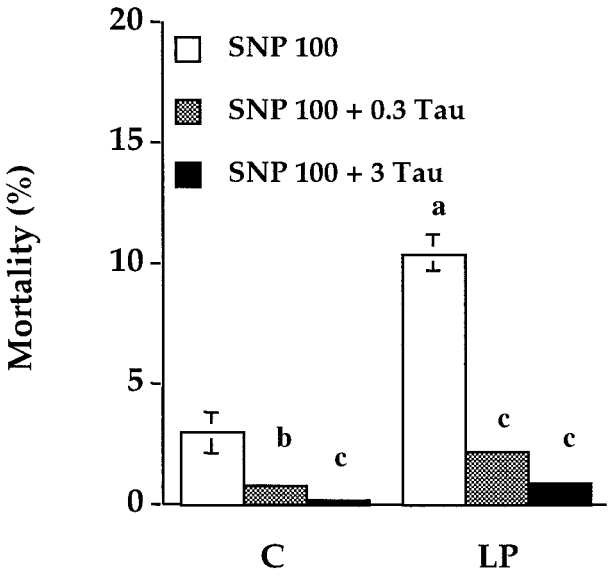

Figure 4 Effect of taurine on the mortality of fetal islet cells induced by SNP. Islets were treated with taurine (Tau, 0·3 or 3 mM) for 42 h. In the last 18 h of taurine treatment, SNP (100 μM) was added. Mortality was quantified by confocal microscopy using permeant probes as described in Materials and Methods. Values are the means of islets pooled from three separate experiments with at least 2400 cells/group. $^{a}P<0{\cdot}01$ compared with control; $^{b}P<0{\cdot}05$, $^{c}P<0{\cdot}01$ compared with 0 mM of taurine.

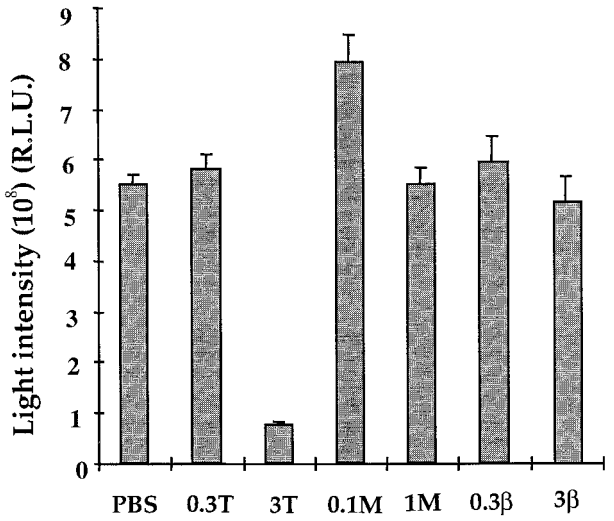

Figure 5 Quenching of peroxynitrite formation by amino acids. After a mixture of luminol and SIN-1 as a source of peroxynitrite, taurine (T, 0·3 or 3 mM), methionine (M, 0·1 or 1 mM) or β-alanine (β, 0·3 or 3 mM) were added. Chemiluminescence of luminol was recorded over 20 min. The figure shows the means of seven replicates.

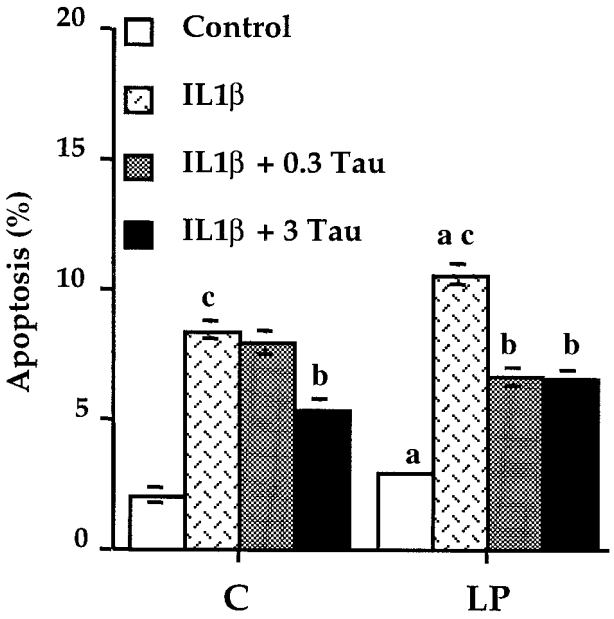

Figure 6 Sensitivity of fetal islets from control (control) and LP groups to IL-1β, and protective effect of taurine (Tau) against this toxicity. On the 5th day of culture, islets were incubated with taurine (0·3 or 3 mM) for 48 h. IL-1β (50 U/ml) was added for the last 24 h. Apoptosis was quantified by confocal microscopy using a TUNEL method. Values are the means of 13–20 islets pooled from three separate experiments with at least 3000 cells/group. $^{a}P<0{\cdot}05$ compared with control; $^{b}P<0{\cdot}01$ compared with IL-1β alone; $^{c}P<0{\cdot}01$ compared with control.

or to SNP for 18 h. In the control group (Fig. 7a), comparison between the results without or with taurine supplementation of the dam's diet shows that this supplementation increased the basal rate of apoptosis. Taurine supplementation of the diet of the mother had no effect on the sensitivity of control fetal islets to SNP. In this case, addition of taurine to the culture medium had the same protective effect on the cytotoxicity of SNP with or without taurine supplementation of the dam's diet. This supplementation, however, significantly decreased the cytotoxicity of IL-1β on the control islets. The protection against IL-1β produced by taurine added to the culture medium was further enhanced when the mother had received taurine in the drinking water. In the LP group fetal islets (Fig. 7b), taurine supplementation of the dam's diet significantly decreased the toxicity of SNP and suppressed the toxic effect of IL-1β. In the case of taurine supplementation of the dam's diet, the addition of taurine to the culture medium had a cumulative protective effect against SNP.

When taurine is supplemented in the diet of the mother, rate of apoptosis after SNP or IL-1β attack is lower in the LP islets than in control islets, notwithstanding the addition of taurine to the culture medium.

Discussion

The present study demonstrates for the first time that malnutrition and, more precisely, protein restriction

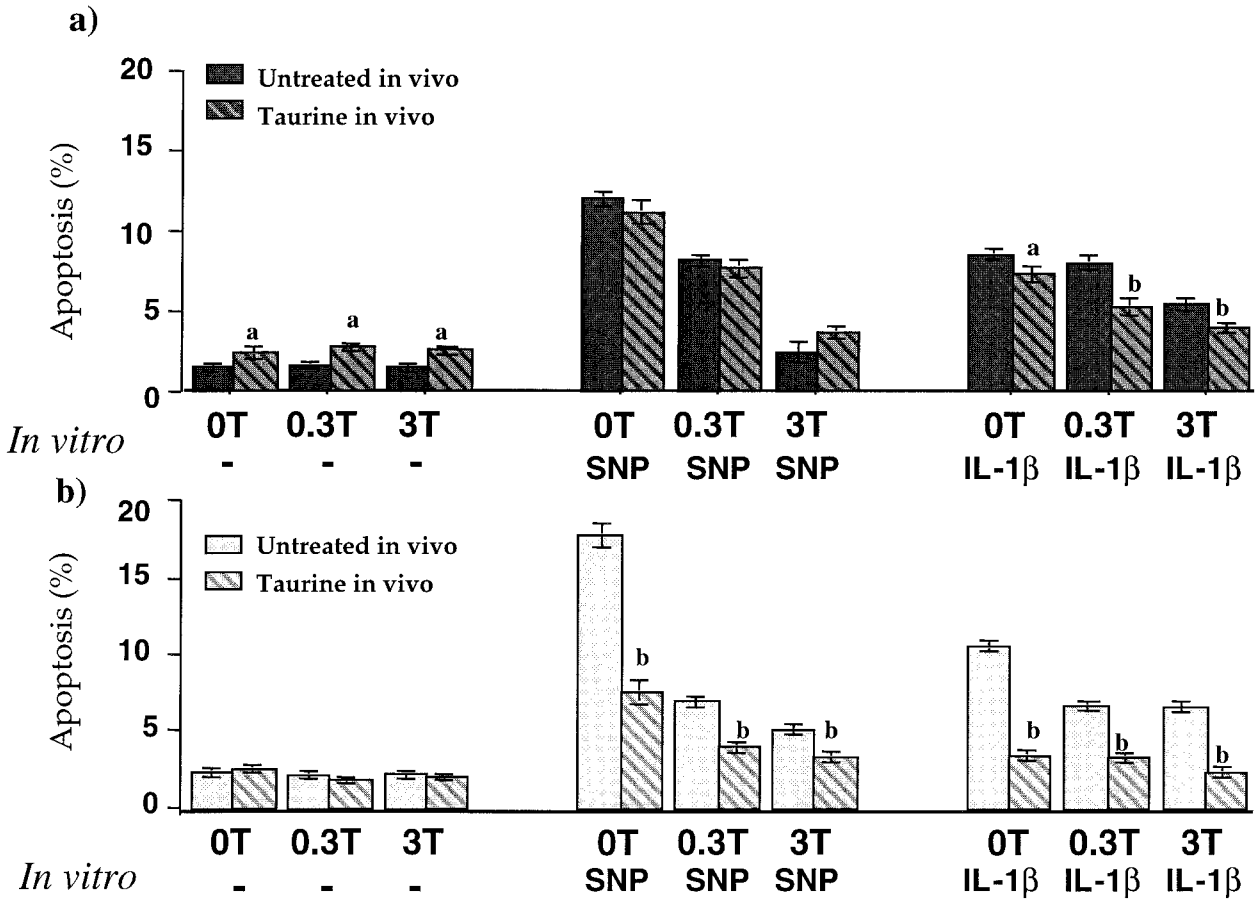

Figure 7 Effect of taurine supplementation on the sensitivity of fetal islet cells from the control group (a) and the LP group (b) to the toxicity of SNP and IL-1β. Taurine (2·5%) was added to the drinking water of the dams during gestation. On the 5th day of culture, islets were pretreated with taurine (0·3 or 3 mM) for 24 h and then treated with IL-1β (50 U/ml) for 24 h or with SNP for 18 h. Apoptosis was quantified by confocal microscopy using a TUNEL method. Values are the means of 19 or 20 islets pooled from three separate experiments with at least 4500 cells/group. $^{a}P<0{\cdot}05$, $^{b}P<0{\cdot}01$, compared with untreated *in vivo*.

during gestation increases the susceptibility of fetal β-cells to the damage caused by IL-1β and NO, and low taurine concentrations are involved in this alteration.

We have shown previously that a low-protein diet given to pregnant dams increases the rate of apoptosis *in vivo* in fetal and neonatal islet cells (Petrik *et al.* 1999). A similarly increased rate of apoptosis in LP fetal islets was also found after 7 days of culture *in vitro* in the same culture conditions as for controls and after withdrawal from the altered maternal environment. This demonstrates a form of 'programming' of the islet cells in conditions of intrauterine protein malnutrition, which induced long-lasting effects. It is known that IL-1β alone or in combination with TNF-α plus IFN-γ induces apoptosis in mouse, rat and human β-cells (Delaney *et al.* 1993, 1997, Cetkovic & Eizirik 1994, Rabinovitch & Suarez-Pinzon 1998). In this study, an increase in apoptosis after exposure to IL-1β was observed in both groups, but the level was greater in LP group islets than in control islets. It is thus clear that a low-protein diet during gestation augments the sensitivity of fetal β-cells to IL 1-β.

One pathway that has been proposed as being the effector for IL-1β-induced apoptosis is the stimulation of inducible NO synthase (Corbett *et al.* 1991, Delaney *et al.* 1993). Therefore, the NO donor SNP was applied in two different concentrations. A dose-dependent increase in apoptosis was observed in islet cells of both groups, but it was more severe in LP islets with high concentrations of SNP. The same was true when another mortality test, based on permeabilization of the cell membrane, was used.

SNP is a complex of ferrous iron (Fe^{2+}) with five cyanide anions (CN^{-}) and a nitrosonium (NO^{+}) ion. It can simultaneously liberate NO and an iron moiety capable of generating $OH^{\bullet}$ radicals via the Fenton reaction (Bates *et al.* 1991). In order to verify that the cytotoxicity of SNP was not mainly attributable to this

highly reactive $OH^{\bullet}$ radical, we added desferrioxamine (DFO), an iron chelator, to the culture medium. DFO partially reduced the rate of apoptosis in both groups of islets, but this reduction accounted only for about 30% of the apoptosis induced by the high dose of SNP (data not shown). The major part of the toxic effect of SNP appears, then, to be mediated by NO. This result shows that consumption of a low-protein diet during gestation generates a β-cell mass with greater sensitivity to nitric oxide. The reasons for this increased sensitivity to NO and IL-1β are unknown, but several explanations could be proposed. The lower concentration of insulin-like growth factors (IGFs)-I and -II in the LP islets could be incriminated in their increased sensitivity. Indeed, maternal low protein induces a lower number of islet cells positive for IGFs-I and -II in the progeny (Petrik *et al.* 1999), and these growth factors have been proposed to be survival factors for the β-cell (Petrik *et al.* 1998). The greater sensitivity can also be related to the fact that a low-protein diet during gestation leads to the formation of an islet cell population that matures differently. It has been reported that the sensitivity of β-cells to IL-1β and streptozotocin is an acquired trait during β-cell maturation (Nielsen *et al.* 1999). It has also been reported that cytokines were consistently found to induce iNOS positively in only a sub-population of β-cells (Eizirik & Pavlovic 1997). In the LP islets, however, the number of cells expressing iNOS appears to be unaffected, but this does not mean that the concentration of iNOS per cell is not modified. A low-protein diet can also perturb the expression of several proteins, among them heat-shock protein 70 (hsp70), a protein known to be very important for the protection of β-cells against the toxicity of NO and IL-1β (Burkart *et al.* 2000). Finally, as taurine has antioxidant properties, the low concentration found in the LP islets could lead to a less efficient defence against reactive oxygen species.

The second part of the study demonstrated that taurine in physiological or supraphysiological concentrations protects fetal islet cells against the cytotoxicity induced by IL-1β and a NO donor. Methionine also demonstrated a protective effect, but it was less marked than that of taurine. These two amino acids are physicochemically different: taurine possesses a more acidic function (pK_a 1·5) than methionine (pK_a 2·28) (Wright *et al.* 1986). This difference is expected as a result of the presence of the sulphonate ion within the taurine acidic function (Wright *et al.* 1986) and this might explain the different biological activities of taurine and methionine. Moreover, β-alanine, a structural analogue of taurine that lacks the sulphur moiety, was tested and no protective effect was observed. This indicates that the sulphur group of taurine is responsible for its protective effect against the fetal β-cell damage induced by NO.

It has been suggested that taurine acts directly as an antioxidant by quenching reactive oxygen species, or indirectly by preventing the increase in membrane permeability resulting from oxidant injury by a mechanism that remains under discussion (Chesney 1985, Banks *et al.* 1992). In the present conditions, the protection by taurine cannot be attributed to an inhibition of NO release from the NO donor, but rather, partially to a blocking of peroxynitrite formation. A decrease in chemiluminescence has, indeed, been observed in an acellular system in the presence of the amino acid, but in a high dose. Other mechanisms must also be evoked. In islets, the cytokine IL-1β induces coexpression of iNOS and cyclooxygenase (COX) (Corbett *et al.* 1993). Taurine chloramine, which is derived from the interaction of taurine with HOCl/$OCl^{\bullet}$, a product of myeloperoxidase activity, has been shown to inhibit iNOS and COX2 expression in activated C6 glial cells (Liu *et al.* 1998). Heat shock protein (hsp)70 has been implicated in the defence of β-cells against cytokine injury (Scarim *et al.* 1998). Taurine might enhance the expression of hsp70 to prevent β-cell destruction by NO and IL-1β. It has been shown that taurine protects liver parenchymal cells against heat injury via regulation of hsp70 expression (Kurz *et al.* 1998).

The effect of taurine on the fetal islets treated by IL-1β differed in the control and LP groups. In control islets, only a high dose of taurine significantly decreased the rate of apoptosis, whereas in LP islets, taurine in both physiological and supraphysiological concentrations significantly decreased the rate of apoptosis. As the LP group islets originated from a low-taurine environment, this could have decreased the threshold of their sensitivity to taurine.

The protective action of taurine *in vitro* is less apparent when islets are treated with IL-1β than with the NO donor. This might be due to the fact that the stimulation of iNOS leading to production of NO mediates in part, but not completely, the cytotoxicity of IL-1β towards β-cells. Indeed, *in vivo*, IL-1β can also stimulate (Yamada *et al.* 1996, Loweth *et al.* 1998, Amrani *et al.* 2000, Zumsteg *et al.* 2000) the apoptosis of β-cells by inducing Fas expression. It has been suggested that, when human islet cells are primed by IL-1β to undergo apoptosis, this involves the close association between cell-surface Fas and its ligand (Loweth *et al.* 1998, Amrani *et al.* 2000). Others report that IL-1β in combination with TNF-α and IFN-γ induces apoptosis of mouse and NIT-1 β-cells by two independent pathways, NO production and Fas surface expression (Zumsteg *et al.* 2000). At first sight, the presence of the Fas ligand is unlikely in our cultures, which did not contain significant elements of the immune system. However, β-cells appear to be able to express FasL themselves, as it has been reported that the destruction of β-cells in non-obese diabetic mice implies an interaction between Fas induced by cytokines and possibly FasL-expressing β-cells themselves (Suarez-Pinzon *et al.* 1999). In contrast, it has been found that cytokines can stimulate apoptosis of cells such as epithelial HT-29 cells, independently of NO formation but via another pathway activating caspases-8 and -3 (Wright *et al.* 1999).

Finally, our findings show that addition of taurine to the drinking water of dams throughout gestation decreases the sensitivity of control fetal islets to IL-1β. In LP islets, taurine supplementation decreased the effect of SNP and suppressed the toxicity of IL-1β. IGFs-I and IGF-II are considered to be survival factors for β-cells, and islets exposed to IL-1β do, indeed, exhibit less apoptosis when treated with IGFs (Petrik *et al.* 1998). We report that supplementation of the maternal diet with taurine restored to normal the number of cells positive for IGF-II in LP islets and enhanced that number in control islets (Boujendar *et al.* 2000). A similar positive action of taurine on IGF-I production has also been described in other experimental conditions, in which taurine supplementation given to elderly rats restored a normal concentration of IGF-I (Dawson *et al.* 1999). Therefore, in our experiments, taurine supplementation could have led to a β-cell population less sensitive or insensitive to IL-1β.

The increased basal rate of apoptosis observed *in vitro* in islets of fetuses from mothers fed a 20% protein diet supplemented with taurine could be attributed to a difference in the fetal β-cell population generated by taurine supplementation during development: this β-cell population could be more mature than that of control fetal islets. Indeed, islets of fetuses from mothers fed a 20% protein diet supplemented with taurine do secrete insulin in response to glucose challenge, whereas normal fetal islets do not (Cherif *et al.* 1998).

In view of our findings, the greater sensitivity of LP group islets and the lower taurine concentration in these islets may suggest that a lack of taurine may be involved in the increased rate of apoptosis.

In conclusion, we have demonstrated that a low-protein diet during development increases the sensitivity of fetal β-cells to NO and IL-1β. Taurine may prevent this damage.

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