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Protective effect of long term high fiber diet consumption on rat exocrine pancreatic function after chronic ethanol intake

María J. Bragado[1,*], Luis J. García[b], María A. López[a], José J. Calvo[a]

[a]*Departamento de Fisiología y Farmacología, Universidad de Salamanca, Cáceres, Spain*
[b]*Departamento de Fisiología, Universidad de Extremadura, Cáceres, Spain*

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Abstract

The effects of ethanol administration on exocrine pancreas have been widely studied, but little is known about the effect of dietary fiber in combination with chronic ethanol on exocrine pancreatic function. The aim of this work was to examine the chronic effects of a high fiber diet, ethanol ingestion, and a combination of both on the function of the rat exocrine pancreas. Four groups of rats were fed for six months the following diets: 1.- NW: standard laboratory diet; 2.- FW: high fiber diet (15% cellulose); 3.- NE: standard laboratory diet and 20% ethanol in the drinking water; and 4.- FE: high fiber diet and 20% ethanol. Cholecystokinin (CCK) and acetylcholine (Ach) effects on amylase release and intracellular calcium mobilization in pancreatic acini were studied. In rats fed a 20% ethanol (NE), both the basal amylase release and the basal $[Ca^{2+}]_i$ were significantly increased; nonetheless, CCK and Ach-induced amylase release were significantly reduced compared with control rats. Ach- but not CCK-stimulated $[Ca^{2+}]_i$ increase in NE rats was significantly decreased compared with NW. In rats fed a combination of ethanol and a high fiber diet (FE) all the parameters under study were not significantly affected compared to control rats (NW). In conclusion, high fiber consumption does not alter the function of the exocrine pancreas. However, it ameliorates the deleterious effect of chronic ethanol consumption on pancreatic amylase secretion and, at least partially, reverses the ethanol-induced alterations on $[Ca^{2+}]_i$ in the rat exocrine pancreas.

Keywords: Fiber; Ethanol; Pancreas; Cholecystokinin; Calcium

1. Introduction

Current studies about physiological responses to a fiber-rich diet and to specific types of fiber suggest that there are some potentially important mechanisms by which fiber can alter the function of digestive organs [1–4]. These mechanisms depend on the nature and on the source of fiber, and include changes in the intestinal transit time [5], small intestine absorption [6], intraluminal pressure [5], bacterial metabolism [7], and fecal excretion of nitrogen and fat [8]. Most of these effects have been explained by the physicochemical properties of fiber. Within the intestinal lumen, fiber may alter pH, and decrease the accessibility of absorbable nutrients to the mucosal surface and, subsequently, altering the availability of enzyme and bile acids in the small intestine [9]. Then, by modifying the digestive enzyme activities, the physiological function of digestive organs can be altered by fiber, in part due to a lower digestibility and slower absorption of nutrients.

The effects of a high fiber diet on the function of the exocrine pancreas are not clear. Some studies reported a stimulatory effect on the exocrine pancreatic secretion in humans after guar gum [10] and pectin [6] consumption. In rats, an increase in exocrine pancreatic secretion has been shown after different periods of treatment [8,11,12]. Some studies reported no effect on pancreatic enzyme secretion [13,14] and in one study the output and concentration of lipase dramatically decreased in the dog [15]. The differences between results can be attributable to the type of fiber used, the duration of treatment, the animal species and the diet composition. Moreover, it has been demonstrated a protector role of high fiber diets in some pancreatic pathologies such as wheat bran dietary supplement [17], or high

* Corresponding author. Tel. and fax: +34 927 257160.
E-mail address: jbragado@unex.es (M.J. Bragado).
[1] Current address: Departamento de Bioquímica, Biología Molecular y Genética, Universidad de Extremadura, Avda Universidad s/n, Cáceres 10071, Spain.

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cellulose fiber, which have an inhibitory effect on the pancreatic carcinogenesis together with low fat intake [16].

The effects of ethanol administration on exocrine pancreatic function have been widely studied. Thus, studies have reported stimulation, inhibition or no change in exocrine pancreatic secretion, depending on the species being used and the mode of ethanol administration [18]. It is well known that ethanol abuse is an important etiologic factor in pancreatitis, although the mechanisms by which ethanol causes pancreatic injury have not been characterized yet. The role of dietary components in the pathogenesis of alcoholic pancreatitis remains controversial, because different studies have shown conflicting results. However, the effect of chronic ingestion of a high fiber diet in combination with chronic ethanol consumption on the exocrine pancreatic function has not yet been studied.

It is generally accepted that the main function of pancreatic acinar cells (the secretion of digestive enzymes) is greatly dependent on intracellular Ca^{2+} [19]. In pancreatic acini, the activation of cholecystokinin (CCK) or acetylcholine (Ach) receptors induce a transient elevation of the cytosolic free calcium. The mechanism of Ca^{2+} elevation is via inositol 1, 4, 5-trisphosphate (IP_3), which is formed as a result of the G protein-dependent activation of phospholipase C. IP_3 elevates intracellular calcium concentration ($[Ca^{2+}]_i$) by releasing Ca^{2+} from intracellular stores. This process, combined with receptor-stimulated Ca^{2+} influx, leads to an increase in $[Ca^{2+}]_i$ that is involved in the secretory response to CCK or Ach in the acinar cell. The aim of this study was to evaluate the effect of chronic ethanol consumption and high fiber (cellulose) ingestion, separately and in combination, on the basal and CCK- and Ach-stimulated amylase release and $[Ca^{2+}]_i$ in rat pancreatic acini.

2. Methods and materials

2.1. Materials

Collagenase type III from *Clostridium histolyticum*, cholecystokinin-octapeptide (CCK-8), soybean trypsin inhibitor (SBTI), bovine serum albumin (BSA), acetylcholine and digitonin were purchased from Sigma Química (Madrid, Spain). Fura-2/AM, fura-2 pentapotasium salt and pluronic acid F127 were purchased from Molecular Probes (Eugene, OR). All the other chemicals were analytical grade reagents.

2.2. Animals and treatments

Diets and treatments of animals were performed exactly as described previously [20]. All experiments were performed using male Wistar rats purchased from CRIFFA (Barcelona, Spain). They were housed in temperature-controlled, 12 h light/dark rooms and they were given free access to food and liquid. They were divided into 4 groups and fed for 6 months the following diets: 1. NW, Standard laboratory diet, A04 PANLAB (Barcelona, Spain), and water; 2. FW, High fiber diet (15% cellulose) and water; 3. NE, Standard laboratory diet, A04 PANLAB, and 20% ethanol; 4. FE, High fiber diet (15% cellulose) and 20% ethanol. In alcohol treated groups (NE and FE), the ethanol was given as 5% concentration (v/v) on the first week, 10% (v/v) on the second week, 15% (v/v) on the third week and 20% (v/v) from the fourth week to the end of the experimental period. The composition of diets, expressed as percentage of total material (including water content) is described previously [20]: 16% protein, 4% lipid, 23.7% starch, 8% minerals, 1% calcium, 0.64% phosporus, 0.3% sodium, 12000 IU/kg vitamin A, 1200 IU/kg vitamin D3, 10 mg/kg vitamin E (alphatocopherol), 4 mg/kg copper (pentahydrated sulfate), traces of antioxidants. The diets differed in their content of fiber; the high fiber diet contained 15% cellulose whereas the standard laboratory diet contained 8% cellulose. The higher percentage of cellulose was obtained by decreasing the amount of other carbohydrates from 23.6% starch in the standard diet to 15.7% starch in the high fiber diet.

After the experimental treatment, rats were killed by decapitation. The pancreas was quickly removed, trimmed of adipose tissue and samples were taken for light microscopy, measurement of pancreatic amylase, protein and liquid content and for isolation of pancreatic acini. A sample of blood was taken to measure plasma amylase concentration.

The National guide for the care and use of laboratory animals was strictly followed in this study.

2.3. Biochemical analysis

To determine the pancreatic content of amylase and total proteins, 100 mg aliquot of pancreatic tissue was homogenized in ice-cold phosphate buffer, 0.1 mol/L, pH 7.4 (with OMNI-1000, at 15,000 rpm for 20 s). Total proteins were measured using the method of Bradford [21] and were expressed as mg protein/100 mg pancreatic tissue. Pancreatic amylase content was measured as described previously, with the Phadebas method, based on Gardner and Jackson, [22], and expressed as Units of amylase/100 mg pancreatic tissue. The pancreatic content of liquid was calculated as the difference in weight of 100 mg fresh pancreatic tissue, before and after being heated at 90°C for 48 h, and was expressed as percentage of tissue weight. To determine the plasma concentration of amylase, blood samples were centrifuged for 10 min at 4°C; plasma was stored at −70°C until determination of amylase activity by a method previously described [23].

2.4. Preparation of isolated pancreatic acini

Acini were isolated by digestion of the rat pancreas with collagenase by a method previously described [23]. The pancreas was placed in modified Krebs-Ringer-Hepes (KRH) solution, pH 7.4, including (in mmol/L): 130 NaCl,

Table 1
Body weight, pancreatic content of liquid, total proteins, pancreatic and plasmatic concentration of amylase for the different experimental groups

	NW	FW	NE	FE
Animal initial weight (g)	197 ± 2	196 ± 2	202 ± 3	202 ± 3
Animal final weight (g)	547 ± 7	511 ± 6[b]	505 ± 12[a,b]	446 ± 8[a]
Liquid in pancreatic tissue (% water)	71.8 ± 0.1	74.5 ± 0.2	74.4 ± 0.3	73.0 ± 0.9
Total proteins in pancreatic tissue (mg/100 mg tissue)	23.1 ± 1.9	23.6 ± 2.1	21.5 ± 2.6	22.9 ± 1.9
Plasma amylase (U/100 mL plasma)	7.8 ± 0.5	6.5 ± 0.2	5.6 ± 0.1	5.8 ± 0.1
Amylase in pancreatic tissue (U/100 mg tissue)	26.8 ± 5.9	27.9 ± 6.5	13.3 ± 3.7[a]	11.3 ± 3.9[a]

NW: animals fed a normal diet and water; NE: animals fed a normal diet and ethanol; FW: animals fed a high fiber diet and water; FE: animals fed a high fiber diet and ethanol. Values are expressed as mean ± SEM; $n = 8$ for each group. After ANOVA resulted statistically significant for body weight ($F = 24,55$) and for amylase in pancreatic tissue ($F = 8.34$), Scheffe test was performed between the different groups.

[a] Statistical differences compared to control group, NW.

[b] Differences compared to FE group. $P < 0.05$ is significant.

5 KCl, 20 Hepes, 1.2 KH_2PO_4, 10 D-Glucose, 1 $CaCl_2$, 2 g/L bovine serum albumin, and 0.1 g/L soybean trypsin inhibitor. The viability of the acini obtained was greater than 95% in all experimental groups, as measured by Trypan blue exclusion.

2.5. Measurement of amylase released in pancreatic acini

Amylase release was determined as described [22] using the Phadebas reagent. Aliquots of 500 μL of the acinar cell suspension were incubated with CCK or Ach at 37°C for 30 min. Amylase release was calculated as the percentage of the total amylase activity in the acini and expressed by subtracting the basal value (without CCK or Ach). Each sample was assayed in duplicate.

2.6. Measurement of free cytosolic Ca^{2+} in pancreatic acini

To measure the $[Ca^{2+}]_i$, cell suspensions were loaded with 4 μM fura-2/AM and 0.025% of pluronic acid, as described previously [23]. Fluorescence of fura-2 was measured in 2 mL aliquots of acinar cell suspension in a Shimadzu RF 5001-fluorescence spectrophotometer. Excitation wavelengths were alternated between 340 nm and 380 nm and emission was monitored at 505 nm. Stimuli were added directly to the cell suspension, which was maintained at 37°C. The effect of CCK-8 was tested, using concentrations ranging from 10 pmol/L to 10 nmol/L and 10 μmol/L acethylcholine was also tested. Maximum and minimum fluorescence ratio values were obtained at the end of each experiment by adding digitonin and EGTA, as described previously [23]. The apparent dissociation constant for the [fura-2-Ca^{2+}] complex, determined under our conditions, was 104 nmol/L, and this value was used in calculating $[Ca^{2+}]_i$ values in nmol/L.

2.7. Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical comparisons between the values of the different groups were performed by analysis of variance (ANOVA). When a statistically significant treatment effect was found, Scheffé's test was applied. Only differences of $P < 0.05$ were accepted as significant.

3. Results

3.1. Animals

At the end of the 6 months treatment, rats appeared healthy. Histological analysis of the rat pancreas from each

Table 2
Values of basal amylase release and intracellular calcium concentration for the different experimental groups

	NW	FW	NE	FE
Basal amylase release (% of total amylase)	5.9 ± 1.1	6.0 ± 0.4	10.2 ± 1.4[a,b]	6.0 ± 0.4
Basal $[Ca^{2+}]_i$ (nmol/L)	75 ± 3	73 ± 5	111 ± 4[a,b]	86 ± 7

NW: animals fed a normal diet and water; NE: animals fed a normal diet and ethanol; FW: animals fed a high fiber diet and water; FE: animals fed a high fiber diet and ethanol. Values are mean ± SEM; $n = 8$ for each group. After ANOVA resulted statistically significant ($F = 5.14$ and $F = 12.32$ respectively), Scheffe test was performed between groups.

[a] Statistical differences compared to control group, NW ($F = 3.26$ and $F = 3.18$, respectively).

[b] Differences compared to group FE ($F = 3.18$ and $F = 3.55$, respectively). $P < 0.05$ is significant.

experimental group showed a similar morphology of the pancreatic tissue as previously described [20] (data not shown). The initial and final body weights are shown in Table 1. Rats fed a high fiber diet, FW group, presented a lower gain in body weight than the rats fed a standard laboratory diet, NW group. As seen in Table 1, rats receiving ethanol, NE, had a significantly reduced gain in body weight than rats receiving water, NW ($P < 0.01$ by ANOVA). A combination of both ethanol and high fiber diet, FE, significantly reduced body weight gain ($P < 0.01$) compared with NW, FW and NE groups.

Regarding the daily food intake (g/100 g body weight) and the daily liquid intake average (mL/100 g body weight) during treatments, there was an effect of the consumption of ethanol as shown in a previous study [20] (data not shown). Thus, rats receiving ethanol (FE and NE) always presented a slightly lower food and liquid intake than rats drinking water (NW and FW). There were no differences in food and liquid intakes attributable to the type of diet (data not shown).

In order to study whether the treatments lead to different effects on the pancreas, we analyzed biochemical parameters in each experimental group. As seen in Table 1, the different treatments had no significant effect on the pancreatic content of liquid, pancreatic content of proteins, and plasma concentration of amylase. However, both groups receiving ethanol, NE and FE, presented a statistically significant reduction in the pancreatic content of amylase ($P < 0.01$).

3.2. Effect of high fiber diet consumption on the function of the exocrine pancreas

The effect of chronic consumption of a high cellulose diet on exocrine pancreatic secretion was evaluated by measuring amylase release in isolated pancreatic acini from rats from the FW group. A high cellulose diet did not affect the basal amylase secretion in pancreatic acini (Table 2). As seen in Fig. 1A, the CCK-stimulated amylase release is not significantly different from the NW group. The 10 μmol/L Ach-induced amylase release is not statistically different from the NW group (Fig. 2A). The effect of a chronic high fiber diet on pancreatic function was also evaluated by determining the concentration of intracellular calcium in isolated pancreatic acini from group FW rats. A cellulose diet affects neither the basal concentration (Table 2) nor $[Ca^{2+}]_i$ stimulated by different concentrations of CCK (Fig. 1B) or Ach (Fig. 2B).

3.3. Effect of chronic ethanol consumption on the function of exocrine pancreas

Long term ethanol consumption markedly altered basal (Table 2) and Ach and CCK-stimulated amylase release from the isolated pancreatic acini (Figs. 2A and 3A). Basal amylase release was significantly elevated in the NE group compared with the control group, NW (Table 2). In addition, in this group of animals, the amylase release in response to maximum and supramaximum concentrations of CCK was markedly decreased (Fig. 3A). In this group, the 10 μmol/L Ach-induced amylase release increase is also markedly reduced compared to the NW group, as seen in Fig. 2A. Chronic ethanol intake significantly increased the basal $[Ca^{2+}]_i$ (Table 2). The dose-response of $[Ca^{2+}]_i$ to CCK was not affected (Fig. 3B); interestingly, the fold increase of $[Ca^{2+}]_i$ after Ach stimulation was markedly, but no significantly, reduced (Fig. 2B).

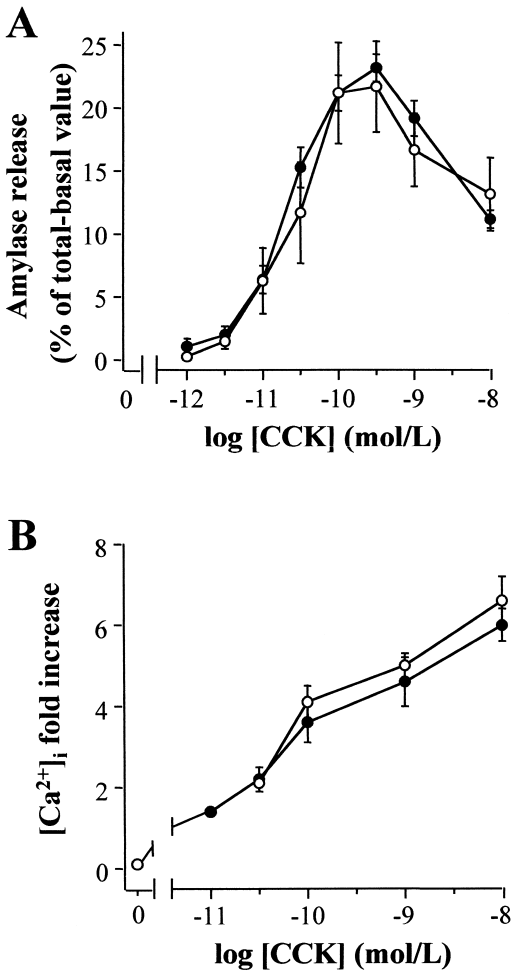

Fig. 1. Effects of high fiber diet consumption (FW, open circles) on CCK-stimulated amylase release (A) or CCK-stimulated intracellular calcium concentration (B) in pancreatic acini. NW (filled circles): animals fed a normal diet and water. Data are expressed as mean ± SEM; $n = 7$. A: Isolated pancreatic acini were incubated with different concentrations of CCK for 30 min and the amylase released in the media was measured as described. B: Pancreatic acini loaded with fura-2 as described were stimulated with different concentrations of CCK. The intracellular calcium concentration was measured as described in the text. No significant differences were found.

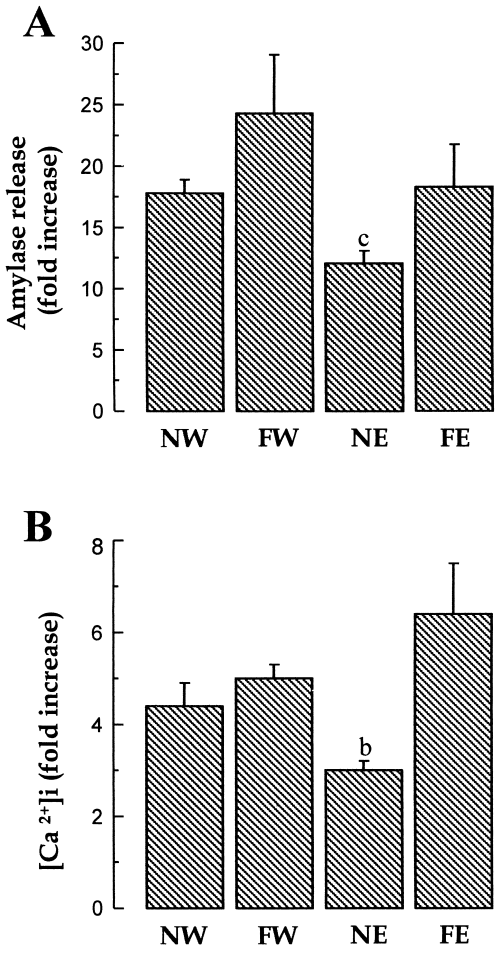

Fig. 2. Acetylcholine-stimulated amylase release (A) or acetylcholine-stimulated intracellular calcium concentration (B) in rat pancreatic acini from different experimental groups. NW: animals fed a normal diet and water; NE: animals fed a normal diet and ethanol; FW: animals fed a high fiber diet and water; FE: animals fed a high fiber diet and ethanol. Data are expressed as mean ± SEM; $n = 6$. A: Pancreatic acini were incubated with 10 μmol/L Ach for 30 min and the amylase released in the media was measured. After ANOVA resulted statistically significant ($F = 3.65$), Scheffe test was performed between groups: [c]Statistical differences compared to FW group ($F = 3.31$). B: Pancreatic acini loaded with fura-2 were stimulated with 10 μmol/L Ach and the concentration of intracellular calcium was measured. After ANOVA resulted statistically significant ($F = 5.00$), Scheffe test was performed. [b]Significant differences compared to FE group ($F = 3.81$). $P < 0.05$ is statistically significant.

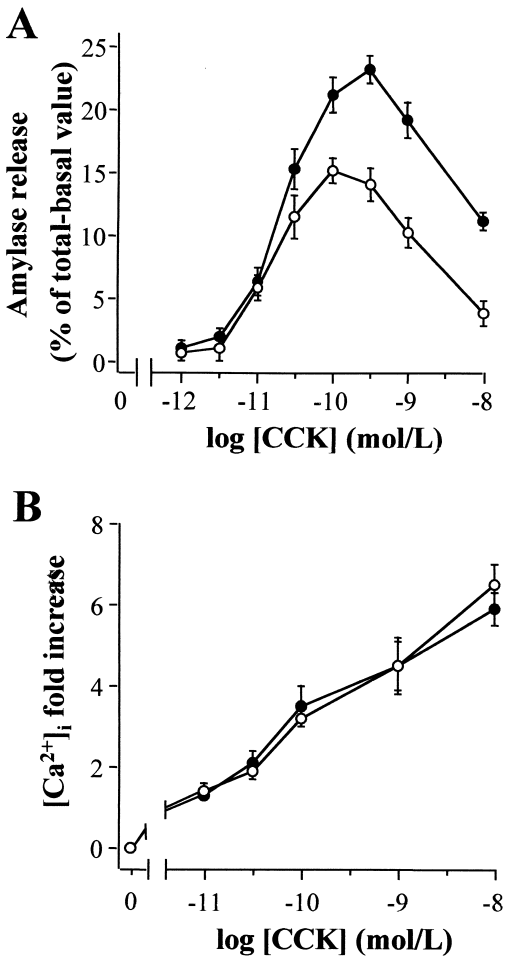

Fig. 3. Effects of long term ethanol consumption (NE, open circles) on CCK-stimulated amylase release (A) or CCK-stimulated intracellular calcium concentration (B) in isolated pancreatic acini. NW (filled circles): animals fed a normal diet and water. Data are expressed as mean ± SEM; $n = 7$. A: Isolated pancreatic acini were incubated with CCK for 30 min and the amylase release in the media was measured as described in the text. B. Pancreatic acini loaded with fura-2 were stimulated with different concentrations of CCK, and intracellular calcium concentration was measured. No significant differences were found between groups.

3.4. Effect of the combination of chronic ethanol and high fiber diet consumption on the function of exocrine pancreas

A combination of both, chronic ethanol and high fiber diet consumption (FE) did not significantly affect the pancreatic acinar function compared to control group. Basal amylase release in the FE group is similar to NW and FW groups, and is statistically lower than in the NE group (Table 2). A combination of a chronic high fiber diet and ethanol completely reversed the decrease in CCK-stimulated amylase release observed in animals fed a control diet and chronic ethanol consumption (Fig. 4A). The CCK-induced pancreatic secretion in animals from the FE group (open circles) is comparable to the control group (filled circles). Interestingly, the 10 μmol/L acetylcholine-induced amylase release is not significantly affected either (Fig. 2A). Regarding the intracellular calcium concentration, a combination of fiber and ethanol had no significant effect on basal $[Ca^{2+}]_i$ (Table 2) although it was slightly increased compared with the control group. In the FE group, neither CCK (Fig. 4B) nor Ach (Fig. 2B) stimulated $[Ca^{2+}]_i$ was significantly affected, when compared to the NW group.

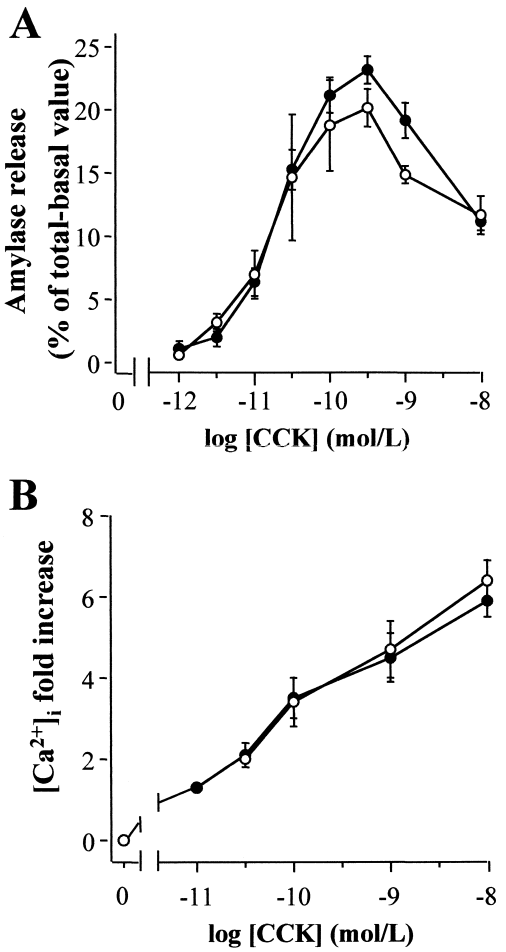

Fig. 4. Effects of the long-term combination of high fiber diet and ethanol consumption (FE, open circles) on the CCK-stimulated amylase release (A) or the CCK-stimulated intracellular calcium concentration (B) in isolated pancreatic acini. NW (filled circles): animals fed a normal diet and water. Data are expressed as mean ± SEM; $n = 7$. A: Pancreatic acini were incubated with CCK for 30 min and the amylase release in the media was measured. B: Isolated acini loaded with fura-2 were stimulated with different concentrations of CCK, and the calcium concentration was measured. No significant differences were found between groups.

4. Discussion

In this study, dietary fiber consumption did not alter the pancreatic function, evaluated by measuring amylase secretion and intracellular calcium concentration in rat isolated pancreatic acini. On the contrary, 6 months ethanol consumption affected the pancreatic function by increasing the basal amylase secretion and decreasing the amylase release after stimulation with CCK or Ach. Chronic ethanol increased calcium concentration under basal conditions and decreased the intracellular calcium concentration after Ach stimulation. When combined with ethanol, fiber prevented both the pancreatic amylase secretion and the basal- and Ach-stimulated intracellular calcium alterations due to the effect of ethanol.

Here we show that a chronic high fiber diet does not affect the biochemical parameters of the pancreas, such as liquid, amylase, and protein pancreatic content, and had no effect on the plasma concentration of amylase. Some of these findings agree with previous results [24,25] that showed that dietary pectin did not change the liquid content of the pancreas. The protein content of the pancreas remained unaffected after high cellulose diet consumption, which is in accordance with previous works [17,24,26], although other authors reported an increase [8]. The plasma concentration of amylase is a parameter used as indicative of damage to pancreatic tissue, as it occurs in pancreatitis, where this index increases dramatically. According to our results, dietary cellulose fed for 6 months did not modify this parameter. Furthermore, the pancreatic amylase content in this group remained unaffected in agreement with previous results [26], also after long term cellulose ingestion in the rat.

Under our conditions, a high cellulose diet did not affect the pancreatic function. First, we showed that neither the basal nor stimulated amylase release in pancreatic acini was modified by consumption of a high cellulose diet. We also demonstrated that the basal and stimulated concentration of intracellular calcium in rats fed a high fiber diet remained in the same range as those from rats fed a control diet. The finding that a high fiber diet does not affect the pancreatic function is also partly supported by the fact that it did not affect the biochemical parameters (Table 1) or morphology of the pancreas. Controversial results about the effect of the fiber on pancreatic secretion have been reported, in part, due to differences in the type of diet, experimental conditions and species. Thus, it was found a stimulatory effect on pancreatic secretion after treatment with different fibers in the rat [1,11], dog [3,15] and pig [27]. A few studies reported an inhibitory role of fiber diet on pancreatic secretion in humans [6,10]. However, experiments in the rat [13,14], and pig [28] showed no effect on enzymatic secretion. In this study, we have shown that dietary cellulose does not modify the concentration of intracellular calcium in pancreatic acinar cells. There are no previous studies analyzing the role of a fiber diet on the level of this intracellular messenger, although $[Ca^{2+}]_i$ is one of the best known intracellular messengers playing a critical role in pancreatic stimulus-secretion coupling. Our results agree with the fact that a high cellulose diet does not affect the pancreatic release of amylase, neither in basal nor in stimulated conditions.

We analyzed the effects of chronic ethanol intake on exocrine pancreas. The biochemical parameters, such as liquid and protein content in the pancreas and plasma concentration of amylase remained unchanged (Table 1). Nevertheless, ethanol did significantly reduce the content of amylase in the pancreas, confirming previous results [29, 30,31]. This effect could be explained by an adaptation to a decrease in the carbohydrate intake in rats consuming chronic ethanol.

Ethanol intake significantly increased the basal amylase

release and significantly reduced the amylase secreted in response to CCK or Ach. Controversial results have been described concerning the effects of long term consumption of ethanol on the pancreas, but in general, an inhibitory effect of ethanol in pancreatic secretion is widely accepted [18]. One explanation can be the lower content of amylase in the pancreas, already described. The fact that basal amylase release is higher than in control rats, which is correlated with a higher basal $[Ca^{2+}]_i$, could indicate an increase of the plasma membrane permeability to calcium, likely due to membrane alterations induced by chronic ethanol consumption. The fact that ethanol affects biological membranes has been widely described [18]. Another potential explanation includes an increase of the sensitivity to endogenous neurotransmitters in rats chronically treated with ethanol [32]; or an increase in the activity of cholinergic neurons, localized in the intrapancreatic ganglia [33]. The relative relevance of this latest hypothesis in our study *in vitro* is questionable, as the preparation of pancreatic acini theoretically did not contain intrapancreatic ganglia.

Long term ethanol intake significantly increased the basal concentration of intracellular calcium only if no fiber is present, perhaps due to the damaging effect on the membranes affecting some of the mechanisms regulating calcium homeostasis in resting conditions [23,34]. This increase in the resting calcium concentration after chronic ethanol may account for the significantly elevated basal amylase release observed in this group (Table 2). It is well known that the pancreatic enzyme secretion is greatly dependent on intracellular calcium concentration in pancreatic acini [21]. Under our conditions, ethanol reduced the Ach-stimulated $[Ca^{2+}]_i$ increase but no the CCK-stimulated $[Ca^{2+}]_i$, increase. Thus, the lower response in amylase release after CCK stimulation was not due to an impairment of CCK-stimulated intracellular calcium rise. However, it may be an explanation for the lower secretory response after Ach stimulation. According to some studies in rats [32] and mice [33], there is an increase in the cholinergic tone in alcoholized animals that is responsible for desensitization in response to cholinergic agents. This desensitization could be produced at different steps in the stimulus-secretion coupling. One potential candidate is the IP_3 receptor of the intracellular Ca^{2+} stores, which is known to be down regulated in hepatocytes exposed to chronic ethanol administration [35]. In rat pancreatic acinar cells, Wojcikiewicz et al. [36] demonstrated that pancreatic secretagogues down regulate IP_3 receptors and other proteins, such as protein kinase C and phospholipase Cβ1, involved in intracellular signaling by a mechanism that partly involves the ubiquitin/proteasome pathway. Loss of these signaling proteins by an increase of cholinergic tone may account for the disruption of Ca^{2+} mobilization that occurs after chronic ethanol consumption. These results can explain, at least partly, the lower secretory response observed in rats from the NE group after Ach stimulation.

Because the fold increase in $[Ca^{2+}]_i$ after CCK is similar to that found in rats fed a normal diet (Fig. 3B), we can postulate that the lower secretion observed in response to CCK in acini from ethanol-treated rats could be due to alterations in the signaling transduction somewhere downstream of the intracellular Ca^{2+} release or parallel to it. One explanation may be the uncoupling of stimulus-secretion in the last step, the exocytosis. Chronic ethanol intake alters the coupling between cells into the acini, by markedly decreasing the number of tight junctions between acinar and centroacinar cells [37], increasing the space between cells and by atrophy of the intercellular processes that couple cells. Altogether, these effects due to ethanol can alter the secretory process.

Although rats fed a high cellulose diet and ethanol showed a lower content of amylase in the pancreas, surprisingly, their dose-response curve to CCK was similar to the control. These results allow the suggestion that a chronic cellulose intake protects the exocrine pancreatic function from deleterious effect of ethanol. Concerning the beneficial effect of the fiber, we have recently described a protective influence of a high cellulose diet on the ethanol-caused damage of the pancreas [20]. We demonstrated that diet cellulose partially avoids the damage of ethanol on the membrane of pancreatic lysosomes, thereby attenuating its adverse effects and reducing the deleterious action of cerulein-induced acute pancreatitis. The mechanism underlying this cellulose effect is not yet known but it might be related to some of the known influences of fiber nutrients on gastrointestinal physiology, which may obstruct the process of ethanol damage on exocrine pancreas, perhaps inhibiting the intestinal ethanol absorption. Though it is clear that more experimental work must be done to clarify these mechanisms, our results show an unknown effect of a high cellulose intake on exocrine pancreatic function, when this fiber is administered chronically in combination with ethanol, attenuating the ethanol damage to the pancreas.

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