Impaired stimulation of intestinal glucose absorption by portal insulin via hepatoenteral nerves in chronically ethanol-intoxicated rats

Tomas Kucera, Kurt Jungermann, Frank Stümpel*

Institute of Biochemistry and Molecular Cell Biology, Georg-August-University, Humboldtallee 23, 37073 Göttingen, Germany

Received 10 April 2000

Edited by Ned Mantei

Abstract In the isolated, jointly perfused small intestine and liver of rats insulin, infused into the portal vein, induced an increase in intestinal glucose absorption via hepatoenteral cholinergic nerves. The possible loss of function of these nerves due to ethanol-induced neuropathy was investigated with 6 weeks ethanol-fed rats. Portal insulin or arterial carbachol failed to increase intestinal glucose absorption but cAMP still did so. The intact stimulatory effect of cAMP indicated an undisturbed capacity of the enterocytes. The loss of action of portal insulin and of arterial carbachol can be explained by the impairment of the hepatoenteral nerves in line with an ethanol-induced neuropathy.

Key words: Glucose absorption; Ethanol-induced neuropathy; Cholinergic nerve; Autonomic nervous system

1. Introduction

The mean daily intake of carbohydrates in the western world amounts to about 300 g. It corresponds to 50% of the supply of calories [1]. After the digestion of dietary carbohydrates, glucose is taken up into the enterocyte from the intestinal lumen via the sodium-dependent glucose cotransporter 1 (SGLT1) located in the apical membrane and then released from the enterocyte into the circulation via the sodium-independent glucose transporter 2 (GLUT2) expressed in the basolateral membrane [2,3]. Recently, an acute modulation of intestinal glucose absorption involving a hepatoenteral nervous signaling chain could be demonstrated. In the isolated perfused small intestine and liver of the rat insulin infused into the portal vein acutely increased intestinal glucose absorption via the SGLT1 in a dose-dependent manner [4]. This stimulatory effect of portal insulin could be inhibited by atropine [4] or tetrodotoxin [5] and mimicked by carbachol [4], all infused into the superior mesenteric artery [4,5]. These findings constituted evidence for a signaling chain beginning with the sensing of insulin in the hepatoportal area, continuing with retrograde transmission via hepatoenteral (from liver to intestine), cholinergic nerves and ending at the absorptive cells of the intestine.

Chronic alcohol abuse, a world-wide disorder and a substantial public health problem, is the cause of a variety of neurologic manifestations [6]. Ethanol neurotoxicity impairs not only the function of the central but also of the peripheral nervous system [7,8]. An impaired function of autonomic nerves due to chronic alcohol abuse in humans has been observed with the sympathetic skin response [9] and the cardiovascular system [10]. Also in chronically ethanol-fed rats a diminished function of sympathetic hepatic nerves [11] and of the vagus nerve [12] has been reported. The underlying mechanism is still not fully understood. However, several studies showed alterations of the membrane lipid composition with a change in membrane fluidity following chronic ethanol consumption [13,14]. The clinical importance of an impaired function of the autonomic nervous system was demonstrated when chronic male alcoholics were followed up for 7 years; the percentage survival with no evidence of vagal neuropathy was 91%. However, with an abnormal function of the vagus nerve survival was reduced to 66%. Hence, ethanol-induced vagal neuropathy was associated with a significantly higher mortality than in the general population of male alcoholics [15].

Ethanol-induced hypoglycemia is a common finding in human and experimental animals [16]. The loss of function of the hepatoenteral nerves and therefore diminished absorption of carbohydrates might contribute to the ethanol-induced hypoglycemia. Therefore, it was the aim of the current study to examine a possible functional impairment of the hepatoenteral nerves, which mediate the insulin-dependent increase in intestinal glucose absorption, in line with an alcohol-induced neuropathy.

2. Materials and methods

2.1. Materials

All chemicals were of reagent grade and from commercial sources. Enzymes were purchased from Boehringer (Mannheim, Germany), insulin and carbachol from Sigma (München, Germany), bovine serum albumin, dextran and db-cAMP from AppliChem (Darmstadt, Germany).

2.2. Animals

Male Wistar rats were obtained from Harlan-Winkelmann (Borchen, Germany). Control rats were weight-matched to the ethanol-treated rats (about 390 g at the time of the perfusion experiments in both groups, Table 1); due to the expected decreased growth rate of ethanol-treated rats initial body weight was 264 ± 24 g in control and 383 ± 18 g in alcohol-fed animals, respectively (Table 1). They were kept on a 12-h day–night rhythm with free access to food and water (standard diet of Ssniff, Soest, Germany). Chronic alcohol feeding was performed for 6 weeks by adding ethanol to the drinking water with stepwise increases within 14 days prior to the 6 weeks to a final concentration of 20% (v/v) [17]. In addition, to overcome the natural aversion of rats to ethanol, the drinking water was sweetened by adding of 0.8 mol/l glucose. Weight of the animals was monitored daily; consumption of the alcohol and glucose containing drinking water and corresponding daily intake of ethanol and glucose as well as the daily intake of laboratory chow were recorded (Table 1). Dur-

*Corresponding author. Fax: (49)-551-395960.
E-mail: fstuemp@gwdg.de

PII: S0014-5793(00)01608-2

ing the preparation of the joint perfusion of small intestine and liver, blood was obtained for subsequent determination of the blood ethanol and glucose concentration. Treatment of animals followed the German Law on the Protection of Animals and was performed with permission of the state animal welfare committee.

2.3. Preparation of the isolated joint perfusion of small intestine and liver

The joint perfusion of small intestine and liver was prepared as described before [4,18]. Rats were anaesthetized by an intraperitoneal injection of pentobarbital (40 g/l in 0.9% NaCl; 60 mg/kg body weight). The abdomen was opened by a midline incision, the superior mesenteric artery (SMA) and coeliac trunc (CT) were cannulated and the inferior vena cava (IVC) opened as outflow. Thus, a non-recirculating perfusion of intestine and liver was started with a hydrostatic pressure of 120 cm H_2O (i.e. 88 mm Hg≅11.77 kPa). Then spleen, pancreas and proximal half of the stomach were removed after ligating their arteries and veins. For the luminal application of glucose, a catheter was positioned in the proximal duodenum. The cecum was cut open and the small intestine washed out with a warm saline solution. A luminal outflow was established by placing a catheter through the cecum into the distal ileum. Afterwards, a cannula for the vascular outflow was introduced into the right atrium, the tip positioned at the inflow of the hepatic vein into the IVC and fixed. Then, intestine and liver were transferred into an organ bath filled with a warmed saline solution and two flexible catheters were introduced into the portal vein (PV), one for obtaining medium samples and the other one for infusion of insulin.

2.4. Determination of vascular flow

The flow rate in the SMA was measured with an ultrasound flow meter T106 (Transonic Systems Inc., Ithaca, NY, USA). Total flow in the IVC was quantified by fractionating the perfusion effluate into calibrated tubes. The flow rate in the CT was calculated as the difference between the flow in the IVC and SMA.

2.5. Perfusion medium, vascular application of effectors and intestinal bolus

The perfusion medium consisted of a Krebs–Henseleit buffer containing 5 mmol/l glucose, 2 mmol/l lactate, 0.2 mmol/l pyruvate, 1 mmol/l glutamine, 1% (w/v) bovine serum albumin and 3% (w/v) dextran. The perfusion medium was equilibrated with a gas mixture of O_2:CO_2 (19:1). Insulin (final concentration 100 nmol/l), carbachol (10 μmol/l) and dibutyryl cAMP (1 μmol/l) were infused as solutions in perfusion medium into the vessels as described in the figure legends. The luminal glucose bolus of 1 g diluted in 1.5 ml 0.9% NaCl was applied within 1 min via the luminal catheter placed into the duodenum.

2.6. Determination of glucose and blood ethanol concentrations

Perfusion samples were taken every minute and immediately chilled on ice. Glucose concentration was measured with standard enzymatic techniques with glucose dehydrogenase (Merck system) [19]. Before CT and SMA were cannulated, blood was drawn from the IVC for subsequent determination of blood alcohol. Ethanol concentration was measured with the indaniline method with horse liver alcohol dehydrogenase [20].

2.7. Statistical analysis

All results are presented as means ± S.E.M. for the indicated number of observations. Data were analyzed by Student's *t*-test for unpaired data. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Characterization of control and chronically ethanol-treated rats

All rats were fed a standard diet. Control rats had free access to normal drinking water; ethanol-treated rats received water with alcohol (20%) and glucose (0.8 mmol/l) added. Following adaptation, ethanol treatment was continued for 6 weeks. Average daily intake during this period was about 9 g ethanol/kg body weight resulting in a blood ethanol concentration of about 18 mmol/l (Table 1). Daily ethanol intake was comparable with previous results obtained with rats fed the ethanol containing Lieber–DeCarli liquid diet; however, blood ethanol concentrations reached were somewhat lower [11,21]. Under this alcohol challenge the growth rate of animals was substantially retarded; this is in agreement with previous data showing a reduced growth rate in animals treated with ethanol in the drinking water [22–24].

3.2. Loss of increase in intestinal glucose absorption by portal insulin in chronically ethanol-intoxicated rats

In the isolated, jointly perfused intestine and liver of rats the rate of basal glucose absorption following the first luminal glucose bolus (5.5 mmol = 1 g) reached a maximum of 2.6 ± 0.9 μmol min^{-1} g^{-1} organ weight (Fig. 1A). Total glucose absorption, corresponding to the area under the rate of glucose absorption vs. time curve (AUC)×organ weight amounted to 251 ± 28 μmol (Fig. 2). The basal rate of glucose absorption with ethanol-treated rats reached a maximum of 2.5 ± 1.3 μmol min^{-1} g^{-1} organ weight (Fig. 1B) corresponding to a total glucose absorption (AUC) of 262 ± 42 μmol (Fig. 2). The difference in basal glucose absorption between control and alcohol-fed rats was statistically not significant.

In the intestine–liver perfusion of control rats portal insulin (100 nmol/l), infused into the portal vein, enhanced the rate of intestinal glucose absorption to a maximum of 14.2 ± 6.7 μmol min^{-1} g^{-1} organ weight (Fig. 1A); total glucose absorption (AUC) was raised from 251 ± 28 μmol to 856 ± 117 μmol (Fig.

Table 1
Characterization of control and chronically (6 weeks) ethanol-fed rats

	Control standard diet[a]	Ethanol water 20% (v/v)+standard diet[a]
Energy intake (kJ day^{-1})	190	240*
Body weight at time of starting ethanol diet (g)	264.3 ± 24.6	342.7 ± 12.3*
Body weight at time of experiment (g)	392.3 ± 31.2	383.4 ± 18.3[n.s.]
Growth (g day^{-1})	+3.2 ± 0.3	+0.9 ± 0.1*
Standard diet intake (g kg^{-1} day^{-1})	62.4 ± 5.8	43.0 ± 1.9*
Ethanol intake with water (g kg^{-1} day^{-1})	–	8.6 ± 0.5
Glucose intake with water (g kg^{-1} day^{-1})	–	7.8 ± 0.3
Glucose intake with diet (g kg^{-1} day^{-1})	40.6 ± 3.2	28.4 ± 2.5
Blood ethanol concentration (mmol l^{-1})	n.d.[b]	19.4 ± 1.7
Blood glucose concentration (mmol l^{-1})	7.8 ± 0.1	7.1 ± 0.5*
Intestinal weight (g)	7.1 ± 0.2	6.8 ± 0.2[n.s.]
Liver weight (g)	12.2 ± 0.5	11.8 ± 0.4[n.s.]

*$P < 0.05$; n.s., not significant.
[a]Ad libitum, 12.7 kJ/g.
[b]Not detectable.

2). After portal infusion of insulin into the liver of ethanol-treated rats, the rate of intestinal glucose absorption was raised only to 5.4 ± 4.2 μmol min^{-1} g^{-1} organ weight (Fig. 1B), the calculated total glucose absorption (AUC) was elevated slightly from 262 ± 42 μmol to 333 ± 73 μmol (Fig. 2). Thus, portal insulin failed to significantly enhance glucose absorption in the perfused intestine and liver from chronically ethanol-fed rats. The flow rates in the SMA and CT remained essentially constant during the entire experiments; they decreased only insignificantly towards the end of the perfusion by 0.23 ml min^{-1} g^{-1} (6.2%) in livers from control rats and by 0.41 ml min^{-1} g^{-1} (11.4%) in livers from ethanol-fed rats, respectively (Fig. 1, lower panels) which cannot have affected absorption.

3.3. Loss of increase in intestinal glucose absorption by arterial carbachol but not by arterial db-cAMP in chronically ethanol-intoxicated rats

The stimulatory effect of portal insulin on intestinal glucose absorption is transmitted from the liver to the enterocytes via cholinergic nerves [4]. The loss of increase in intestinal glucose absorption by portal insulin following chronic ethanol intoxication could be due to an impairment of the hepatoenteral nerves (including insulin sensing, signal propagating and effectory nerve cells) and/or of the absorptive enterocytes. The nerve action can be mimicked by carbachol [4] and the enterocytic glucose absorption can be stimulated by db-cAMP [25–29]. Therefore, in another series of experiments the influence of chronic ethanol intoxication on the stimulation of glucose

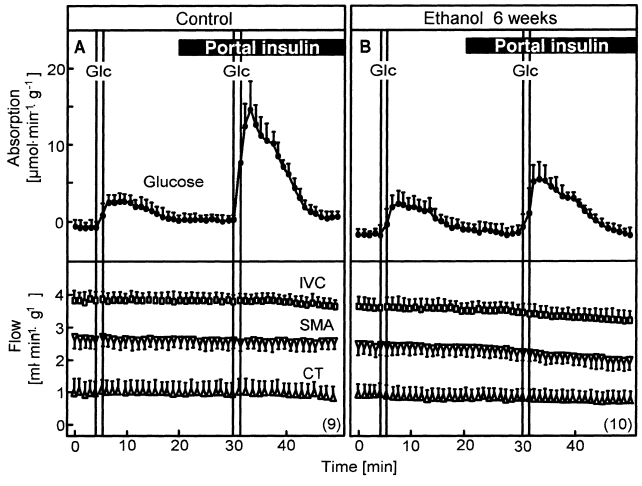

Fig. 1. Impairment of the increase in intestinal glucose absorption by portal insulin in the isolated, jointly perfused small intestine and liver of rats following chronic ethanol intoxication. Rats were subjected to chronic alcohol feeding by addition of ethanol to the drinking water (final concentration 20% (v/v)) in combination with the standard diet. Small intestine and liver were jointly perfused as described previously [4,18] with a Krebs–Henseleit bicarbonate buffer containing 5 mmol/l glucose, 2 mmol/l lactate, 0.2 mmol/l pyruvate, 1 mmol/l glutamine, 1% bovine serum albumin and 3% dextran. A glucose bolus (1 g diluted in 1.5 ml 0.9% NaCl) was applied into the intestinal lumen in the 6th and 31st min. Insulin (100 nmol/l) was infused into the portal vein from the 21st min onwards. Flow in the superior mesenteric artery (SMA) was measured by a flow meter and in the inferior vena cava (IVC) by fractionated sampling. Flow in the celiac trunc (CT) was calculated from the difference between flow in the IVC and SMA. Values are means ± S.E.M. of the number of experiments given in parentheses.

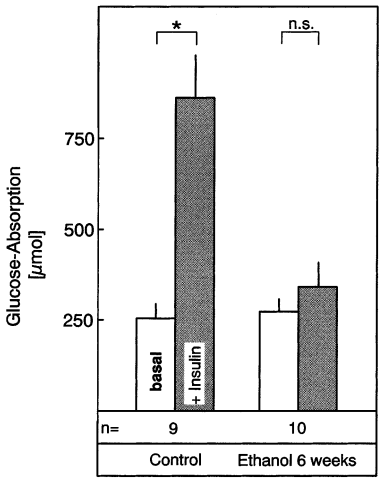

Fig. 2. Loss of increase in intestinal glucose absorption by portal insulin in the isolated, jointly perfused intestine and liver of chronically ethanol-fed rats. Experiments were performed as described in the legend to Fig. 1. Total absorption of glucose (μmol) was determined as the area under the absorption versus time curve (μmol min^{-1} g^{-1} × min) multiplied by organ weight (g). Columns show basal glucose absorption following the first luminal glucose bolus (6th min, 1 g diluted in 1.5 ml 0.9% NaCl) and insulin-stimulated glucose absorption following a second glucose bolus (26th min) with infusion of insulin (100 nmol/l) into the portal vein. Values are means ± S.E.M. of 4–5 experiments. $*P < 0.05$; n.s. = not significant.

absorption by carbachol and by the membrane permeable cAMP analogue db-cAMP was investigated.

In the isolated perfused intestine and liver of control rats and ethanol-fed rats of the second series basal glucose absorption amounted to 271 ± 28 μmol and 289 ± 62 μmol. Infusion of carbachol into the SMA raised total glucose absorption (AUC) to 796 ± 63 μmol with control animals (Fig. 3). With 6 weeks alcohol-fed animals, carbachol completely failed to enhance glucose absorption (Fig. 3). In the same perfused organ system the infusion of db-cAMP into the SMA caused a significant increase in total glucose absorption curve (AUC) from 271 ± 28 μmol to 890 ± 31 μmol with control animals (Fig. 3). With rats fed alcohol for 6 weeks db-cAMP still enhanced total glucose absorption (AUC) from 289 ± 62 μmol to 750 ± 89 μmol (Fig. 3). Thus, chronic ethanol intoxication entirely abrogated the carbachol-induced but not the cAMP-dependent increase in intestinal glucose absorption, i.e. it substantially impaired the signaling chain 'portal insulin–hepatoenteral nerves–enterocytes–glucose absorption' but not the absorptive process in the enterocytes.

4. Discussion

In the present investigation it was demonstrated that in the joint perfusion of small intestine and liver of rats following 6 weeks of alcohol feeding the stimulatory action of portal insulin on intestinal glucose absorption was completely abolished and that this abolition was due to an impairment of the hepatoenteral signal chain involving cholinergic nerves and not of the absorptive enterocytes.

4.1. Unaltered capacity for intestinal glucose absorption following chronic ethanol intoxication

Chronic ethanol feeding might have impaired the function

of the intestinal glucose transporters involved, the apical SGLT1 and/or the basolateral GLUT2. However, in the isolated, jointly perfused small intestine and liver basal glucose absorption did not differ significantly in preparations of control and chronically ethanol-fed rats (Figs. 1–3). These findings indicated an unaltered capacity of both glucose transporters, the SGLT1 and the GLUT2. Previous studies on the chronic effects of ethanol on glucose absorption yielded contradictory results: Following long-term ethanol treatment glucose absorption in intact segments of the rat small intestine was found to be increased [30], unaltered [31] or decreased [32]. In brush-border membrane vesicles prepared from chronically ethanol-fed rats glucose uptake via the SGLT1 was enhanced [31]; however, in the same preparation from guinea pigs the driving force of the SGLT1, the sodium gradient, was decreased [33].

It was shown previously with different experimental systems that cAMP acutely stimulated glucose absorption via the SGLT1 [25–29]. In the present study with the isolated, jointly perfused small intestine and liver db-cAMP acutely enhanced glucose absorption in preparations of control and chronically ethanol-fed rats (Fig. 3). This also indicated that the absorptive capacity via the SGLT1 and GLUT2 of the enterocytes was unaltered in the chronically ethanol-intoxicated rats.

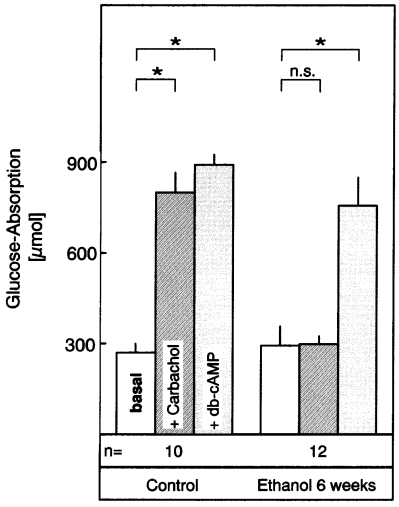

Fig. 3. Loss of increase in intestinal glucose absorption by arterial carbachol, but not by arterial db-cAMP in the isolated, jointly perfused small intestine and liver of chronically ethanol-fed rats. Experiments were performed as described in the legend to Fig. 1. Total glucose absorption (µmol), which is the area under curve (AUC) of the rate of glucose absorption vs. time curve multiplied by the organ weight (Fig. 2), is depicted. Columns show the basal glucose absorption following the first luminal glucose bolus (6th min, 1 g diluted in 1.5 ml 0.9% NaCl), the other columns the stimulated glucose absorption following a second glucose bolus (26th min) with infusion of carbachol (10 µmol/l) or db-cAMP (10 µmol/l), both into the arteries. Values are means ± S.E.M. of 4–5 experiments. $*P < 0.05$; n.s. = not significant.

4.2. *Impaired function of the signaling chain involving hepatoenteral nerves in chronically ethanol-intoxicated rats*

The anatomical basis of the hepatoenteral nervous pathway mediating the increase in intestinal glucose absorption by portal insulin is unknown so far. It must start with the sensing of insulin in the hepatoportal area. Electrophysiological studies demonstrated a sensing in the portal vein of the concentrations of glucose [34], glucagon and insulin [35]. Therefore, the nerves between liver and small intestine could originate in the hepatoportal area and end in the small intestine, where they would release acetylcholine to a cell expressing muscarinic receptors. Since cAMP is the intracellular messenger in the enterocytes which stimulates glucose absorption [25–29], and muscarinic receptors are known to decrease cAMP or to increase IP_3 but not to elevate cAMP [36], acetylcholine cannot act directly on the enterocytes but via an additional intervening cell. Since in the chronically ethanol-intoxicated rats arterial cAMP was still able to increase intestinal glucose absorption (Fig. 3), the absorptive function of the enterocytes was not impaired. Thus, the loss of the stimulatory effect of portal insulin and arterial carbachol was due to a functional impairment in the signal chain from the liver to the small intestine. This signal chain is composed of several steps. Of the different single steps, sensing the insulin concentration in the hepatoportal area by a sensory cell, transmitting the signal to the small intestine via cholinergic nerves, releasing acetylcholine to a muscarinic receptor or an intervening cell and forwarding the signal by the intervening cell, one alone or a combination of two or all can be compromised in function. At least the last step was impaired, but the first and second might also have been affected. Thus ultimately ethanol intoxication resulted in a failure of portal insulin to stimulate glucose absorption via hepatoenteral nerves. This impairment corresponds to a neuropathy, which is a well known complication of chronic alcohol abuse in humans [7,8]. A loss of function of the hepatoenteral nerves would be in line with a general impairment of autonomic nerves as found with the thermoregulatory response [37], the cardiovascular system [10], the sympathetic skin response [9] and effectory hepatic nerves [11]. An impaired function due to a diabetic neuropathy of these hepatoenteral nervous signal chain has also been observed in streptozotocin-diabetic rats [5].

4.3. *Possible pathophysiological role of the impairment of the signaling chain via hepatoenteral nerves in chronic ethanol intoxication*

Ethanol-induced hypoglycemia is a common finding in humans and in animals under experimental conditions [38–40]. In the postabsorptive state the organism maintains euglycemia by hepatic gluconeogenesis and glycogenolysis. Hypoglycemia with chronic ethanol abuse is mainly due to the inhibition by ethanol of gluconeogenesis [41–43] and of the sympathetic nerve-dependent glycogenolysis in the liver [11]. In the absorptive phase the organism normally has to handle more glucose than it actually needs for the supply of glucose-dependent cells; part of the glucose offered in excess is used to replenish the hepatic glycogen reserves [44] and thus prevents a postprandial hyperglycemia exceeding the reabsorptive capacity of the kidney. Since the ethanol-induced impairment of the hepatoenteral signal chain would result in a delayed and even reduced intestinal absorption of ingested glucose, it would help to prevent a postprandial hyperglycemia. This could contribute to ethanol-induced hypoglycemia only if in the meals of the alcoholics ethanol replaces glucose to a high extend, so that glucose intake is smaller than the actual demands. With heavy drinkers this may often be the case.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft through the Sonderforschungsbereich 402: 'Mo-

lekulare und Zelluläre Hepatogastro-enterologie', Teilprojekt B3. We wish to thank Angela Hunger, Birgit Döring and Frank Rhode for their excellent technical assistance.

References

[1] Elsenhans, B. and Caspary, W.F. (1987) in: Structure and Function of the Small Intestine (Caspary, W.F., Ed.), Curr. Clin. Pract. Ser. Vol. 46, pp. 139–159, Excerpta Medica, Amsterdam.
[2] Hediger, M.A., Turk, E. and Wright, E.M. (1989) Proc. Natl. Acad. Sci. USA 86, 5748–5752.
[3] Philpott, D.J., Butzner, J.D. and Meddings, J.B. (1992) Can. J. Physiol. Pharmacol. 70, 1201–1207.
[4] Stümpel, F., Kucera, T., Gardemann, A. and Jungermann, K. (1996) Gastroenterology 110, 1863–1869.
[5] Stümpel, F., Kucera, T. and Jungermann, K. (1999) Am. J. Physiol. 277, G285–G291.
[6] Cloninger, C.R., Dinwiddie, S.H. and Reich, T. (1989) Annu. Rev. Psychiatr. 8, 331–346.
[7] Diamond, I. and Messig, R.O. (1994) West. J. Med. 161, 279–287.
[8] Villalta, J., Estruch, R., Antunez, E., Valls, J. and Urbano-Marquez, A. (1989) Alcohol. Alcoholism 24, 421–428.
[9] Novak, D.J. and Victor, M. (1974) Arch. Neurol. 30, 273–284.
[10] Low, P.A., Walsh, J.C., Huang, C.Y. and McLeod, J.G. (1975) Brain 98, 357–364.
[11] Kucera, T., Stümpel, F. and Jungermann, K. (1997) J. Hepatol. 26, 183–190.
[12] Guo, Y.P., McLeod, J.G. and Baverstock, J. (1987) J. Neurol. Neurosurg. Psychiatr. 50, 1449–1453.
[13] Chin, J.H. and Goldstein, D.B. (1981) Mol. Pharmacol. 19, 425–431.
[14] Kukielka, E., Dicker, E. and Cederbaum, A.I. (1994) Arch. Biochem. Biophys. 309, 377–386.
[15] Johnson, R.H. and Robinson, B.J. (1988) J. Neurol. Neurosurg. Psychiatr. 51, 476–480.
[16] Arky, R.A. (1979) in: Endocrinology (DeGroot, L.J., Cahill, G.F., Odell, W.D., Martini, L., Potts, J.T., Nelson, D.H., Steinberger, E. and Winegrad, A.I., Eds.), pp. 1099–1123, Grune and Stratton, New York.
[17] Keegan, A., Martini, R. and Batey, R. (1995) J. Hepatol. 23, 591–600.
[18] Gardemann, A., Watanabe, Y., Große, V., Hesse, S. and Jungermann, K. (1992) Biochem. J. 283, 759–765.
[19] Banauch, D., Brümmer, W., Ebeling, W., Metz, H., Rindfrey, H., Lang, H., Leybold, K., Rick, W. and Staudinger, H.J. (1975) Z. Klin. Chem. Klin. Biochem. 13, 101–107.
[20] Kovar, J., Schneider, J., Skursky, L. and Dubsky, H. (1984) Anal. Biochem. 137, 74–79.
[21] Lieber, C.S. and deCarli, L.M. (1982) Alcoholism 6, 523–531.
[22] Sankaran, H., Deveney, C.W. and Larkin, E.C. (1983) Alcohol. Clin. Exp. Res. 7, 369–371.
[23] Fields, M., Lure, M.D. and Lewis, C.G. (1995) Alcohol 12, 65–70.
[24] Furuya, H., Aikawa, H., Yoshida, T. and Okazaki, I. (1996) Alcohol. Clin. Exp. Res. 20, 305A–310A.
[25] Sharp, P.H. and Debnam, E.S. (1994) Exp. Physiol. 79, 203–214.
[26] Stümpel, F., Scholtka, B. and Jungermann, K. (1997) FEBS Lett. 410, 515–519.
[27] Stümpel, F., Scholtka, B., Hunger, A. and Jungermann, K. (1998) Gastroenterology 115, 1163–1171.
[28] Scholtka, B., Stümpel, F. and Jungermann, K. (1999) Gut 44, 490–496.
[29] Grubb, B.R. (1995) Am. J. Physiol. 268, G505–G513.
[30] Thomson, A.B.R. (1984) Am. J. Physiol. 246, G120–G129.
[31] al-Balool, F. and Debnam, E.S. (1989) Q. J. Exp. Physiol. 74, 751–753.
[32] Kaur, J., Jaswal, V.M., Nagpaul, J.P. and Mahmood, A. (1993) Alcohol 10, 299–302.
[33] O'Neill, B., Weber, F., Hornig, D. and Semenza, G. (1988) FEBS Lett. 194, 183–188.
[34] Niijima, A. (1982) J. Physiol. 332, 315–323.
[35] Niijima, A. (1988) Progr. Brain Res. 74, 155–160.
[36] Caulfield, M.P. (1993) Pharmac. Ther. 58, 319–379.
[37] Robinson, B.J., Johnson, R.H., Lambie, D.G. and Palmer, K.T. (1985) Austr. Alcohol Drug Rev. 4, 157–161.
[38] Forsander, O.A., Räihä, N., Salaspuro, M. and Mäenpää, P. (1965) Biochem. J. 94, 259–265.
[39] Wilson, N.M., Brown, P.M., Juul, S.M., Prestwich, S.A. and Sonksen, P.H. (1981) Br. Med. J. Clin. Res. 282, 849–853.
[40] Pandit, M.K., Burke, J., Gustafson, A.B., Minocha, A. and Peiris, A.N. (1993) Ann. Intern. Med. 118, 529–539.
[41] Krebs, H.A., Freedland, R.A., Hems, R. and Stubbs, M.F. (1969) Biochem. J. 112, 117–124.
[42] Deaciuc, I.V., D'Souza, N.B. and Lang, C.H. (1992) Biochem. Pharmacol. 44, 1617–1624.
[43] Berry, M.N., Gregory, R.B., Grivell, A.R., Phillips, J.W. and Schon, A. (1994) Eur. J. Biochem. 225, 557–564.
[44] Jungermann, K. and Barth, C. (1996) in: Human Physiology (Greger, R. and Windhorst, V., Eds.), pp. 1425–1457, Springer, Berlin.