Results. Neither single SNP-, nor haplotype analysis could detect significant associations with alcohol dependence. Additionally, we could not detect any relation to Cloninger’s Type 1/2 or Babor’s Type A/B classification, to withdrawal symptoms, to the age of onset or to the amount of alcohol intake.

Conclusions. In conclusion, our results suggest that the analyzed SNPs, as well as the corresponding haplotypes of the SNP gene are unlikely to play a major role in the pathophysiology of alcohol dependence. Further analyses are needed to confirm the present results.

PA3-8
CHANGES IN THE NEUROPEPTIDE Y SYSTEM DURING ETHANOL INTOXICATION AND WITHDRAWAL IN RATS
Olling JD, Ulrichsen J, Woldbye DP (Denmark)

Aims. Ethanol withdrawal is associated with neuronal hyperexcitability and increased hippocampal glutamate release. Neuropeptide Y (NPY) appears to play an important role in the regulation of neuronal excitability in the hippocampal formation by reducing release of glutamate. Central administration of NPY reduces symptoms of ethanol withdrawal in rats. To further understand the role of NPY in ethanol withdrawal, the present study examined the effects of ethanol intoxication and withdrawal on the expression of NPY in the hippocampus.

Methods. Male Wistar rats were exposed to chronic ethanol intoxication for four days and decapitated at 0, 16, 76, or 168 hours after the last ethanol dose. Ethanol was administered intragastrically five times daily. Rats receiving isocaloric sucrose served as controls. NPY mRNA were examined using in situ hybridisation, NPY receptor binding were studied using autoradiography, and finally NPY-stimulated [35S]GTPγS functional binding were also studied.

Results. Decreased NPY gene expression was found in hippocampal areas in the ethanol-treated group compared to control rats at 16 hours (peak withdrawal) after the last ethanol dose. In contrast, at 76 hours (late withdrawal) a prominent increase in NPY gene expression was observed. Surprisingly, decreased NPY gene expression was accompanied by increased total and functional NPY receptor binding.

Conclusions. These data show that the NPY system undergoes substantial plastic changes during ethanol treatment and withdrawal, and these neuronal adaptations could play an important role in the development of cerebral hyperexcitability during withdrawal.

PA3-9
EFFECT OF STRESS ON ETHANOL DRINKING BEHAVIOR IN PER1 MUTANT MICE
Bilbao-Leis A, Leonard-Essmann F, Rodríguez de Fonseca F, Spanagel R (Germany)

Aims. Neurotransmission mediated via opioid receptors is believed to be involved in the reinforcing and/or rewarding effects of ethanol consumption as well as in the response to stress. Our preliminary data showed decreased levels of proenkephalin mRNA expression in the amygdala of mice lacking the clock period gene per1 (per1 brdm1). Therefore our aim was to study the effect of stress on free-choice ethanol consumption in per1 brdm1 mice.

Methods. per1 brdm1 and wild type male mice were given continual free-choice access to a 12% ethanol solution or water (8 weeks). At the end of this period, mice received 3 consecutive days of different stressors and the ethanol intake was tested.

Results. per1 brdm1 mice lacked any light-dark cyclic pattern of ethanol intake, clearly indicating the role of per1 in diurnal intake of ethanol. When stress was given to wild type mice, an increase in ethanol intake was observed, an effect which was potentiated in per1 brdm1 mice, suggesting a role of per1 in stress-induced ethanol consumption. This potentiation in stress-induced ethanol intake showed by per1 brdm1 mice may reflect a compensatory response to a highly increased threshold in the anxiolytic and/or rewarding effects of ethanol under stress.

Conclusions. The data support the putative role of per1 in the effect of stress in ethanol intake and suggest that per 1-induced changes in proenkephalin within the amygdala may be a locus associated to this effect.

PA3-10
HAPLOTYPES ANALYSES OF THE GHR ELIN AND GHSR-1A GENES IN ALCOHOL DEPENDENCE

Background/Aims. There is a genetic influence on the development of dependence, but the genes involved still needs to be elucidated. Ghrelin is a novel orexigenic peptide suggested to be involved in the reward systems (see Jerlhag et al. 2006). Ghrelin also stimulates appetite, increases food intake and causes adiposity by mechanisms that include direct actions on the brain. Ghrelin, like ethanol, increases locomotor activity and accumbal dopamine overflow, implicating that ghrelin is a part of the neurochemical overlap between the reward systems and those that regulate energy balance. Furthermore, ghrelin levels are higher in alcoholics and abstainers as well as in smokers compared to healthy controls. SNP rs572169 in the ghrelin receptor (GHSR) has been associated with obesity and SNPs Leu72Met and Arg51Gln in the pro-ghrelin gene have been associated with bulimia, diabetes type 2, obesity and methamphetamine withdrawal while others could not repeat these findings. The overlap between drug addiction and eating disorders prompted as to investigate these genes in these haplotype analyses in alcohol dependent individuals.

Methods. 78 well characterized individuals (51 patients recruited from a Swedish Minnesota treatment unit) and 415 Spanish individuals (138 patients admitted to the hospital) were investigated in haplotype analyses of the ghrelin and ghrelin receptor genes. Six tag SNPs in the proghrelin gene (rs696217, rs3491141, rs4684677, rs35680, rs24351 and rs26802) and four tag SNPs in the GHSR gene (rs495225, rs2232165, rs572169 and rs2948694) were chosen using HapMap data and the Tagger and Haploview softwares. These SNPs were then genotyped using TaqMan Allelic Discrimination. Individuals with alcohol-dependence are separated into two groups, (1) individuals with alcohol-dependence without concomitant tobacco-use and (2) individuals with alcohol-dependence and concomitant tobacco-use. Coding parts of these two genes have also been sequenced in 32 individuals, confirming known SNPs.

Results. In preliminary statistical analyses of the genotyping/haplotyping, we have found an association between SNP rs495225 (p = 0.0298), as well as with one haplotype (GAGCCC) (p = 0.0396) of the GHSR gene and alcohol dependence in the Swedish population and association with SNP rs2232165 (p = 0.036) in the Spanish population. We will continue the statistical analyses of the quantitative traits and the haplotypes using more complex softwares, also performing a proper permutation test.

Conclusions. Even though the functional significance of these SNPs remains to be clarified the present findings are the first to disclose an association between the GHSR gene and alcohol dependence.
Results. Arginase activity (p < 0.01) and plasma levels of urea (p < 0.05) were elevated in-group of animals treated with ethanol compared to controls. L-arginine supplementation increases ammonia detoxification in alcoholic rats by increase of arginase activity and urea synthesis (p < 0.05).

Conclusions. L-arginine increases arginase activity and urea synthesis in alcohol-treated rats. Supplementation with arginine may be beneficial in ethanol-induced hyperammonemia.

PA4-2

EFFECT OF THE PERFLUOROCARBONIC COMPOUNDS (PERTOFRAN) ON THE ANTIOXIDANT STATUS IN THE LIVER AND NITRIC OXIDE LEVEL OF RATS UNDER ETHANOL INTOXICATION

Miskевич D, Petushok N, Borodinsky A, Gerazimchyk P (Belarus)

Aims. Perftoran(PF) (perfluorocarbon compound) known as ‘blue blood’ exhibited immunomodulating, antioxidant, membrane-stabilizing and disin-toxicking properties. We evaluated whether PF can be used for the correction of metabolic changes in the liver caused by chronic ethanol administration.

Methods. Experiments were performed on male Wistar rats (180 g). Animals fed ethanol in drug dose (3.5 g/kg, intragastrically, 25% solution) twofold per day during 42 days. I group was sacrificed after 1 day, II–III groups were sacrificed after 7 days following the last alcohol injection. During the last 7 days of experiment, animals of II group were two fold PF injected (1 ml/100 gr. i.v.). III group were two fold NaCl 0.9% solution (injected 1 ml/100 gr. i.v.). In the liver homogenates the activities of superoxide dismutase, catalase (CAT), glutathione peroxidase, alaminase transferase, level of glutathione, thiorbarbituric acid-reactive substances (TBARS) and nitrite (NOx) were measured. The activity of γ-gluamyrampeptidase (GGTP), level of nitrite (NOx) in plasma was determined using Greiss reagent assay.

Results. The animals of I and III group have strongly pronounced oxidative stress (free radical processes activated, increased level of TBARS, greatly microvesicular whereas lymphocytic infiltration and necrotic area compared to the control group. Fatty dystrophy in rats treated with diglycine and triglyceride contents. Liver triglycerides were elevated about 3-fold compared to the control group. Feeding of the diet significantly activated serum AlAT and alkaline phosphatase, serum TNF content and significantly decreased liver triglycerides content.

Conclusions. These results demonstrated a clear hepatoprotective activity of diglycine in rat alcoholic liver damage. The present investigation is a pilot study of diglycine as a promising hepatoprotector envisaging further development.

PA4-4

CYTOKINE AND CHEMOKINE EXPRESSION DURING THE DEVELOPMENT OF STEATOHEPATITIS IN RATS FED A LOW-CARBOHYDRATE DIET CONTAINING ETHANOL


Aims. To determine the temporal relationship of cytokine and chemokine expression to the development of alcohol-induced liver pathology. Male Sprague-Dawley rats were fed low carbohydrate ethanol diets via total enteral nutrition for up to 49 days. Rats were sacrificed at regular intervals in relation to urine ethanol concentrations. Steatosis preceded significant oxidative stress, inflammation and cell death. Reduced expression of IL-4 and IL-1β mRNAs occurred at all times following the start of ethanol treatment.

Two peaks of TNFα mRNAs were observed, at 14 days and at 35–49 days, the latter occurring when hepatocyte proliferation was stimulated. Expression of chemokines CINC-1 and CXCL-2 increased after 28 days and correlated with inflammation. Steatosis and changes in cytokine profile were acute responses to ethanol treatment; whereas the majority of oxidative stress, necrosis, apoptosis, CINC-1 and CXCL-2 mRNA expression and a late peak of TNFα expression occurred after 4–5 weeks of ethanol. The late effects coincided with appearance of inflammatory infiltrates and were associated with stimulation of hepatocyte proliferation. It has been suggested that TNFα is derived from Kupffer cells, and produced in response to endotoxin which enters the circulation as the result of ethanol-induced gut permeabilization.

However, in this model, we previously reported little evidence of endotoxin-dependent Kupffer cell activation. Still, we see induction of TNFα and changes in production of other cytokines and chemokines.

Taken together these data demonstrate complex patterns of hepatic cytokine and chemokine synthesis in response to chronic ethanol exposure, and further clarify their relationship to development of liver pathology and proliferative repair responses.

PA4-5

ETHANOL ADMINISTRATION GENERATES OXIDATIVE STRESS AND INCREASES OXIDATION OF PROTEINS IN THE LIVER

Cvetkovic T (Serbia)

Aims. Acute and chronic ethanol treatment has been shown to increase the production of reactive oxygen species, lower cellular antioxidant levels, and enhance oxidative stress in many tissues. Ethanol-induced oxidative stress plays a major role in the mechanisms of ethanol toxicity. Mitochondria and microsomes have a main contribution in the process of free radical generation. We have studied the lipid peroxidation levels, as MDA, glutathione levels and catalase activity in liver tissues in the evaluation of oxidative stress in mechanisms of ethanol-induced liver injury. We have also studied the importance of protein carbonyl content and possible modulatory effect of L-arginine on investigated parameters.

Methods. Male Sprague Dawley rats were divided into 4 groups: 1-control rats, 2-treated with ethanol (as 15% solution in drinking water), 3-arginine treated rats (150 mg/kg daily), 4-ethanol + arginine. Animals were killed after three weeks. We have measured malondialdehyde (MDA) level, reactive carboxyl groups (RCG), glutathione (GSH) and catalase (CAT) activity in liver homogenates.

Results. Obtained results demonstrated that MDA was significantly increased in ethanol-treated group of animals, 4.54 ± 0.43nmol/mg proteins compared to control (3.18 ± 0.33, p < 0.001) and ethanol plus arginine group (3.04 ± 0.59). The protein carbonyl content in the liver was increased in the ethanol-treated rats (9.26 ± 1.51nmol/mg proteins) compared with the control (7.41 ± 0.85, p < 0.05). The levels of GSH as well as CAT activities were increased in the ethanol treated group (p < 0.001) compared to control. L-arginine supplementation with ethanol elevated glutathione level and catalase activity compared to ethanol group and to the group of animals treated with arginine alone (p < 0.001).

Conclusions. Chronic ethanol treatment leads to oxidative stress and to the damage of proteins. L-arginine supplementation to ethanol treated rats decreases the lipid peroxidation level, protein oxidation and increases content of glutathione and activity of catalase. The beneficial effects of arginine are probably the consequence of increased level of nitric oxide but the other effects of arginine cannot be excluded.
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PA4-6
PROFIBROGENIC TGF-β/ALK5 SIGNALING VIA CTGF EXPRESSION IN HEPATOCYTES

Aims. Connective tissue growth factor (CTGF) is important for TGF-β-induced liver fibrogenesis. Hepatic stellate cells (HSCs) were recognized as its major cellular source in liver. CTGF is increased in damaged liver, however, TGF-β treatment does not induce CTGF in HSCs. In the present study, we displayed TGF-β enhanced CTGF expression in hepatocytes.

Methods. We measured CTGF expression in liver tissue from TGF-β transgenic mice, CCl4 treated Smad7 transgenic and knock out mice and patients with chronic HBV by immunohistochemistry. In primary cultured hepatocytes, we used western blot to detect TGF-β-dependent CTGF expression and its dependent signaling pathway. Alternatively we measured CTGF expression in patients with chronic HBV infection before and after nine-months of IFN-γ treatment.

Results. We demonstrate induction of CTGF expression in hepatocytes of damaged liver and identify a molecular mechanism responsible. CTGF expression was found by immunohistochemistry in bile duct epithelial cells, HSCs and hepatocytes in fibrotic liver tissue from patients with chronic hepatitis B infection. Similarly, CTGF expression was also induced in hepatocytes of CCl4 treated FVB mice. CTGF expression and secretion was detected spontaneously in the medium of hepatocytes after 3 days of culture, which was enhanced by stimulation with TGF-β. TGF-β-induced CTGF expression was mediated through the ALK5/Smad3 pathway, while ALK1 activation antagonised this effect. CTGF expression in liver tissue of TGF-β transgenic mice correlated with serum TGF-β levels. Smad7 overexpression in cultured hepatocytes abrogated TGF-β-dependent and intrinsic CTGF expression, indicating that TGF-β signaling is required. In line with these data, hepatocyte specific transgenic Smad7 reduced CTGF expression in CCl4 treated animals, whereas in Smad7 knock out mice it was enhanced. Furthermore, IFN-γ treatment of patients with chronic HBV infection induced Smad7 expression in hepatocytes leading to decreased CTGF expression and fibrogenesis.

Conclusions. In conclusion, our data provide evidence for pro-fibrogenic activity of TGF-β directed to hepatocytes and mediated via upregulation of CTGF. We identify ALK5 dependent Smad3 signaling as a responsible pathway inducing CTGF expression, which can be interfered by an activated ALK1 pathway and completely inhibited by TGF-β antagonist Smad7.

PA4-7
EFFECT OF CHRONIC ETHANOL CONSUMPTION ON THE EXPRESSION OF COMPLEMENT COMPONENTS AND ACUTE PHASE PROTEINS IN LIVERS OF COMPLEMENT C3-DEFICIENT MICE
Bykov II, Jumiikkaa S, Pekna M, Lindros KO, Meri S. (Finland)

Aims. The complement system contributes to inflammation and steatosis in alcohol-induced liver injury. Recent evidence suggests that a deficient terminal complement pathway predisposes to tissue injury and promotes a pro-inflammatory cytokine response, whereas C3 deficiency can protect against alcohol and diet-induced fatty infiltration. Here we investigated the effect of chronic ethanol on liver gene expression of complement components and acute phase proteins.

Methods. Complement C3-deficient (C3−/−) and wild-type (C3+/+) mice were fed a high-fat/ethanol liquid diet for six weeks. The hepatic mRNA expression was analyzed by micro-array analysis.

Results. The expression of transcripts for complement factor B, C1qB-chain, C6 and factor I was lower in the livers of C3−/− mice as compared to C3+/+ mice, while the opposite was true for factor D (adipsin) and Masp-2A. Ethanol down-regulated mRNA levels of factor D and of the terminal components C6, C8 and C9K, and C9. Factor B, C1qA-chain, C2 and clusterin mRNA were up-regulated and of factor H and C4bp were down-regulated by ethanol significantly in C3+/+ mice. However, ethanol down-regulated transcripts of C1qB-chain and vonmorselin and down-regulated Masp2 expression specifically in C3+/− mice. Chronic ethanol consumption also up-regulated liver SPARC and Lipocalin-2 mRNA expression which showed the highest fold increase among all acute phase proteins studied.

Conclusions. The induction of classical and alternative pathway components and suppression of terminal pathway components and soluble regulators by chronic ethanol intake may contribute to alcohol-induced liver injury.

Monitoring increased liver expression of lipocalin-2 and SPARC might serve as new markers of ethanol-induced hepatic inflammation and fibrogenesis.

PA4-8
THE EFFECTS OF ETHANOL ON GENE EXPRESSION IN SKELETAL MUSCLE USING MICROARRAYS
Arno MJ, Lin JCI, Wiseman H, Preedy VR. (UK)

Aims. We tested the hypothesis that skeletal muscle gene expression is perturbed by ethanol.

Methods. To address this, we injected Male Wistar rats with either ethanol (75 mmol/kg body weight) or saline (0.15 mol/l). Food was withdrawn from both groups of rats to circumvent potential difficulties in interpreting the data due to ethanol-induced anorexia. At the end of 24 hours, rats were killed and gastrocnemius muscle dissected for subsequent RNA extraction and measurement of mRNA using the Affymetrix gene chip array.

Results. Of the 15,000 genes on the array, about 10,000 were shown to be significantly expressed in most of the samples. Of these 666 was either up- or down regulated 1.5 fold. After correcting for multiple comparisons, 269 were shown to be significant increased and 159 were decreased at the P<0.05 level or less. There were about 140 pathways or cellular processes affected which included (number of genes affected in parenthesis), cell adhesion (17 genes), apoptosis (15 genes), protein folding (11 genes), ubiquitin-dependent protein catabolism (9 genes), anti-apoptosis (8 genes), ribosome biogenesis (6), regulation of translational initiation (5), regulation of translational initiation (5), RNA processing (4 genes), regulation of muscle contraction (4 genes) and positive regulation of I-B kinase/NF-κB cascade (4 genes). Focusing on a single pathway, all 8 anti-apoptotic genes shown to be perturbed by ethanol were actually up-regulated. Essentially, this supports some studies suggesting that, whilst some genes relating to apoptosis are up-regulated by ethanol, this is not evident in terminal measures of the apoptotic process (i.e., immuno-histochemical studies with the TUNEL assays). There were also changes in unusually ascribed genes such as those pertaining to skeletal development and hearing, indicating possible new functions of previously ascribed genes.

Conclusions. In conclusion, alcohol perturbs the expression of numerous skeletal muscle genes, reflecting a multifaceted aetiology.

POSTER SESSION PA5

PA5-1
FIB-4 AND APRI HAVE POOR ACCURACY FOR THE DIAGNOSIS OF ADVANCED FIBROSIS IN PATIENTS WITH ALCOHOLIC LIVER DISEASE

Aims. Invasiveness and cost of liver biopsy have led to the development of alternative methods for the determination of fibrosis in patient with chronic liver disease. FIB-4 and APRI are simple tests based on routine laboratory values that have been shown to be good predictors of liver fibrosis in patients with hepatitis C. The present study addresses the diagnostic performance of FIB-4 and APRI in patients with alcoholic liver disease.

Methods. We calculated the FIB-4 (age( yr) × AST/UL/L)/Platelets(10⁹/UL) and ALT (UL/L) and APRI (AST/UL/L)/Platelets(10⁹/UL) values in 159 patients who had a liver biopsy during a hospitalisation for alcohol withdrawal. Patients with viral hepatitis or HIV infection were excluded. Advanced fibrosis defined as the presence of numerous septa or cirrhosis (equivalent to stage F3 or F4 in the Metavir scoring system) was present in 35 (22%) patients.

Results. The AUROC for FIB-4 for the diagnosis of advanced fibrosis was 0.65. For a cut-off of <1.56, the negative predictive value to exclude advanced fibrosis was 87.5% with a sensitivity of 83%. A cut-off of ≥5.77 had a positive predictive value of 41% and a specificity of 85%. AUROC of APRI for the diagnosis of advanced fibrosis was 0.52.

Conclusions. FIB-4 and APRI have insufficient accuracy for the diagnosis of advanced fibrosis in alcoholic liver disease and should not be used in this context.