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Changes in adipose tissue hormone-sensitive lipase activity and cAMP during ethanol withdrawal

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Abstract

The time course of the effects of ethanol withdrawal on brown and white adipose tissue hormone-sensitive lipase, cAMP production, and phosphodiesterase have been investigated after chronic drinking or liquid diet schedules. Chronic drinking significantly reduced brown adipose tissue hormone-sensitive lipase activity and cAMP levels from control. During withdrawal, there was a rebound increase to 200% control, peaking 9 h into withdrawal. White adipose tissue hormone-sensitive lipase activity and cAMP accumulation were significantly raised by both treatment schedules. Ethanol liquid diet produced a significant fall in adipose tissue hormone-sensitive lipase activity and cAMP accumulation. In brown fat, there was a rebound increase in hormone-sensitive lipase activity and cAMP; in white fat, no rebound was observed. In brown fat, the reductions in hormone-sensitive lipase activity and cAMP accumulation after chronic drinking coincided with an increase in phosphodiesterase activity. In white fat, the rise in cAMP and hormone-sensitive lipase activation coincided with a decrease in phosphodiesterase activity. We conclude that the effects of chronic ethanol on hormone-sensitive lipase activity are cAMP-dependent and mediated via alterations in phosphodiesterase activity.

Keywords: Ethanol withdrawal; Adipose tissue; Hormone-sensitive lipase, cAMP; Phosphodiesterase

1. Introduction

Ethanol produces both acute and chronic changes in plasma lipid levels and lipoprotein metabolism (Baraona and Lieber, 1979; Taskinen et al., 1985; Parkes et al., 1990). These effects have been widely reported in both human and animal studies and are very much dose-dependent. In human subjects, when ethanol is consumed regularly but moderately, the increase in plasma triglycerides is comparatively less than when ethanol is consumed to excess (Ginsberg et al., 1974; Taskinen et al., 1987). More interestingly, the concomitant increase in plasma high-density lipoproteins (the lipoprotein fraction responsible for the transport and disposal of cholesterol) is strongly associated with the reduced mortality rate from chronic heart disease observed amongst light to moderate drinkers (Suh et al., 1992). Although behavioural and neurophysiological studies in animals, in which ethanol tolerance and dependence have been induced by the chronic administration of ethanol in the food or water, have demonstrated a distinctive abstinence syndrome with a well-defined time course, which is characterised by epileptiform activity in the electroencephalogram (Walker and Zornetzer, 1974), neuronal hyperexcitability in tissues ex vivo (Whittington and Little, 1990) and a reduced convulsive threshold (Watson and Little, 1995), the changes in lipid metabolism during withdrawal from chronic ethanol have not so far been investigated.

The central neuronal effects of chronic ethanol have attracted most attention, since it is in the central nervous system that there is most direct evidence for adaptive changes in excitability and receptor function; for example, cerebral cortical membranes from mice chronically fed ethanol show diminished adenylyl cyclase activity (Tabakoff et al., 1995). The adaptive changes, which occur gradually in response to the chronic presence of ethanol, revert to the normal pre-alcohol state during the period

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immediately following the withdrawal of alcohol (Littleton, 1983). Since the effects of ethanol are ubiquitous, and there is good evidence that peripheral receptors such as the β-adrenoceptor can also exhibit downregulation of expression and changes in functional responsiveness (Benovic et al., 1988; Whaley et al., 1995), it is reasonable to suppose that adaptive changes to chronic ethanol may also occur outside the central nervous system. Adipocyte hormone-sensitive lipase, whose activity is regulated by a cAMP-dependent protein kinase, is the key enzyme controlling lipolysis. Activation of adipose tissue β-adrenoceptors stimulates adenylyl cyclase activity through a Gs protein to increase cAMP accumulation, thereby activating hormone-sensitive lipase and increasing lipolysis (Strålfors et al., 1984). The level of cAMP in adipose tissue is regulated, in turn, by the activities of adenylyl cyclase and the low Km cGMP-inhibited phosphodiesterase type3B (Degerman et al., 1997).

Our earlier in vivo studies in mice have demonstrated that increases in cAMP accumulation and hormone-sensitive lipase activation are closely correlated following β-adrenoceptor activation (Shih and Taberner, 1995a). In addition, we have previously shown that relatively low circulating levels of ethanol (1–5 mM) during chronic drinking can stimulate cAMP production and increase hormone-sensitive lipase activity in white adipose tissue, whilst the higher levels (15–20 mM) present during ethanol liquid diet treatment have no such effect (Shih and Taberner, 1997). In the same animals, both treatments suppressed brown adipose tissue hormone-sensitive lipase activity and cAMP production.

The key enzymes responsible for the release of lipids into, and the removal of lipids from, the blood stream are hormone-sensitive lipase and lipoprotein lipase, respectively. Since we have already shown that white adipose tissue lipoprotein lipase activity is unaffected by chronic ethanol treatment (Shih and Taberner, 1997), we have focused on adipose tissue hormone-sensitive lipase activity. It is well established that chronic ethanol is capable of upregulating protein expression; for example, dihydropyridine-sensitive Ca^{2+} channels (Messing et al., 1990) and that rebound changes can be observed during the withdrawal phase (Watson and Little, 1999). For this reason, measuring enzyme activity during the ethanol withdrawal phase should provide important clues to the mechanism whereby these adaptive changes in lipid metabolism are brought about. We have, therefore, used the same ethanol treatment schedules as before to compare the effects of ethanol withdrawal on brown and white adipose tissue hormone-sensitive lipase activity and cAMP production. These same dosing schedules have previously been used to demonstrate the correlation between the behavioural and neurophysiological changes, which occur during the withdrawal period (Watson and Little, 1999). By monitoring hormone-sensitive lipase activity, cAMP production and phosphodiesterase activity during the withdrawal phase, the possible biochemical mechanism of this adaptive process can be investigated.

2. Materials and methods

2.1. Materials

Triolein, oleic acid, phosphatidylcholine, leupeptin, pepstatin A, DL-dithiothreitol, collagenase (type I), cAMP, 5′-nucleotidase, Dowex ion exchange 1×2-400, were all obtained from Sigma (Poole, Dorset, UK). Glycerol tri [9,10 (*n*)-^{3}H] oleate, [1-^{14}C] oleic acid and [8-^{3}H] cAMP, ammonium salt were obtained from Amersham International (Aylesbury, Bucks, UK).

2.2. Animals

Male CBA/Ca mice, bred within the University of Bristol Medical School, were 4–5 months old with body weight 30–34 g and housed at 20–22°C with a 12 h light cycle (0900–2100 h). They were provided with ad libitum access to tap water and laboratory rodent chow (CRM, special Diet Services or standard mouse and rat diet, Bantin and Kingman).

2.3. Ethanol treatment schedules

Chronic ethanol drinking: mice were given a 4% then 8% (w/v) solution for 2 consecutive days, followed by a 12% (w/v) solution for 3 days, then maintained on 20% (w/v) solution for an average of 4 weeks as sole drinking fluid. The drinking solutions were prepared from 95% v/v ethanol (Hayman) by dilution with tap water. Subchronic ethanol liquid diet: ad libitum access to tap water was provided, but food was solely a liquid diet (Lieber and DeCarli liquid rat diet, Dyets, Pennsylvania), provided freshly each day at 1700 h. Two diets were used: control liquid diet containing 18% protein, 46% carbohydrate and 35% fat, and ethanol liquid diet, which was calorifically balanced with the former by the addition of 7% v/v ethanol, which contributed 36% of total energy (1.0 kJ ml^{-1} diet). Initially, both ethanol and control groups received the control liquid diet for 3 days. The ethanol treatment groups were then given 3.5% v/v ethanol liquid diet for 2 days. The treatment was completed by giving 7% ethanol liquid diet for a further 5 days. Control groups continued to receive the control liquid diet in a pair-fed regime. In both ethanol treatment schedules, ethanol was withdrawn at midnight for studies at the 9 and 12 h withdrawal time points and in the early morning for 3 and 6 h withdrawal. Thus, all mice were killed between 0930 and 1100 h for tissue assays.

2.4. Assay of ethanol concentrations

Interscapular brown adipose tissue and 250–300 mg samples of epididymal white adipose tissue were dissected, immediately frozen on solid CO_2, then weighed and homogenised in 1.0 ml of 1.2 M H_2S0_4 using a teflon-glass homogeniser (six strokes, 2000 rpm). The homogenate was neutralised with 5 M KOH then sodium pyrophosphate buffer (pH 8.8) added to make a final volume of 2 ml. After centrifugation at 6000 rpm for 15 min at 0°C, the supernatants were stored at −20°C until assayed. Ethanol levels were measured in duplicate supernatant (100 μl) and plasma (50 μl) samples using a standard NAD-coupled enzyme assay (Bonnichsen and Brink, 1955).

2.5. Assay of hormone-sensitive lipase

Interscapular brown adipose tissue was dissected out and cleaned, then homogenised in 10 volumes of pH 7.0 medium containing 0.25 M sucrose, 1 mM EDTA, 4 mg ml^{-1} leupeptin, 1 mg ml^{-1} pepstatin A, and 1 mM dithiothreitol. After centrifugation at $105{,}000 \times g$ for 45 min at 4°C the clear infranatant fraction was decanted for the assay of hormone-sensitive lipase activity (Shih and Taberner, 1997). Hormone-sensitive lipase from epididymal white adipose tissue was prepared similarly except that 2 volumes of medium were used. Triolein substrate was prepared freshly each day as described by Nilsson-Ehle and Schotz (1976). The assay medium consisted of 100 mM Tris–HCl (pH 7.0); 5 mg ml^{-1} bovine serum albumin (fatty acid-free, fraction V) and 4.58 mM triolein. The omission of apolipoprotein CII and the addition of NaCl (1 M) prevented any lipoprotein lipase activity.

Hormone-sensitive lipase activity was estimated by subtracting hormone-sensitive lipase inhibited activity (assayed in the presence of 100 mM NaF) from the total lipase activity. After 15-min incubation at 37°C, the reaction was stopped by adding 3.25 ml methanol:chloroform: heptane (1.41:1.25:1). The [^{3}H] oleate product was extracted into 100 mM K_2CO_3-borate buffer pH 10.5. [^{14}C] oleic acid was added as internal standard for estimating the recovery. Protein concentrations were determined by the Coomassie Blue method of Bradford (1976).

2.6. cAMP determination

Basal cAMP levels in fat cells were measured as described previously (Shih and Taberner, 1997). Briefly, adipocytes were prepared from brown and white adipose tissue according to Rodbell (1964) but using Krebs-Ringer phosphate buffer (pH 7.4) containing 128 mM NaCl, 1.4 mM $CaCl_2$, 1.4 mM $MgSO_4$, 5.2 mM KCl, and 10 mM Na_2HPO_4, and collagenase (3 mg ml^{-1} for BAT; 1 mg ml^{-1} for white adipose tissue). After 30-min incubation at 37°C, the suspended fat cells were washed with collagenase-free Krebs-Ringer buffer containing 4% BSA. Ice-cold 2 M HCl (20 ml) was added to 200 ml of suspended fat cells. This was centrifuged at $3000 \times g$ for 5 min at 4°C, and 150 ml of the resultant supernatant collected for cAMP measurement.

A standard competitive binding assay was used to measure tissue cAMP (Farndale et al., 1992). Standard or sample cAMP was mixed with [^{3}H]-cAMP (40 nCi) in 50 mM Tris–HCl buffer (pH 7.4) containing 4 mM EDTA. The reaction was started by adding 100 ml of cAMP binding protein. After 90-min incubation at 4°C, 200 ml of charcoal (0.25 g mixed with 0.1 g BSA in 50 ml of Tris–HCl/EDTA buffer) was added and incubated for a further 20 min at 4°C. The mixture was centrifuged at $3000 \times g$ for 15 min at 4°C and the supernatant decanted into scintillation vials for counting. Each assay was performed in triplicate and standard curve data was fitted to a logistic expression. The protein content of cell suspensions was determined as described above and the results expressed as nmol of cAMP mg $protein^{-1}$.

2.7. Measurement of cAMP phosphodiesterase type 3B

Brown and white adipose tissue were dissected out as described above. The clean tissue was homogenised with either 10 volumes (brown adipose tissue) or 2 volumes (white adipose tissue) of 10 mM Tris–HCl (pH 7.4) containing 0.25 M sucrose, 6 mM $MgCl_2$, 1 mM dithiothreitol, and 1 mg l^{-1} BSA (fraction V), pH 7.4. The homogenate was centrifuged at $20{,}000 \times g$ for 2 min at 4°C. The supernatant (S1) was then centrifuged for further 30 min and the P2 pellet resuspended in 1 ml of ice-cold buffer, containing 0.25 M sucrose and 2 mM Tris–HCl, pH 7.4 (Kono et al., 1975). The assay was carried out in 80 μl 50 mM Tris–HCl pH 7.4 containing 6 mM $MgCl_2$, 2.5 mM dithiothreitol, 0.05 mg ml^{-1} 5′-nucleotidase (powdered extract of *Crotalus adamanteus* snake venom, Sigma), 0.23 mg ml^{-1} bovine serum albumin, 1 μM unlabelled cAMP , and 10 μl [^{3}H] cAMP (10 nCi). The reaction was started by adding 10 μl of phosphodiesterase P2 suspension (see above) to each tube and incubating at 30°C for 10 min. The reaction was stopped by addition of 1.2 ml of Dowex 1 ion-exchange slurry (200–400 mesh). The assay tubes were then centrifuged at $15{,}000 \times g$ for 5 min, and 200 μl aliquots of the supernatant added to 3.5 ml of scintillation liquid (Packard LSC for aqueous samples) for counting. phosphodiesterase activity was calculated as pmol cAMP hydrolyzed per min per mg protein.

2.8. Statistical analysis

Results are shown as mean ± S.E.M. combined from three or four different experimental days. Statistical signif-

icances of differences were analysed by Student's *t*-test for independent samples or paired samples where appropriate.

3. Results

3.1. Plasma and adipose tissue ethanol levels

The average daily ethanol consumption of the mice was 14.8 ± 1.4 g kg body weight^{-1}on the chronic-drinking schedule and 21.2 ± 0.6 g kg body weight^{-1} on the ethanol liquid diet ($n = 16$). The highest plasma ethanol concentrations occurred during the hours of darkness, the period when the mice are most active. The mice exhibited no overt behavioural signs of sedation (decreased locomotor activity or ataxia) during the ethanol treatment schedules. On the chronic-drinking schedule, the initial concentration (blood collected at 0900 h) was between 4 and 5 mM, and fell to zero by 6 h withdrawal (Fig. 1A). On the ethanol liquid diet schedule, the initial concentration (blood collected at 0900 h) was between 14 and 16 mM and after withdrawal fell rapidly, also approaching zero within 6 h

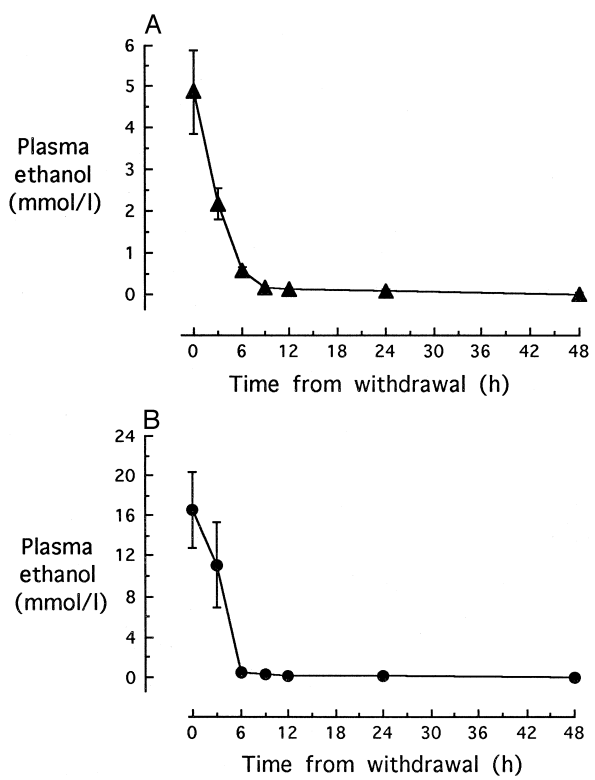

Fig. 1. Plasma ethanol concentration (mmol l^{-1}) in mice on either (A) the chronic drinking schedule, 20% ethanol in the drinking water for 4 weeks, or (B) the ethanol liquid diet schedule following withdrawal of alcohol at $t = 0$. Results are shown as means ± S.E.M. from $n = 16$–20 mice. Each assay was performed in duplicate.

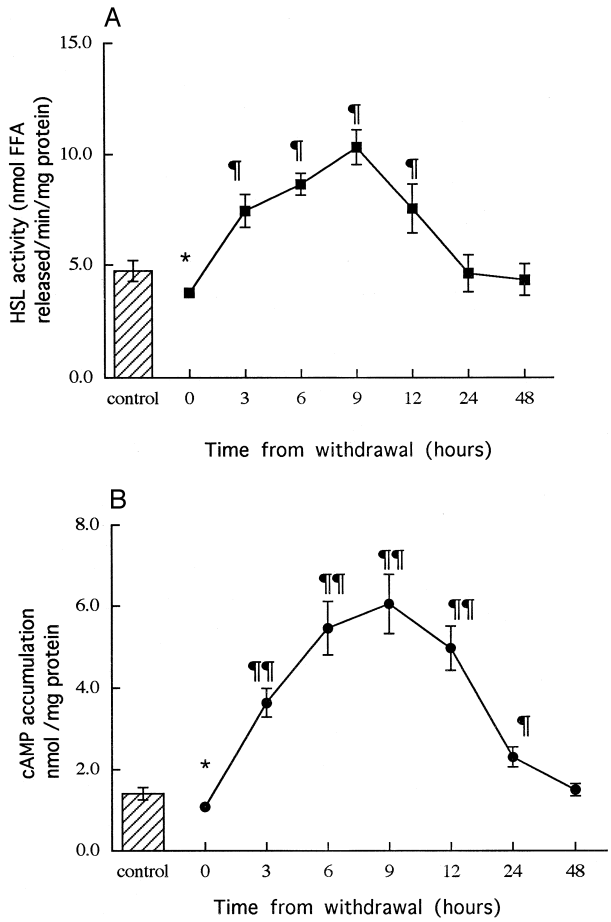

Fig. 2. Effects of withdrawal from the chronic drinking schedule on (A) hormone-sensitive lipase activity (HSL) expressed as nmol free fatty acids released per min per mg tissue protein, and (B) cAMP accumulation in brown adipose tissue. The results are shown as means ± S.E.M. from $n = 20$–24 mice (control groups) and $n = 8$–10 for each withdrawal time point. The results were combined from groups of mice randomized between different experimental days. Each assay was performed in triplicate and the median value used. Hormone-sensitive lipase activity or cAMP accumulation < control: $^{*}P < 0.05$. Hormone-sensitive lipase activity or cAMP accumulation > non-withdrawal (zero time point): ¶ $P < 0.05$, ¶¶ $P < 0.01$.

(Fig. 1B). In both cases, the decline in plasma concentration was linear, appearing to follow zero order kinetics, so that the half-life could not be calculated On the basis of this pattern, the time points selected for the assays of hormone-sensitive lipase, cAMP and phosphodiesterase were 3, 6, 9, 12, 24 and 48 h from the time of withdrawal.

In a separate experiment, groups of six mice on the ethanol drinking and ethanol liquid diet schedules were killed at the time the alcohol was withdrawn (0900 h) for simultaneous measurement of plasma and adipose tissue ethanol levels. The mean ethanol levels in the ethanol-drinking mice were blood plasma: 4.6 ± 1.1 mM, brown adipose tissue: 3.8 ± 0.7 mM, white adipose tissue: 4.0 ± 0.6 mM. In the ethanol liquid diet group, the mean levels

were 15.2 ± 2.8 mM, 12.8 ± 2.1 mM, and14.0 ± 2.5 mM in the blood plasma and brown and white adipose tissues, respectively. The adipose tissue ethanol levels were consistently lower than the plasma level in each animal, and, although the differences were statistically significant ($P < 0.025$, paired *t*-test), there was no significant difference between the levels in the brown and white adipose tissue.

3.2. Effects of withdrawal from the chronic ethanol-drinking schedule

Prior to withdrawal from the chronic ethanol-drinking hormone-sensitive lipase activity in brown adipose tissue was significantly reduced ($P < 0.05$) compared to untreated controls (Fig. 2A). During the withdrawal period, there was a highly significant rebound increase to above the control level. There was a gradual increase, which peaked at 9 h into withdrawal ($P < 0.01$, *t*-test), followed by a return to the control level by 24 h withdrawal (Fig. 2A). cAMP accumulation in brown adipose tissue showed a very similar pattern of changes, being significantly increased at 3, 6, 9 and 12 h ($P < 0.01$), and returning to control levels after 24 h (Fig. 2B). In contrast, during the same chronic ethanol treatment hormone-sensitive lipase

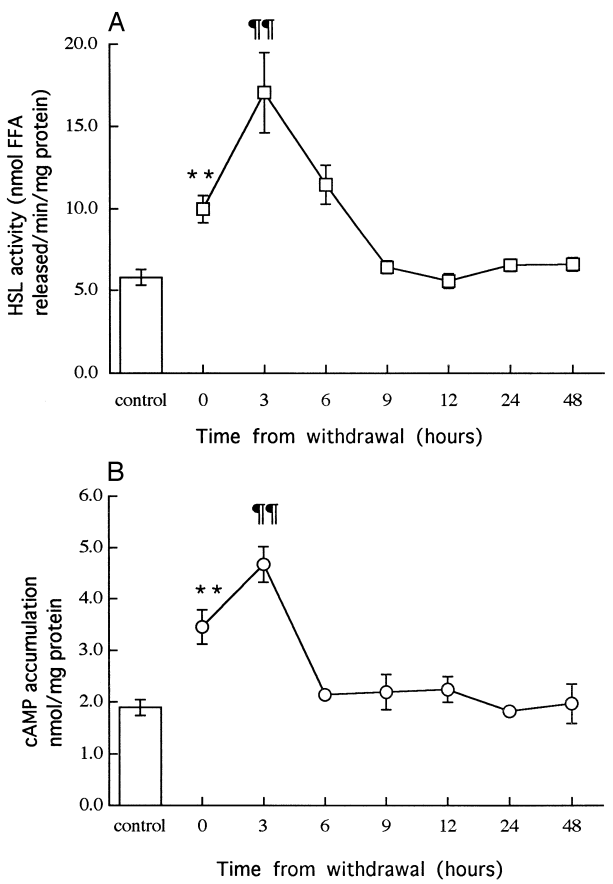

Fig. 3. Effects of withdrawal from the chronic drinking schedule on (A) hormone-sensitive lipase activity (HSL) expressed as nmol free fatty acids released per min per mg tissue protein, and (B) cAMP accumulation in white adipose tissue. The results are shown as means ± S.E.M. from $n = 20–24$ mice (control groups) and $n = 8–10$ for each withdrawal time point. The results were combined from groups of mice randomized between different experimental days. Each assay was performed in triplicate and the median value used. Hormone-sensitive lipase activity or cAMP accumulation > control: $^{**}P < 0.01$. Hormone-sensitive lipase activity or cAMP accumulation > non-withdrawal (zero time point): ¶¶ $P < 0.01$.

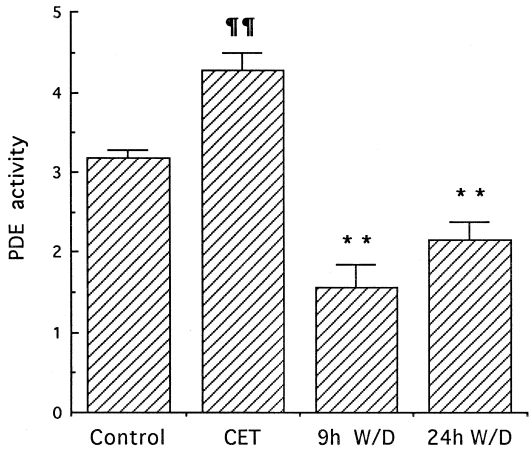

Fig. 4. Effects of withdrawal from the chronic drinking schedule (CET) on phosphodiesterase type 3B (PDE) activity (expressed as pmol cAMP hydrolyzed per min per mg protein) in brown adipose tissue. The results are shown as means ± S.E.M. from $n = 16–20$ mice (control group) and $n = 8$ for each withdrawal time point. The results were combined from groups of mice randomized between different experimental days. Each assay was performed in triplicate and the median value used. Phosphodiesterase activity > control: ¶¶ $P < 0.01$; Phosphodiesterase activity < control $^{**}P < 0.01$.

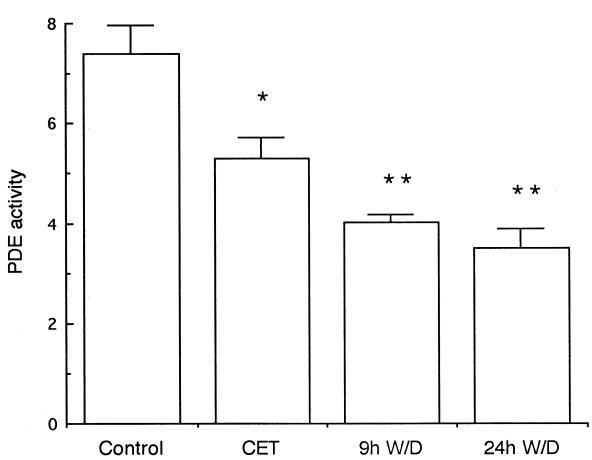

Fig. 5. Effects of withdrawal from the chronic drinking schedule (CET) on phosphodiesterase type 3B (PDE) activity (expressed as pmol cAMP hydrolyzed per min per mg protein) in white adipose tissue. The results are shown as means ± S.E.M. from $n = 16–20$ mice (control group) and $n = 8$ for each withdrawal time point. The results were combined from groups of mice randomized between different experimental days. Each assay was performed in triplicate and the median value used. Phosphodiesterase activity < control $^{*}P < 0.05$, $^{**}P < 0.01$.

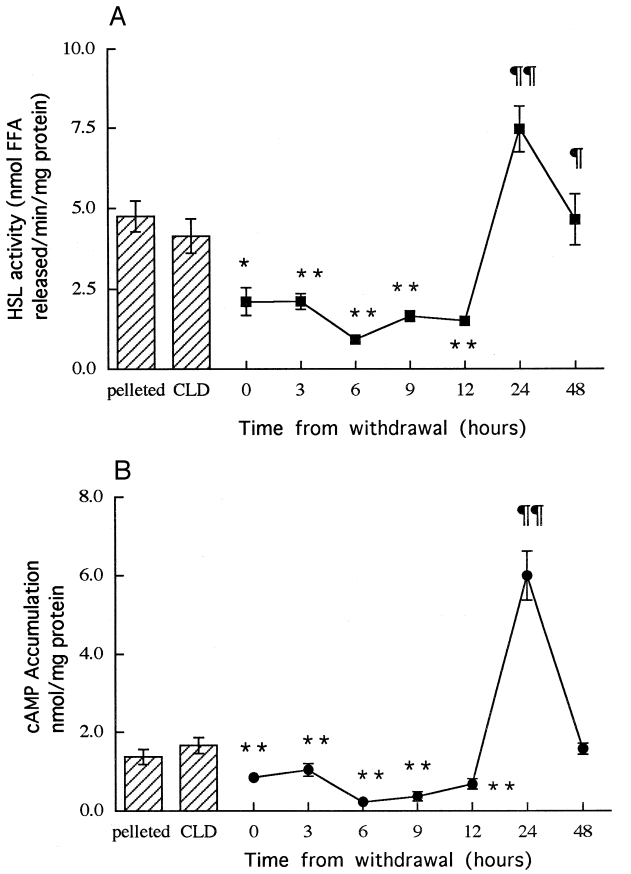

Fig. 6. Effects of withdrawal from the ethanol liquid diet on (A) hormone-sensitive lipase activity (HSL) expressed as nmol free fatty acids released per min per mg tissue protein, and (B) cAMP accumulation in brown adipose tissue. The results are shown as means ± S.E.M. from $n = 24$ mice (control groups) and $n = 8$ for each withdrawal time point. The control groups (non-ethanol-treated) shown correspond to normal pelleted diet and control liquid diet (CLD). The results were combined from groups of mice randomized between different experimental days. Each assay was performed in triplicate and the median value used. Hormone-sensitive lipase activity or cAMP accumulation < control liquid diet group: $^{*}P < 0.05$, $^{**}P < 0.01$. Hormone-sensitive lipase activity or cAMP accumulation > non-withdrawal (zero time point): ¶ $P < 0.05$, ¶¶ $P < 0.01$.

activity in white adipose tissue was significantly increased ($P < 0.01$, see Fig. 3A). During the ethanol withdrawal phase, the hormone-sensitive lipase activity increased even further, reaching a peak within the first 3 h of withdrawal ($P < 0.01$, *t*-test), returning to the control level by 9 h withdrawal (Fig. 3A). The cAMP level in white adipose tissue also rose at 3 h withdrawal ($P < 0.01$, *t*-test) and returned to the control level after 6 h withdrawal (Fig. 3B).

During the chronic ethanol-drinking schedule, phosphodiesterase activity in brown adipose tissue was significantly increased compared to control ($P < 0.005$, Fig. 4). There was a rebound fall in activity, which was maximal after 9 h ethanol withdrawal ($P < 0.01$, *t*-test). At the same time, however, phosphodiesterase activity in white adipose tissue was decreased ($P < 0.05$, Fig. 5). This fall in activity was even greater after 9 and 24 h withdrawal ($P < 0.01$).

3.3. Effects of withdrawal from the ethanol liquid diet

Withdrawal from the ethanol liquid diet schedule also produced parallel reductions in hormone-sensitive lipase activity and cAMP accumulation in brown adipose tissue (see Fig. 6A and B, respectively), although the time course of the effects of withdrawal from ethanol on hormone-sen-

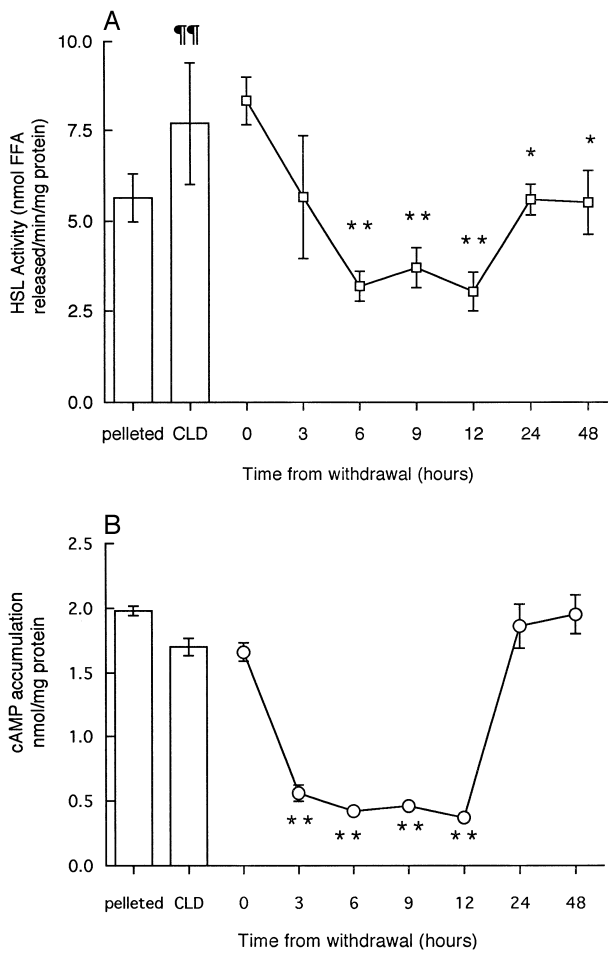

Fig. 7. Effects of withdrawal from the ethanol liquid diet on (A) hormone-sensitive lipase activity (HSL) expressed as nmol free fatty acids released per min per mg tissue protein, and (B) cAMP accumulation in white adipose tissue. The results are shown as means ± S.E.M. from $n = 24$ mice (control groups) and $n = 8$ for each withdrawal time point. The control groups (non-ethanol-treated) shown correspond to normal pelleted diet and control liquid diet (CLD). The results were combined from groups of mice randomized between different experimental days. Each assay was performed in triplicate and the median value used. Hormone-sensitive lipase activity after control liquid diet > control pelleted diet group: ¶¶$P < 0.01$. Hormone-sensitive lipase activity or cAMP accumulation < non-withdrawal (zero time point): $^{*}P < 0.05$, $^{**}P < 0.01$.

sitive lipase activity and cAMP were slightly different from those observed after withdrawal from chronic ethanol drinking. In brown adipose tissue, hormone-sensitive lipase activity (Fig. 6A) and cAMP levels (Fig. 6B) fell significantly throughout the first 12 h of withdrawal ($P < 0.01$, *t*-test), then rose dramatically at 24 h withdrawal before returning back to control level after 48 h withdrawal. It should be noted that the control liquid diet in itself did not alter the levels of hormone-sensitive lipase or cAMP compared to control mice fed on pelleted diet.

In contrast, the control liquid diet significantly increased hormone-sensitive lipase activity in white adipose tissue compared to pellet-fed controls (Fig. 7A) and, following the chronic ethanol-drinking schedule, no increase in hormone-sensitive lipase activity was seen. The changes in hormone-sensitive lipase activity (Fig. 7A) and cAMP production (Fig. 7B) after withdrawal were initially similar to those observed in brown adipose tissue over 12 h; however, in this tissue, there was no rebound increase in enzyme activity or cAMP accumulation at 24 h withdrawal.

In order to determine whether the observed changes in cAMP were related to changes in phosphodiesterase, the activity of the enzyme was measured at the time of ethanol withdrawal ($t = 0$) and 9 and 24 h subsequently. The ethanol liquid diet schedule did not alter phosphodiesterase activity in either brown (Fig. 8) or white adipose tissue (Fig. 9) compared to untreated controls. However, enzyme activity was significantly increased after 9 and 24 h withdrawal in brown adipose tissue ($P < 0.01$, Fig. 8). In white adipose tissue, phosphodiesterase activity was also increased significantly ($P < 0.01$) at 9 h withdrawal (Fig. 9).

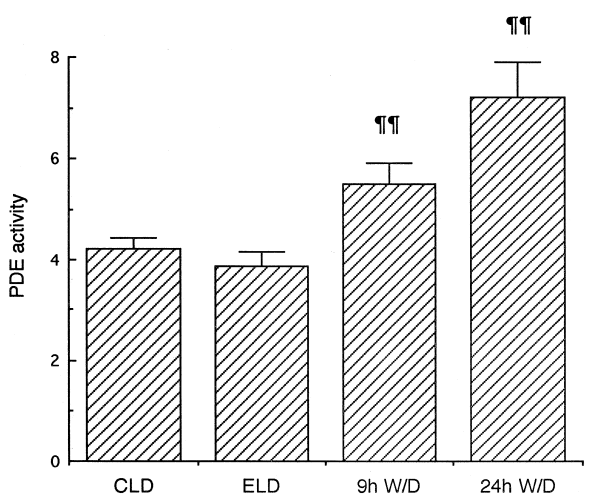

Fig. 8. Effects of withdrawal from the ethanol liquid diet on phosphodiesterase (PDE) activity (expressed as pmol cAMP hydrolysed per min per mg protein) in brown adipose tissue. The results are shown as means ± S.E.M. from $n = 16–20$ mice (control group) and $n = 8$ for each withdrawal time point. The results were combined from groups of mice randomized between different experimental days. Each assay was performed in triplicate and the median value used. Phosphodiesterase activity at 9 and 24 h withdrawal > control or ethanol liquid diet groups: ¶¶ $P < 0.01$.

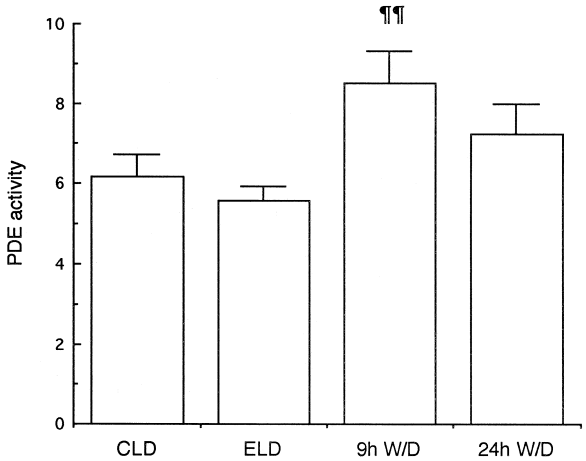

Fig. 9. Effect of withdrawal from the ethanol liquid diet on phosphodiesterase (PDE) activity (expressed as pmol cAMP hydrolysed per min per mg protein) in white adipose tissue. The results are shown as means ± S.E.M. from $n = 16–20$ mice (control group) and $n = 8$ for each withdrawal time point. The results were combined from groups of mice randomized between different experimental days. Each assay was performed in triplicate and the median value used. Phosphodiesterase activity at 9 h withdrawal > control or ethanol liquid diet groups: ¶¶ $P < 0.01$.

4. Discussion

The severity of the withdrawal syndrome following chronic ethanol is an indication of the level of ethanol induced dependence and is a function of the dose level and duration of the chronic treatment. In the present studies, the plasma levels of ethanol during the two treatment schedules were much lower than those associated with sedation or anaesthesia following acute ethanol injection (Unwin and Taberner, 1982). These concentrations have no direct effect on hormone-sensitive lipase activity in vitro (Shih and Taberner, 1997). Studies on the diurnal variation in ethanol consumption, and the subsequent ethanol levels in mice during the same treatment schedules, have indicated that peak plasma concentrations tend to occur in the middle of the dark phase, i.e. 2200–0500 h (Jelic et al., 1998). Therefore, in the present studies, the 2400 or 0300 h time points were selected for withdrawal of the ethanol. The relatively rapid disappearance of ethanol within 6 h suggests that the biochemical changes observed at subsequent times post-withdrawal are post-ethanol adaptive changes rather than concentration-dependent effects.

The effects of withdrawal from the ethanol-drinking schedule on brown and white adipose tissue hormone-sensitive lipase activity and cAMP production were quantitatively similar; i.e. hormone-sensitive lipase activity and cAMP production initially increased after removal of ethanol then gradually returned toward control levels by 48

h. However, the time course of the changes induced by ethanol withdrawal differed between brown and white adipose tissue, even though the two tissues were obtained from the same animals. This would seem to preclude a pharmacokinetic effect since, although brown adipose tissue does have a higher vascular perfusion rate than white adipose tissue, the equilibrium fat:water partition coefficient for ethanol is only 1:1.2. When plasma and adipose tissue ethanol concentrations were assayed in parallel, the slightly lower levels observed in the adipose tissues were consistent with this partition ratio. The different time courses of changes in hormone-sensitive lipase activity and cAMP level seen in brown and white adipose tissue after the disappearance of ethanol are, therefore, more likely to be due to different tissue responses to ethanol. The effects of withdrawal from 20% w/v ethanol solution on brain cAMP level have been reported to show similar patterns but varying time courses, according to the brain areas examined (Shen et al., 1983).

The rebound change in brown fat hormone-sensitive lipase activity occurred at 9 h withdrawal, when the ethanol had effectively disappeared from the circulation. This time point coincides with the rebound changes that have previously been observed in both neuropharmacological and behavioural studies employing similar ethanol treatment schedules (Whittington and Little, 1990, Watson and Little, 1995). These changes could be related to intracellular Ca^{2+} levels, since the upregulation of dihydropyridine-sensitive Ca^{2+} channels, which occurs after chronic ethanol treatment (Harper et al., 1989), can be prevented by treatment with dihydropyridine-sensitive Ca^{2+} channel antagonists (Whittington et al., 1991). Dihydropyridines can also inhibit the development of tolerance and attenuate the ethanol withdrawal syndrome in vivo (Watson et al., 1994).

In the present study, the effects of withdrawal from ethanol liquid diet on hormone-sensitive lipase activity and cAMP production were markedly different from those observed following the drinking schedule. Although both the chronic-drinking schedule and ethanol liquid diet suppressed hormone-sensitive lipase activity in brown adipose tissue (see Figs. 2 and 6, respectively); following ethanol withdrawal, hormone-sensitive lipase activity was stimulated after chronic drinking but suppressed even further after ethanol liquid diet (see Fig. 6A). In addition, phosphodiesterase activity was suppressed at 9 and 24 h ethanol withdrawal from the drinking schedule, but was stimulated at 9 and 24 h ethanol withdrawal from the ethanol liquid diet schedule. This could be a dose-dependent effect, since plasma levels were higher after the ethanol liquid diet schedule compared to the ethanol-drinking schedule. It should be noted that cAMP production in the brain is also affected differently by different ethanol treatment schedules; for example, it has been shown that after different drinking schedules cAMP production in brain decreases (Saffey et al., 1988), increases (Kuriyama and Israel, 1973) or shows no change (Saito et al., 1987). Hoffman and Tabakoff (1990) have reviewed a number of ethanol studies using different tissues or cell cultures. The disparate findings emphasise the fact that different cells respond to chronic ethanol exposure in different ways. If individual cell types can respond differentially to the chronic presence of ethanol, it is perhaps not surprising that different types of adipose tissue should show variable changes after chronic ethanol ingestion by animals, and that some of these changes may differ from those seen in transformed cells in culture.

Although we have demonstrated that the differential changes in brown and white adipose tissue hormone-sensitive lipase activity and cAMP levels brought about by the chronic ethanol treatment can be associated with alterations in phosphodiesterase activity, these effects may also involve an activation of dihydropyridine-sensitive Ca^{2+} channels. It has already been shown that upregulation of the dihydropyridine binding site in chronic ethanol treatment involves an activation of protein kinase C (Harper et al., 1989, Messing et al., 1990). Increased α_2-adrenoceptor sensitivity after the drinking schedule could be the consequence of increased Giα protein expression (Wand et al, 1993). Sensitisation of α_2-adrenoceptor activity after this ethanol-drinking schedule was observed previously (Shih and Taberner, 1995b), suggesting that increases in Gi protein expression might be involved. Although it has not been directly demonstrated that there are dihydropyridine-sensitive Ca^{2+} channels present in adipose tissues, previous studies in this laboratory have shown that the dihydropyridine-sensitive Ca^{2+} channel antagonist PN 200-110 (isradipine) prevented the inhibitory effects of ethanol withdrawal on lipogenesis (Hughes et al., 1995) and hormone-sensitive lipase activity (Jelic and Taberner, 1996). Further studies of dihydropyridine-sensitive Ca^{2+} channel and α_2-adrenoceptor binding, assay of protein kinase C activity, together with Giα subunit protein expression, may clarify the cellular basis for the activation and suppression of phosphodiesterase by relatively low levels of chronic ethanol.

The present study confirms that the effects of chronic ethanol consumption and its withdrawal on the regulation of lipolysis in brown and white adipose tissue are clearly dependent upon the dose and duration of the treatment. The different rebound changes in hormone-sensitive lipase activity in brown and white adipose tissue observed during the withdrawal phase appear to be mediated via changes in tissue cAMP levels as a consequence of short-term alterations in the activity of phosphodiesterase activity, which may represent a key cellular target in the ethanol withdrawal syndrome.

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