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THE INFLUENCE OF BRAIN ACETALDEHYDE ON OXIDATIVE STATUS, DOPAMINE METABOLISM AND VISUAL DISCRIMINATION TASK

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Abstract—The toxic effect of acetaldehyde on brain oxidative capacity and dopamine metabolism has been investigated in rat brains after a single intraperitoneal injection of acetaldehyde (5 mmol/kg) and the results compared with those from chronically ethanol fed rats. Acetaldehyde was present in rat brain 120 hr after a single dose of acetaldehyde, confirming that it is able to cross the blood–brain barrier. Brain catalase increased significantly after acetaldehyde or chronic ethanol administration although there were no other significant changes in the total brain activity of superoxide dismutase, glutathione peroxidase or glutathione reductase. Dopamine turnover was increased in both experimental groups. The acute dose of acetaldehyde reduced the ability of the rats to relearn a computer visual discrimination task.

Keywords: acetaldehyde; oxidative stress; dopamine metabolism; liver; brain

Ethanol-related neurological disorders occur in a large number of alcohol abusers producing a diverse and often devastating series of medical complications [1]. Approximately 50–70% of detoxified alcohol misusers show the presence of mild cognitive impairment [2]. Ethanol and especially its oxidative metabolite, acetaldehyde, may directly damage the developing and mature nervous system [3–7].

It is likely that the highly reactive nature of ACH‖, will cause perturbations of normal brain function either directly by altering cellular functions, e.g. inhibiting the ion transferring ATPases (Na^{2+}, K^+ and Mg^{2+} activated) in cellular membranes [8] or indirectly, e.g. by altering the metabolism of biogenic amines [9], forming adducts with neurotransmitters [10] or generating bioactive derivatives such as tetrahydroisoquinolones and tetrahydropapaverine after interaction with catecholamines [11], the latter compounds being implicated in dependency [12].

Early studies questioned if ACH would [13] or would not [14] traverse the blood–brain barrier, and whether any detectable residual brain acetaldehyde was merely as a result of local ethanol metabolism either by the inducible P450IIEI system [15] or by brain catalase [16]. The presence of ACH in the brain will also be dependent on the speed at which it is removed from the brain tissues by ALDH. Pettersson and Kiessling [17] showed that a low K_m ALDH was important for the oxidation of brain ACH. Several brain ALDH isoenzymes are present; their substrate specificities include DOPAL [18] and noradrenaline as well as ACH [19].

It remains unclear whether acetaldehyde can act as a substrate for xanthine oxidase to produce free radicals generated during its oxidation [20, 21]. However, if radicals are generated these would have devastating effects on the brain tissue by virtue of the high levels of polyunsaturated fatty acids, and to a lesser extent iron, present in brain [22]. In addition the low capacity of the brain to detoxify reactive oxygen species may be insufficient to scavenge excessive amounts of these species [23, 24].

In these present studies we have investigated firstly if acetaldehyde is present in the brain after a single acute intraperitoneal dose. The capacity of such brain acetaldehyde levels to interfere with normal brain function has been assessed by the assay of dopamine metabolism and cellular cytoprotective enzyme activities and compared to such parameters after chronic ethanol administration. Finally, the ability of such rats to learn a computer visual-discrimination task after the acute dose of ACH was measured by a simplified version of Field's VDA [25].

MATERIALS AND METHODS

Animal studies. Male Wistar rats (200–250 g), N = 54, were administered one intraperitoneal injection of acetaldehyde (5 mM/kg body weight)

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‖ Abbreviations: ACH, acetaldehyde; ALDH, aldehyde dehydrogenase; DOPAL, dihydroxyphenyl-acetaldehyde; VDA, visual discrimination test; DIH, diphenylacetyl-1,3-inandione-1-hydrazone; HVA, homovanillic acid; DHBA, dihydroxybenzylamine; 5HT, 5-hydroxytryptamine; 5HIAA, 5-hydroxyindole-3-acetic acid; dopa, 3,4-dihydroxyphenylalanine.

and nine animals killed at each of the time point, 4 hr, 12 hr, 24 hr, 48 hr, 72 hr and 96 hr. Prior to killing, blood was removed by cardiac puncture into heparinized tubes for biochemical analyses and ACH measurements. The livers were removed from each rat for assay of cytoprotective enzymes and ACH content. The brains were dissected from the cranium on ice for the assay of ACH content, activities of cytoprotective enzymes and studies of dopamine metabolism.

Male Wistar rats (70–100 g), N = 6, were administered ethanol by the adapted Lieber-de Carli diet for 6 weeks [26]. At the end of this time period the brains were dissected on ice, the striatum removed and dopamine and its metabolites assayed. The remaining brain was homogenized in 0.25 M sucrose (10%, w/v) for assay of cytoprotective enzymes.

Detection of acetaldehyde in the brain. Blood, 1 mL, was immediately mixed with the acetaldehyde trapping reagent DIH, 2 mL, while a portion of the liver (approx. 1 g) and the whole brain (approx. 1 g) from three rats at each time point from the acute ACH experiment, were polytroned with the ACH trapping reagent DIH (4 mL). The homogenized tissues or blood were centrifuged at 1000 *g* for 15 min at 4°, and the supernatant removed. This was acidified with 1 N hydrochloric acid, incubated at 37° for 20 min and stored at −70° prior to its analysis by HPLC [27].

Assessment of oxidative stress by assay of antioxidant content and activities of cytoprotective enzymes. In three rats from the ACH experiment one half of the brain was freeze clamped for assay of oxidized, GSSG, and reduced, GSH, glutathione content, while the remainder was homogenized in 0.25 M sucrose (10%, w/v) and aliquots stored at −70° for analyses of cytoprotective enzymes.

Oxidized and reduced glutathione were assayed by a modification of the fluorometric method of Hissen and Hilf [28]. Freeze-clamped portions (0.1 g) were homogenized in an ice cold mixture of phosphate–EDTA buffer (1.5 mL) and orthophosphoric acid, 0.54 M (0.4 mL) followed by centrifugation at 100,000 *g* at 4° for 30 min. For GSH analysis a portion of the supernatant (100 μL) was mixed with phosphate–EDTA buffer (1.8 mL) and *o*-phthaldehyde, 7.5 mM (100 μL). After 15 min at room temperature the fluorescence was measured at E_x 350 nm and E_m 420 nm. For GSSG a portion of the supernatant (100 μL) was incubated with *N*-ethylmaleimide, 40 mM (40 μL) for 30 min at room temperature followed by the addition of sodium hydroxide, 0.1 M (860 μL). A portion of this diluted supernatant (100 μL) was mixed with sodium hydroxide, 0.1 M (1.8 mL) and *o*-phthaldehyde, 7.5 mM (100 μL). The mixture was left for 15 min at room temperature and fluorescence measured as described above. Standard curves for GSH and GSSG were prepared in the ranges 0.32–6.5 mM and 0.15–3.0 mM, respectively.

α-Tocopherol was assayed in tissue homogenates by reverse-phase HPLC [29]. Homogenates, 10% (1.5 mL) were mixed with the antioxidant pyrogallol, 1.5% in ethanol (3.0 mL) preincubated at 70° for 5 min prior to the addition of potassium hydroxide, 10 M (0.45 mL) to saponify the lipids. After cooling, hexane (2 mL) was added and the samples mixed for 1 min before centrifugation at 1000 *g* for 5 min at 4°. The hexane layer containing α-tocopherol was removed to a glass tube, dried at room temperature under oxygen-free nitrogen and redissolved in methanol (200 μL). Aliquots were injected onto an MOS-2 column, 250 × 4.6 mm and eluted with 95% methanol, 5% ammonium acetate, 10 M. The eluting α-tocopherol was detected fluorometrically by a Merck-Hitachi F-1050 fluorescence spectrophotometer and the peak height compared with those of α-tocopherol standards in the range 0.232–1.16 mM.

Superoxide dismutase was assayed by the method of Beauchamp and Fridovich [30]. Suitably diluted tissue homogenates (200 μL) were mixed with the buffered substrate containing nitroblue tetrazolium, 2.5×10^{-5} M, xanthine 1×10^{-4} M, EDTA 1×10^{-4} M (1275 μL) in a UV disposable cuvette at 25°C. The absorbance at 560 nm was recorded at 1 and 4 min after the addition of xanthine oxidase, 8 U/mL (25 μL). Bovine liver SOD standard was prepared in the range 0–5 U/L and a reagent blank of 0.25 M sucrose was assayed. One unit of enzyme activity was equivalent to 50% inhibition of the reduction rate of nitroblue tetrazolium.

Catalase assay was according to the method of Peters and Batt [31] which was a micro-modification of Baudhuin [32]. Suitably diluted tissue homogenates (100 μL) were incubated for 30 min at 25° with freshly prepared buffered substrate (100 μL) containing imidazole, 0.2 M, pH 7.0, 2% Triton-100, BSA 0.015 mM and hydrogen peroxide 30%, w/v. The enzyme–substrate reaction was stopped by the addition of titanium oxysulphate (2 mL) and the remaining hydrogen peroxide measured spectrophotometrically as the yellow peroxy titanium sulphate at 405 nm. One unit of catalase activity is defined as the amount of enzyme to cause the destruction of 90% substrate in 1 min in a volume of 50 mL under the assay conditions.

Glutathione peroxidase was assayed by a modification of the method of Paglia and Valentine [33]. Suitably diluted tissue homogenates (100 μL) were mixed with the buffered substrate (1.5 mL) containing 50 mM Tris-HCl pH 6, 0.1 mM EDTA, 0.1 mg/mL NADPH, 0.25 M reduced glutathione and glutathione reductase 10 U/mL, and preincubated at 37° for 5 min. Cumene hydroperoxide (50 μL) was added to start the reaction and the rate of NADPH oxidation recorded at 1 and 4 min. One unit of activity of glutathione peroxidase was defined as the amount of enzyme to catalyse the transformation of 1 mmol of NADPH per minute under the assay conditions.

Glutathione reductase assay was adapted from the method of Worthington and Rosemeyer [34]. The tissue homogenate (5 mL) was mixed with the glutathione reductase substrate (1.5 mL) containing KCl 0.2 M, EDTA 1.0 mM, oxidized glutathione 1.0 mM, and 1 mg NADPH-Na_4 in phosphate buffer, 50 mM pH 7.0. The rate of oxidation of NADPH per minute was measured after 4 min at 340 nm at room temperature. A blank of 0.25 M sucrose was assayed exactly as the test sample. After subtraction

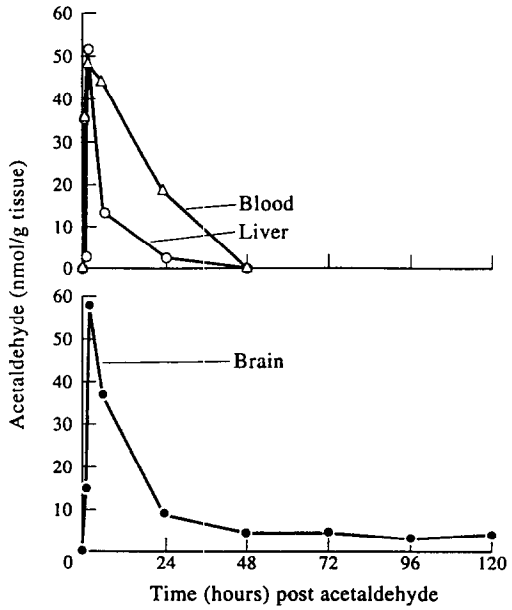

Fig. 1. Acetaldehyde content of liver, blood and brain in rat after an acute intraperitoneal dose of acetaldehyde (5 mM/kg).

of the blank the activity of glutathione reductase was calculated.

Dopamine assay. From three rats the striatum was dissected from the brain and analysed for both its dopamine content and metabolites HVA and DOPAC by HPLC with electrochemical detection by a modification of the method of Rose *et al.* [35]. The striated region of the brain was dissected on ice, weighed and sonicated in 10 volumes (w/v) of 0.4 M perchloric acid containing 1 mM EDTA and 0.5% sodium metabisulphite by a Microson tissue disruptor. The resulting homogenate was added to a solution of the internal standard, dihydroxybenzylamine in a 9:1 (v/v) ratio to give a final concentration of DHBA of 100 ng/mL. The homogenate was centrifuged at 15,000 rpm for 10 min at 4°. An aliquot of the supernatant was injected onto a Spherisorb ODS-2 reverse-phase column and chromatographic peaks detected by a BAS LC-4B amperometric detector with a thin-layer electrochemical cell fitted with a glassy carbon working electrode and Ag/AgCl reference electrode.

Visual discrimination test. Twelve Male Lister Hooded rats (450–550 g) were given an intraperitoneal injection of acetaldehyde (5 mM/kg body weight) after learning the complex visual discrimination task to criterion. A simplified version of Field VDS [25] with four panels, each with five escape doors, four locked doors marked with a white circle on a black background, and the open door bearing a white triangle on a black background, was used for the studies. The reward was 2 min in a complex play area with the cage mates plus some sunflower seeds. The rat was given three runs through the apparatus in each test session. The position of the stimuli was varied in each run using random numbers. This gave four scores: 1, correct first decisions; 2, errors (pushing locked doors); 3, total time for a run-through four panels; and 4, running time for first panel only.

All the rats were tested 2 days before being administered ACH, four were retested 24 hr after injection, four were retested at 72 hr post-injection. There were also two controls at each time point which had been administered saline intraperitoneally. The final figure was lower than the planned experiment as three rats were not well enough to test and one had failed to learn the criterion. All of the rats were killed at the conclusion of the test criterion, blood removed for biochemical testing and acetaldehyde assay, in addition to the brain for measurement of its acetaldehyde content.

RESULTS

Biochemical assays

Assay of the plasma from the rats administered either acute ACH or chronic ethanol did not show any consistent changes in markers of liver function or electrolyte balance.

ACH content

The ACH content of brain, liver and blood, after an intraperitoneal injection of ACH, is shown in Fig. 1. There was a rapid rise in the ACH content of each of the tissues, a maximum concentration of between 50 and 60 nmol ACH/g tissue being discernible 4 hr post-injection. After this time point there was a rapid decline in acetaldehyde concentration in both the liver and brain, which at 24 hr was 3 nmol/g tissue and 10 nmol/g tissue, respectively. In contrast, the blood levels of acetaldehyde declined at a slower rate, approximately 40 nmol/g tissue being assayed 24 hr post-ACH injection. After 48 hr no ACH was detectable in either the blood or the liver. However, the brain showed detectable levels of acetaldehyde, approximately 4 nmol/g tissue at each time point assayed up to 120 hr. Similar brain ACH contents were assayed in the Male Lister Hooded rats at 72 hr used for the VDA.

Assessment of oxidative stress by assay of antioxidant content and activities of cytoprotective enzymes after ADH administration

Table 1 shows the activities of the cytoprotective enzymes together with the antioxidant content in both the liver and brain. The oxidative capacity of the liver was clearly superior to that of brain, as exemplified by the high activities of superoxide dismutase, glutathione peroxidase and catalase (6×, 100× and 1000×, respectively, greater than brain). Although the α-tocopherol content were similar in these two organs, reduced glutathione was approximately 2-fold higher in the liver than the brain. Despite the high acetaldehyde content significant changes were only observed in brain glutathione peroxidase and catalase. Glutathione peroxidase showed significant increases at 6 and

Table 1. Oxidative protection in liver and brain

	Cytoprotective enzymes					
	SOD		Catalase		GPx	
	U/g	U/mg protein	mU/g	mU/mg protein	mU/g	mU/mg protein
Liver	14,416 ± 2509	77.5 ± 8.3	60,253 ± 5035	329 ± 46	41,260 ± 6700	222 ± 21
Brain	1679 ± 247	13.1 ± 2.2	42.2 ± 2.7	0.329 ± 0.03	693 ± 80	5.39 ± 0.67

Antioxidants			
α-Tocopherol		GSH	GSSG
nmol/g	nmol/mg protein	nmol/g	nmol/g
33.3 ± 2.4	0.18 ± 0.02	2502 ± 65.0	543 ± 95
22.4 ± 1.4	0.17 ± 0.02	1042 ± 10	278 ± 35

24 hr post-ACH injection (Fig. 2). Brain catalase activity had increased significantly 6 hr post-ACH and remained elevated for almost all time points assayed for the duration of the study (Fig. 2). In contrast, superoxide dismutase and glutathione reductase showed no significant changes in their activities at any of the time points assayed. Apart from an early reciprocal alteration in reduced and oxidized glutathione, at 2.5 and 6 hr, there were no other significant changes in antioxidant content (Fig. 2).

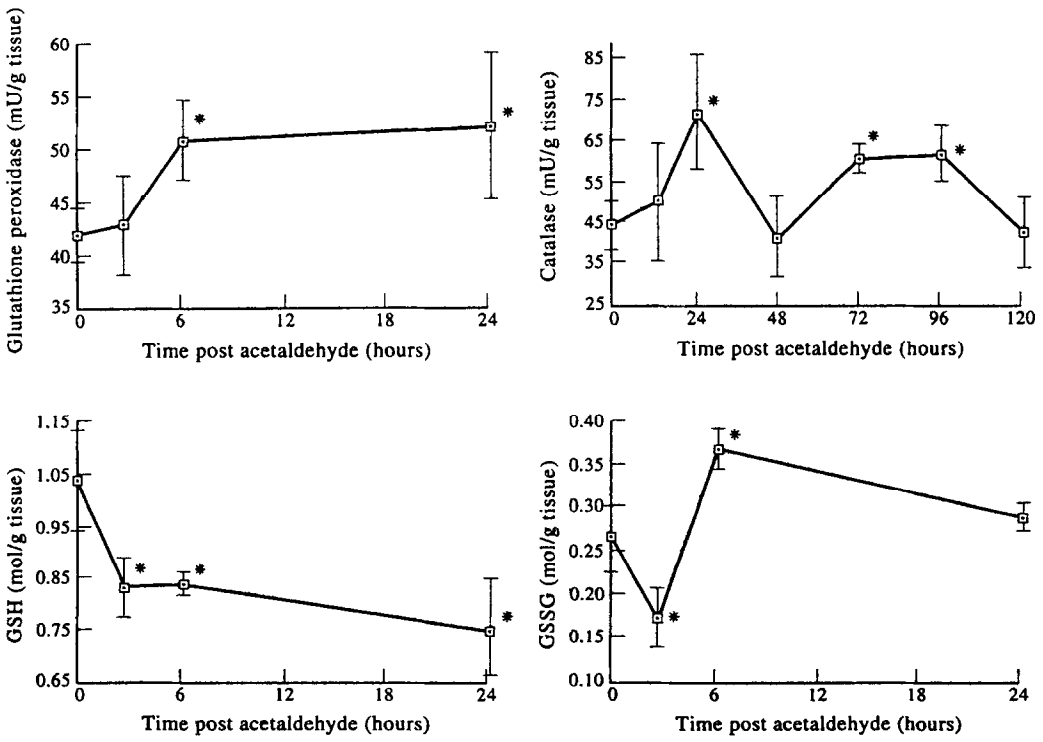

Fig. 2. Changes in brain catalase and glutathione peroxidase activities, and oxidized and reduced glutathione content for up to 120 hr after an acute intraperitoneal dose of acetaldehyde (5 mM/kg).

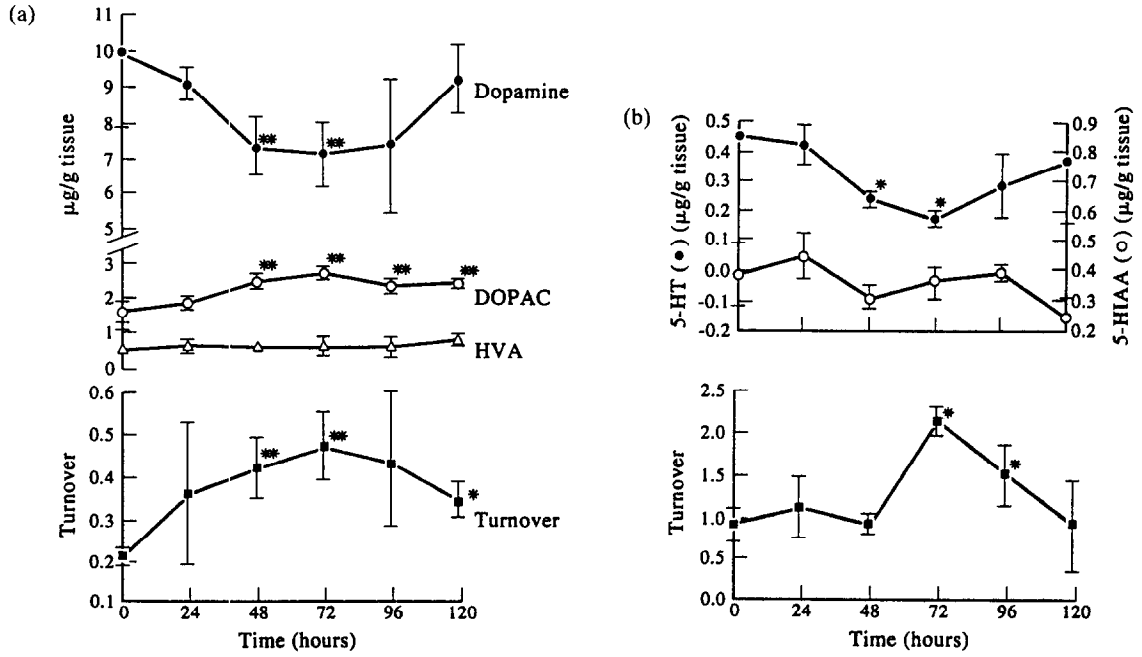

Fig. 3. Changes in striatal dopamine, DOPAC, HVA and dopamine turnover (a), and striatal 5-hydroxytryptamine (b) for up to 120 hr after an acute intraperitoneal dose of acetaldehyde (5 mM/kg).

Assessment of oxidative stress in brain, by assay of antioxidant content and activities of cytoprotective enzymes, after chronic ethanol ingestion

The activities of the cytoprotective enzymes in the brains of the chronically ethanol fed rats are shown in Table 2. Apart from a significant increase in the mean brain catalase activity none of the other enzymes showed any significant changes.

Response of dopamine and its metabolites

To acute dose of ACH. There was a steady decrease in the striatal dopamine content after acute acetaldehyde injection, which reached significance at both 48 hr and 72 hr (Fig. 3a). After these time points dopamine levels recovered such that at 120 hr the levels were similar to the basal striatal content. DOPAC levels, the major metabolite of dopamine in rats, increased significantly at 48 hr, 72 hr, 96 hr and 120 hr. In contrast the levels of the minor metabolite HVA did not change significantly during the test period. Dopamine turnover increased during this period, reaching significance at 48 hr, 72 hr, and 120 hr. The levels of striatal 5HT levels gradually decreased after ACH injection, reaching significance at 48 hr and 72 hr post-injection (Fig. 3b). After this time it gradually increased. In contrast its metabolite, 5HIAA, showed little change at each time point assayed during the study.

Chronic ethanol loading. There were small but insignificant changes in dopamine, DOPAC, HVA 5HT and 5HIAA after chronic ethanol loading. However there was a significant increase in dopamine turnover in the alcohol-fed rats in comparison to that of the rats fed the liquid diet without ethanol supplementation (Table 3).

Table 2. Cytoprotective enzymes in chronically ethanol-fed rats

	Controls (N = 5)	Ethanol-fed (N = 4)
Glutathione peroxidase (mU/mg protein)	21.8 ± 10.2	18.7 ± 11.6
Glutathione reductase (U/mg protein)	0.46 ± 0.18	0.42 ± 0.15
Catalase (mU/mg protein)	0.30 ± 0.10	0.54 ± 0.19
Superoxide dismutase (U/mg protein)	8.1 ± 1.8	6.3 ± 0.74

Visual discrimination test

The results indicated that those rats which were injected with ACH had their learning process disrupted, even though the rats appeared well and were normally active in the reward area. The experimental animals behaved in a hesitant manner in the first panel of the first run, while not making random errors, hesitated and ran up and down in a confused manner. Comparing the running times for the first run only, the rats which had received acetaldehyde demonstrated a significant slowing down in running times by comparison to the control animals (Mann–Whitney, $P < 0.05$). A previous pilot study had indicated that this interference was temporary and recovery was seen within 5–7 days.

DISCUSSION

In this present study a pharmacological dose rather

Table 3. Dopamine metabolism in chronically ethanol-fed rats

	Dopamine	DOPAC	HVA	Turnover	5HT	5HIAA
Controls (N = 5)	14.3 ± 1.35	1.063 ± 0.117	1.57 ± 0.4	0.173 ± 0.02	0.57 ± 0.1	0.46 ± 0.04
Ethanol-fed (N = 4)	13.7 ± 1.36	1.009 ± 0.093	2.06 ± 0.34	0.231 ± 0.036	0.52 ± 0.04	0.416 ± 0.07

than a physiological dose of ACH was administered to the rats. Such a dose was used so that it could be ascertained if ACH was able to cross the blood–brain barrier. It was then possible to investigate the effects of this single acute dose of ACH on brain function, assessed by assay of neurotransmitters and oxidative capacity, as well as behavioral aspects. By giving ACH directly intraperitoneally, this has minimized many criticisms which have been directed at previous studies where ethanol alone was administered, either intraperitoneally or by feeding, such that it was difficult to assess the contribution of ethanol and ACH to various changing parameters. In addition any ACH detected within the tissues or blood in these present studies was unlikely to reflect an artefact of the analytical method [36, 37]. The method utilized for the assay of ACH was extremely sensitive, with a detection limit of 0.1 μM with the advantage that all of the ACH present is immediately trapped by the reagent. Earlier studies which have used headspace GC analysis for ACH determination often experience losses of ACH. Any brain ACH detected in these present studies must have crossed the blood–brain barrier, and was not present as a result of ethanol metabolism either by P450IIEI system in brain cells or of its enzymatic oxidation by catalase.

The content of ACH was only assayed in the liver and brain in this present study. It is likely that ACH will be taken up by other tissues, notably adipose tissues, where large amounts accumulate (unpublished data). ACH was present in the brain at 120 hr after a single acute intraperitoneal dose of ACH; during the first 48 hr the concentration of ACH in the brain was comparable to that of liver. Although the dose of ACH was high, 5 mM/kg, it clearly traversed the blood–brain barrier to enter the brain. A previous *in vivo* study [38] claimed that blood ACH concentration needed to be greater than 250 nmol/L before it would be detected in the brain. Our results differed from this study, the blood concentration being of similar content to that of brain during the first 12 hr, namely 50 nmol/L. In agreement with the study of Ref. 39 the levels of ACH in the brain appeared to be determined principally by blood ACH levels especially during the first 24 hr. However later time points indicated the presence of residual brain ACH. Although further analytical techniques to verify that the HPLC peak in these brain samples was ACH (i.e. mass spectrometry) have not been utilized, the HPLC peak showed a comparable retention time and co-eluted with the ACH standard. The rate at which this ACH will be oxidized will be dependent upon the activity of ALDH.

There is regional variation of ALDH in the brain, the extra nuclear cytoplasm of neuronal and glia cell bodies showing the strongest affinity to an anti-sera to low K_m ALDH. The subcellular localization of the enzyme remains controversial, mitochondria having the highest activity in some studies [40] or evenly distributed between cytosol, microsomes and mitochondria in other studies [41]. It remains unclear where the metabolism of acetaldehyde occurs in the brain although it is clear that it is important that ADH and ALDH should co-exist such that oxidation of ethanol to acetate and CO_2 and water occurs rapidly. The presence of increased P450IIEI expression or of catalase activity may cause an increased oxidation of ethanol, with the net result of high brain acetaldehyde levels.

The brain was noteworthy in its poor oxidative capacity, reflected by the low activities of cyto-protective enzymes in comparison to liver. Oxidative stress could disturb synaptosomal membrane fluidity as well as the metabolism of some neurotransmitters. The mean activity of catalase had increased in the brain of rats 6 hr after administration of ACH, and remained elevated for several days, i.e. up to 96 hr. An increase in the mean catalase activity was also evident in the brains of rats chronically administered ethanol. The explanation for the change in catalase activity remains unclear. There were no significant changes in brain superoxide dismutase activity after either acute ACH or chronically ethanol fed rats although a previous study identified decreases in cytosolic superoxide dismutase activity in the brains of chronically ethanol-fed rats [42]. However, it remains unclear as to whether such decreases reflect free radical generation. It might be expected that the response of the cell to increased generation of superoxide would be to upregulate mRNA synthesis of superoxide dismutase with the net result of an increase in its activity within the cell [43]. Assay of subcellular fractions rather than total brain homogenate may be necessary to observe such changes.

During the first 24 hr after ACH administration there were reciprocal changes in reduced glutathione and oxidized glutathione suggesting some alteration in the reducing poise of the cell. However, the lack of change in glutathione reductase, an important enzyme which facilitates the oxidation of NADPH to $NADP^+$ when oxidative stress is encountered by the cell, might indicate the minimal oxidative stress is imposed on the cell by this concentration of brain ACH. The relatively high activity of glutathione reductase in the brain would help to ensure the reducing environment within the brain in the absence of high amounts of GSH [23]. Although GSH and GSSG were not assayed in the brains of the

chronically ethanol fed rats, a previous study has shown a decrease in brain glutathione content [44].

Studies which have utilized acute high doses of ethanol demonstrated elevated levels of dopa together with increased turnover of both 5HT and dopamine [45], or only dopamine metabolism (synthesis, release and turnover) [46–49]. The increased turnover of dopamine after chronic ethanol ingestion was confirmed in this present study. Dopamine has been implicated in the ethanol-induced tolerance, dependence and withdrawal symptoms often identified in alcohol misusers. However it was unknown whether such changes in dopamine metabolism is caused by ethanol or ACH. Our present study clearly shows that ACH is capable of initiating changes in dopamine and 5HT metabolism in the striatum in a similar fashion to that previously reported for small doses of ethanol. Combining our two sets of observations it may indicate that it is the metabolite of ethanol, rather than ethanol itself having an effect on the dopaminergic and serotonin systems. Precisely how ACH induces such changes is unclear, although the changes were reversible.

The visual discrimination task was selected in these present studies as it has a rough analogue to that offered to the alcoholic patients and in which they show a significant deviation. A single dose of ACH given to rats showed that established learning is not affected but further learning is at least temporarily impeded. Such an effect was noteworthy since at the time point that the rats were required to learn a new schedule, the brain ACH levels were relatively low. Further studies are clearly needed to investigate changes in various neurotransmitters after ACH administration.

These studies have clearly identified the toxic effects of acetaldehyde on various brain functions, which may be responsible for many of the adverse effects identified after chronic ethanol administration. Further investigations of the brain distribution of ACH, together with that of ALDH activity, are warranted which may identify which regions of the brain are vulnerable to the toxic effects of acetaldehyde. It was noteworthy that behavioural changes were apparent in the rat after only one dose of ACH further which further substantiated the toxicity of this ethanol metabolite.

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