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Brain Research Bulletin 68 (2005) 171–178

RESEARCH BULLETIN

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The hypothalamopituitary–adrenal axis and alcohol preference

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Received 11 May 2005; received in revised form 2 August 2005; accepted 17 August 2005
Available online 8 September 2005

Abstract

Effects of alterations in stress hormones and their actions were investigated on alcohol preference, by intraperitoneal administration of RU38486 (a Type II glucocorticoid receptor antagonist, also given by the intracerebroventricular route), spironolactone (a Type I glucocorticoid receptor antagonist), metyrapone (a corticosterone synthesis inhibitor), corticosterone, adrenocorticotropin (ACTH1-39), or intracerebroventricular injection of corticotropin releasing factor (CRF) or a CRF antagonist (alpha-helical CRF9–41). Intracerebroventricular or intraperitoneal administration of RU38486 did not alter the alcohol consumption of mice with high preference for alcohol, or, on first administration, the intake of those with low alcohol preference. When given by repeated intraperitoneal injection however this drug prevented the increase in alcohol consumption seen in "low preference" mice after 3 weeks vehicle injections. Spironolactone did not alter alcohol preference when given by intracerebroventricular or intraperitoneal routes. Repeated, but not single, administration of metyrapone reduced alcohol preference in both high and low preference animals and prevented the increase from low alcohol preference caused by repeated vehicle injections. ACTH1–39 or corticosterone administered by single or repeated intraperitoneal injection, or CRF given i.c.v., did not alter alcohol preference, but the CRF antagonist, alpha-helical CRF9–41, caused a transient increase from low alcohol preference. Blood corticosterone concentrations prior to preference measurements did not correlate with the alcohol preference of the mice. The results indicate that delayed consequences of corticosterone acting on Type II glucocorticoid receptors may be involved in the increases in alcohol preference after injection stress. They also suggest that central actions of CRF may influence the low alcohol consumption of the low alcohol-preferring mice.

Keywords: Alcohol preference; Corticosterone; CRF

1. Introduction

Much evidence now implicates hypothalamic–pituitary–adrenal (HPA) hormones in drug dependence. Corticosterone has been found to increase self-administration of psychostimulants [12]. Metyrapone, that inhibits corticosterone synthesis, reduced the reinstatement of self-administration in rats which had undergone extinction of self-administration of cocaine [28]. Footshock stress reinstated operant self-administration of alcohol [17]. Considerable evidence has been presented by Fahlke and co-workers for the involvement of corticosterone in voluntary alcohol consumption by rats [7–10]. In addition, rats will self-administer corticosterone to achieve plasma concentrations (1–1.5 μM) in the stress range [27] and will also self-administer adrenocorticotropin (ACTH [16]). The alcohol withdrawal syndrome involves increased release of corticosterone [31] and raised corticotropin releasing factor (CRF) concentrations [21,30].

Early work demonstrated increases in voluntary alcohol drinking after stress produced by immobilization [22,32] or footshock [34], although not all studies have found such an effect with these types of stress (e.g. [11,23]). Rodents vary considerably in the amount of alcohol that they consume voluntarily. A consistent pattern in the effects of stress has been the demonstration of increased alcohol consumption after stress in low preference animals, with less, or no, effect on the consumption of individuals with high preference for alcohol prior to the stress. This pattern was seen by Volpicelli et al. (1990) and Rockman et al. [32] and in our previous work [18,24,25].

Our previous studies on alcohol consumption by the C57BL/10 (line ScSn) line of mice [18] showed they have a wide range of preference for alcohol, when tested in a two bottle, free choice model. Although the C57 strain of mouse has been widely used for many years as an alcohol-preferring strain

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doi:10.1016/j.brainresbull.2005.08.006

[1,20,29], when mice from our breeding line are placed in a free choice situation with a bottle of dilute alcohol (8%, v/v) and a bottle of tap water, a considerable percentage (10–40% of mice in any tested group) had a low preference for alcohol, consuming the majority (70–100%) of their fluid in the form of water [18]. The preference was consistent for individuals, and a biphasic distribution was seen, with the large majority of the individuals having either high or low alcohol preference and very few falling in the intermediate range. The differences in alcohol preference did not appear to be inherited and were not due to differences in alcohol metabolism [18]. Our previous work also showed that once daily intraperitoneal injections of saline for 3 weeks significantly increased the alcohol preference of mice characterised as "low preference" (i.e. which showed consumption ratios of dilute alcohol to total fluid of less than 0.35, in a free choice two bottle preference test choice of 8% alcohol and tap water [18,24]). This increase in preference was seen only after at least a week of daily injections. Preference for a sucrose solution was unaffected. The change was prevented by administration of a CCK_B receptor antagonist, but unaffected by diazepam [18]. The effect of the multiple saline injections was shown to be caused by the actual injection procedure, not the administration of fluid, as it was apparent after sham injections, when no fluid was injected, but not when mice were only handled [24]. We also found that repeated injections of the vehicle used for administration of non-aqueous soluble drugs, 0.5% (v/v) Tween 80, increased the alcohol preference of the low preference C57 mice, with a delayed effect as was seen in the effects of the daily saline injections [18].

In the present study, the involvement of the components of the HPA axis in the alcohol preference of these mice was examined. The studies measured the effects of drugs acting on each of these components in turn, the glucocorticoid receptors, glucocorticoid synthesis and the effects of ACTH, and of CRF and a CRF antagonist. The studies aimed specifically to examine the effects of the drugs on the raised alcohol preference in low preference animals caused by repeated vehicle injections and on the innate high alcohol preference in other animals. The effects of the Type II glucocorticoid receptor antagonist, RU38486, the Type I glucocorticoid receptor antagonist, spironolactone, and the corticosterone synthesis inhibitor, metyrapone, were studied in order to determine whether or not glucocorticoid receptors were involved in the range of alcohol preference. Effects of the receptor antagonists, and of metyrapone, on the action of repeated vehicle injections in increasing the alcohol preference of low preferring mice were investigated, to determine whether the increase in preference could be prevented by these drugs. The short term effects of the receptor antagonists when given by the intracerebroventricular (i.c.v.) route were also examined.

The effects on the alcohol consumption of low alcohol-preferring mice of intraperitoneal administration of corticosterone and of ACTH1–39 were studied in order to determine whether or not the innate low preference could be increased by exogenous administration of these hormones in the short term. Effects of CRF and a CRF antagonist were measured in both high and low alcohol-preferring mice, i.c.v. administration being used because these compounds do not enter the CNS after systemic administration.

Owing to the limited availability of the mice in this colony it was not possible to examine a range of doses of each drug. Single doses were therefore chosen that have been shown to cause behavioural changes in other studies [2,13–15]. In addition to the effects of drugs on alcohol preference, we measured the circulating blood corticosterone concentrations prior to alcohol preference screening, and after 3 weeks once daily saline injections to determine whether or not there was any correlation between circulating hormone levels and alcohol preference.

2. Methods

All animals in these investigations were bred "in house". The animals originated from the Bristol Medical School animal facility, and breeding has continued for 15 years with no introduction of new stock. The bimodal distribution of alcohol preference in these mice [18,24,25] has been maintained throughout this time.

All mice were housed at $21 \pm 1\,^{\circ}C$, with $55 \pm 10\%$ relative humidity, and a 12 h light/dark cycle, with the light phase between 08.00 and 20.00 h, and free access to tap water and laboratory rodent chow (CRM) at all times. The conditions of housing of the breeding pairs were kept consistent among the pairs. The mean litter size was seven, and the pups were weaned at 19–21 days, at which point they were transferred to new cages in single sex groups of 10 per cage. In these, mice from different litters were mixed and this housing was kept consistent between this time and the alcohol screening or other tests. As our initial studies had shown that there was no influence of gender on the alcohol preference [18], both male and female mice were used in the present studies.

2.1. Alcohol preference measurements

All tests of preference were made on mice housed individually. In the preference measurements, two fluid bottles were made available to the animals, for the whole of every 24 h period. For all the studies, one bottle contained tap water and the other, alcohol diluted to 8% (v/v). with tap water. The positions of the bottles in the different cages were randomised with respect to which side of the cages they were placed. In all experiments, the ratio of alcohol to water consumed, and the total fluid consumption, were calculated.

To establish the alcohol preference of each animal prior to the studies on the effects of drugs, a screening procedure was used in which the animals were housed singly, and two bottles, one containing 8% alcohol (v/v) and one containing tap water, were made continuously available for 3 weeks. Measurements of fluid intake were made, three times per week (Monday, Wednesday and Friday, at 09.00–10.00 h), and the amount drunk from each bottle used to calculate the preference for 8% alcohol compared with water. The mean ratios for the last week of measurements were used to allocate mice to the preference categories. Mice with a ratio of 0.75 and above for the consumption of 8% alcohol to total fluid intake were classed as "high" drinkers, and those showing a ratio of 0.34 and lower were classed as "low" drinkers. The cages were not cleaned during the last week of the screening procedure, in case this altered the alcohol consumption.

This standard screening procedure was carried out on all animals prior to the tests described below (with the exception of the experiment in which blood samples were taken prior to the screening, see below). From this mice were then classed as "high preference" or "low preference", the small numbers of "intermediate" preference animals (alcohol preference 0.35–0.74) not being used.

2.2. Drug administration

In the studies on the glucocorticoid receptor antagonists, RU38486 and spironolactone, the aims were to determine whether or not the drugs decreased the alcohol preference of high preference mice, when given over a period of

1 week, and whether or not the drugs prevented the increase in preference caused by vehicle injections previously demonstrated to occur over a period of 3 weeks. The effects of ACTH and of corticosterone were examined to determine if the low alcohol preference could be directly increased by acute effects of these hormones. CRF and its antagonist, alpha-helical CRF, could only be examined after i.c.v. injection, as these compounds do not penetrate the CNS after systemic administration. Because the i.c.v. injection method is suitable only for single administration, the short term effects of these compounds were studied.

2.3. Administration of glucocorticoid receptor antagonists

The effects of the glucocorticoid Type II receptor antagonist, RU38486, and of the Type I receptor antagonist, spironolactone, were investigated on alcohol preference by once daily intraperitoneal injection in mice with low and high alcohol preference, between 17.00 and 18.00 h, just prior to the nocturnal activation phase. RU38486 was suspended in its vehicle (Tween 80, 0.5% (v/v) in distilled water). Once daily injections of either RU38486, 100 mg/kg, or its vehicle, or spironolactone, 50 mg/kg, were given for 3 weeks. The fluid consumption was measured three times per week (Monday, Wednesday, Friday), starting on Day 0 (Friday) when the first drug injections were given. The first intake measurements were therefore obtained on Day 3 (Monday). *N* values were 8 per treatment group.

In a separate study, a single injection of either RU38486 or spironolactone was given by the i.c.v. route. The injections were made in conscious mice, using a custom made apparatus that enables injection into the third ventricle [36]. Each antagonist was given at 150 μg/mouse in 2 μL. The vehicle was 0.05% Tween 80 in distilled water, and control animals received corresponding i.c.v. injections of the vehicle. Previous studies showed that i.c.v. injection of this Tween vehicle had no effect on the behaviour of mice in volumes up to 5 μL [35]. *N* values were 6 per treatment group. The injections were carried out between 17.00 and 18.00 h, just prior to the nocturnal activation phase, and the fluid drunk from each bottle was measured 6 h after the injections.

2.4. Administration of metyrapone

Effects of the corticosterone synthesis inhibitor, metyrapone, were determined by single and repeated intraperitoneal injection in mice with low and high preference for alcohol. Metyrapone was suspended in Tween 80 (0.5%, v/v), and the suspension sonicated prior to injection. A preliminary study showed that a dose of 50 mg/kg metyrapone had no effect on alcohol preference or alcohol intake. These experiments therefore used a dose of 100 mg/kg metyrapone, given twice daily (09.00–10.00 h and 16.00–17.00 h). The drug administration was continued for seven days for the high alcohol preference mice, with the first injections on Day 0 (Friday) and fluid consumption measured once daily thereafter. For the low preference animals, metyrapone administration started on Day 0 (Friday) and continued for 3 weeks, with the first intake values obtained on Day 3 (Monday). Control animals received corresponding vehicle injections throughout, *N* values were 8 per treatment group.

2.5. Administration of ACTH1–39

This experiment examined the actions of the ACTH fragment 1–39 (Sigma) on alcohol preference of mice with low preference for alcohol. The peptide was dissolved in sterile saline and given intraperitoneally once daily between 17.00 and 18.00 h, for 4 days. The fluid intake of all animals was measured on the Friday (Day 0) and Monday (Day 3) preceding the start of injections and then once daily for 4 days (Days 4–7). Control animals received corresponding saline injections; *N* values were 9 per treatment group.

2.6. Administration of corticosterone

Corticosterone was given by the intraperitoneal route to low alcohol preference mice, at 20 mg/kg, once daily between 17.00 and 18.00 h, for 1 week; *N* values were 9 per treatment group. Fluid intake was measured on the day prior to (Day 0) and each day during the administration (Days 1–8).

2.7. Administration of CRF and of a CRF antagonist

Effects of h/rCRF (Sigma) and of the CRF antagonist, alpha-helical CRF9–41 (Sigma) were examined on the alcohol intake of mice with low and with high alcohol preference. The compounds were injected i.c.v. (in conscious mice, see above), at 17.00 h, both at doses of 5 μg per mouse, in 2 μL. The compounds were dissolved in isotonic saline and controls received corresponding saline injections. *N* values were 6 per treatment group. Fluid intake was measured 12, 24 and 36 h after the injections.

2.8. Measurements of corticosterone concentrations

Samples of tail vein blood were taken prior to screening for alcohol preference, in order to determine whether or not the plasma corticosterone concentrations differed in high and low preference mice prior to their exposure to alcohol. Samples were taken from 48 mice, 25–30 g. Of these, 24 were sampled at 08.00–08.30 h, and 24 at 20.00–20.30 h, to provide information on both morning and evening corticosterone levels. The method of Durschlag et al. [6] was used for obtaining the blood samples from the base of the tail vein.

After the blood sampling, the mice were placed in single cages, in a different stock room, and screened for their alcohol preference for the next 3 weeks, as above. Comparison was made with the results of alcohol preference screening on a different set of 48 mice which were not subjected to the sampling procedure, but which were otherwise treated concurrently in exactly the same way, in order to check whether or not the sampling procedure affected the subsequent alcohol preference. These animals were not used for further experiments.

In a separate set of animals, the effects of repeated intraperitoneal saline injections were examined on circulating corticosterone concentrations in mice with low alcohol preference. The mice were first screened for alcohol preference, as described above. Two groups, $N = 6$ per treatment group were selected that had low alcohol preference, as defined above, their alcohol bottle was removed and they were supplied with normal tap water only for the remainder of the experiment. One group was injected once daily, between 17.00 and 18.00 h, with isotonic saline for 3 weeks; the other group were handled, once daily, in the same way for the injection procedure but no injection was made. At the end of the 3 weeks, blood samples were then taken from the tail vein, between 20.00 and 21.00 h and blood corticosterone concentrations assayed by radioimmunoassay.

Blood corticosterone concentrations were measured by radioimmunoassay. Corticosterone concentrations were determined by radioimmunoassay using a specific corticosterone polycolonal antiserum raised in rabbits from ICN diagnostics. The antibody had a cross-reactivity of <3% to deoxycorticosterone and 0.01–0.3% to other steroids. Plasma samples were diluted 0.05 M Tris–HCl, pH 8, 0.1 M NaCl, 0.1% bovine serum albumen and 0.1% sodium azide. Aliquots of diluted sample were heated to 98 °C in a water bath for 10 min to inactivate the corticosterone binding globulin, then [3*H*]-corticosterone (10,000 cpm/0.1 ml) and 0.1 ml of anti-corticosterone (ICN) were added and incubated at 4 °C overnight. The following day, ice-cold 0.5% dextran coated charcoal suspended in Tris–HCl buffer was added, then the tubes were incubated for a further 20 min, centrifuged ($2500 \times g$) for 15 min at 4 °C, the supernatant decanted and scintillation fluid added before liquid scintillation counting.

2.9. Statistical analysis

The results were compared by two-way analysis of variance (drug treatment versus days), followed by Bonferonni post hoc tests for comparisons between drug and control for each day and between measurements on first day compared with subsequent days.

3. Results

Total fluid intake was not significantly altered by any of the drug treatments and consequently, throughout the results the alcohol intake (measured in g/kg alcohol) changed in parallel with the alcohol preference (measured as the ratio of volume of 8% alcohol consumed to total fluid). The ethanol consumption of the low preference mice prior to drug treat-

ments was 1–3 g/kg/day and that of the high preference animals 14–16 g/kg/day (blood and brain alcohol concentrations after these levels of consumption were given in [25].

3.1. Effects of glucocorticoid antagonists

The Type I glucocorticoid receptor antagonist, spironolactone, did not alter the alcohol preference or intake of mice with high or low preference for alcohol (data not shown), whether given by the intraperitoneal or intracerebroventricular route.

The effects of intraperitoneal administration of the glucocorticoid Type II receptor antagonist, RU38486 are illustrated in Fig. 1. Once daily injections of RU38486 for 3 weeks, or the corresponding vehicle, did not significantly alter the alcohol intake of the high preference mice (Fig. 1a, $P > 0.1$). For the experiment using low preference mice, there was a significant effect of both day ($F[7,112] = 3.83$; $P < 0.001$) and drug treatment ($F[1,112] = 53.9$; $P < 0.0001$). The schedule of daily injections of the Tween vehicle significantly increased the alcohol preference of low preference mice by the third week of treatment, as illustrated in Fig. 1b ($P < 0.01$ for comparison between Day 1 and Days 15, 17 or 19), but in the mice that received corresponding injections of RU38486, 100 mg/kg, no change in preference was seen over time (Fig. 1b). The difference between the preference after RU38486 or vehicle administration was significant on Days 15, 17 and 19 ($P < 0.001$).

i.c.v. injection of RU38486 did not alter the alcohol preference measured 6 h after the treatment (data not shown).

3.2. Effects of metyrapone

Administration of metyrapone, 100 mg/kg twice daily for 7 days, significantly reduced the alcohol preference of mice with high alcohol preference (Fig. 2a), compared with the preference after vehicle injections ($F[1,112] = 52.03$, $P < 0.0001$ for drug treatment; no significant effect of day). The preference was significantly lower on Days 3–7 of the experiment ($P < 0.01$, $P < 0.05$, $P < 0.01$ and $P < 0.001$) in animals receiving the glucocorticoid synthesis inhibitor, compared with that after vehicle injections on same day.

In mice with low alcohol preference (Fig. 2b), in the metyrapone experiment the effects of both day ($F[7,112] = 9.52$, $P < 0.0001$) and drug treatment were significant ($F[1,112] = 226.2$, $P < 0.0001$). The vehicle injections caused an increase

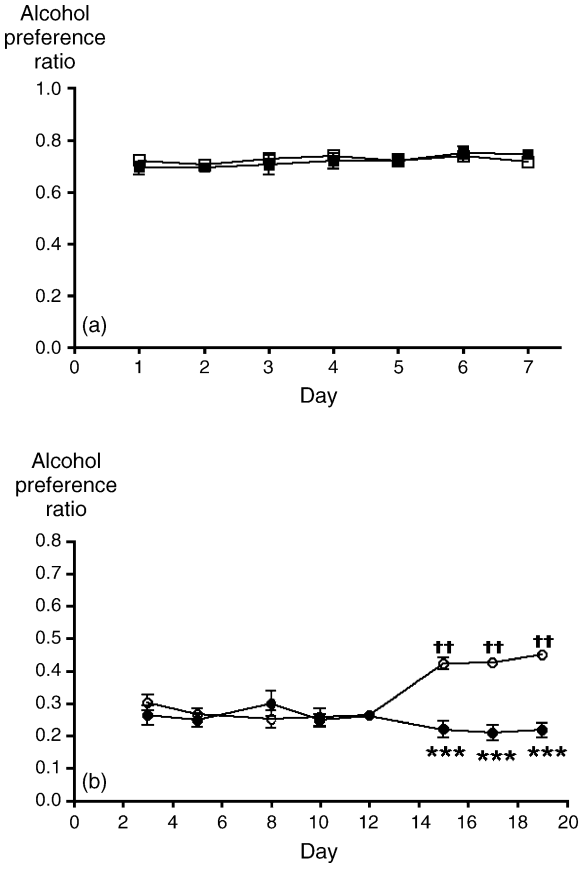

Fig. 1. Effects of repeated intraperitoneal injection of the Type II glucocorticoid receptor antagonist, RU38486, on alcohol preference in "high preference" mice (a) and "low preference mice" (b). RU38486 was given once daily from the beginning of the experiments (i.e. starting on Day 0). Open squares and circles = vehicle injections, black squares and circles = RU38486, 100 mg/kg. $^{\dagger\dagger}P < 0.01$ for comparison with Day 1; $^{***}P < 0.001$, comparison with vehicle injections on same day.

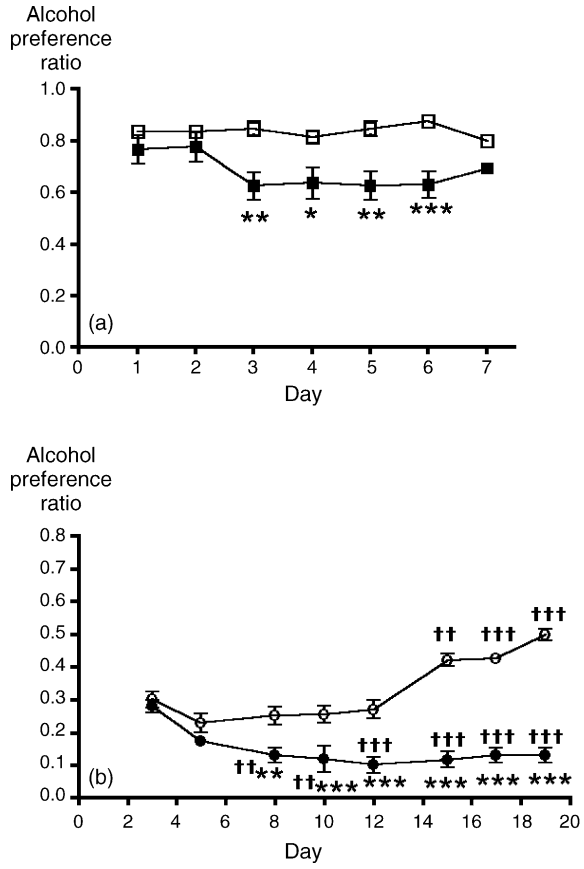

Fig. 2. Effects of repeated intraperitoneal injection of metyrapone on alcohol preference in "high preference" mice (a) and "low preference mice" (b). Metyrapone was given twice daily from the beginning of the experiments (i.e. starting on Day 0). Open squares and circles = vehicle injections, black squares and circles = metyrapone, 100 mg/kg. $^{\dagger\dagger}P < 0.01$; $^{\dagger\dagger\dagger}P < 0.001$ for comparison with first day of corresponding injections; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, comparison between vehicle and metyrapone injections on each day.

in alcohol preference that was significant during the third week of treatment, on Days 15, 17, and 19 ($P < 0.01$, $P < 0.001$ and $P < 0.001$, for comparison with first day). The mice that were given metyrapone showed alcohol preference that was significantly lower ($P < 0.01$ Days 8 and 10, $P < 0.001$ other days) than those receiving vehicle injections in both the second and third weeks of the study. In addition, the alcohol preference of the low preferring mice given RU38486 was significantly lower than on the first day from Day 8 onwards ($P < 0.01$ Day 8, other days $P < 0.001$, for comparison with first day of measuring).

3.3. Effects of ACTH1–39

ACTH1–39 did not alter the alcohol preference of low alcohol-preferring mice, when given once daily for 4 days (Fig. 3a).

3.4. Effects of corticosterone

Administration of corticosterone for 1 week did not alter the alcohol preference of low alcohol-preferring mice (Fig. 3b).

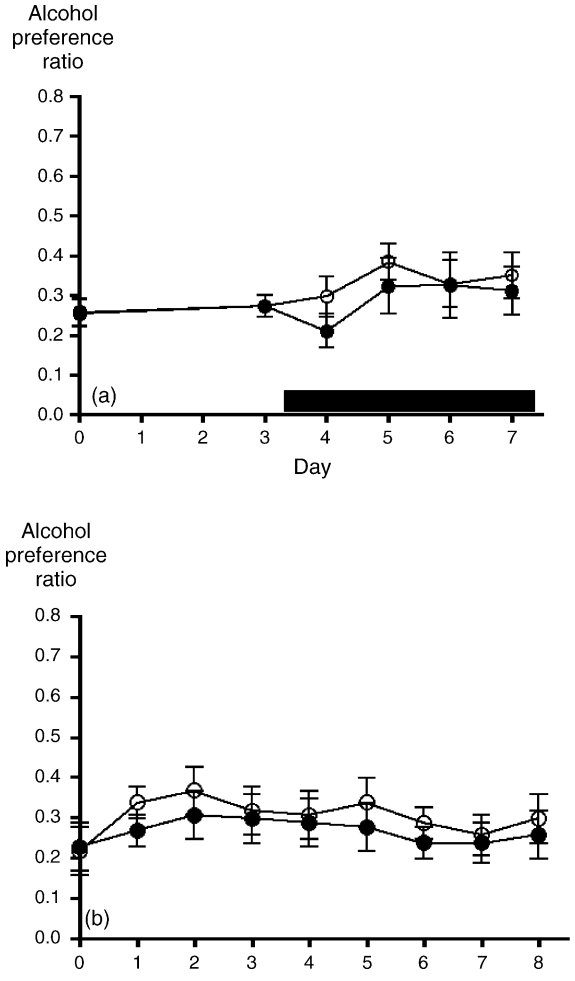

Fig. 3. Effects of repeated intraperitoneal injection of ACTH1–39 (a) or of corticosterone (b) on the alcohol preference of low preference mice. Open circles = vehicle injections, black circles = ACTH (a) or corticosterone (b). Grey bar in (a) indicates duration of once daily injections of ACTH; hatched bar in (b) indicates duration of once daily injections of corticosterone.

3.5. Effects of CRF and the CRF antagonist

i.c.v. injection of CRF did not alter the alcohol preference of either high or low alcohol-preferring mice (data not shown).

The preference of high alcohol-preferring mice given alpha-helical CRF remained unaltered during the measurements (Fig. 4a), compared with both control values and with the baseline measurements.

In low alcohol-preferring mice (Fig. 4b), there were significant effects of time ($F[3,56] = 9.0$, $P < 0.0001$) and drug treatment ($F[1,56] = 2.9$, $P < 0.05$). The CRF antagonist, alpha-helical CRF, increased the alcohol preference significantly at the 12 h measurement compared with vehicle injection ($P < 0.01$). By the subsequent two measurements, the preference of the low preferring mice given alpha-helical CRF had decreased and was no longer significantly different from baseline values. The preference of control animals, however, increased during this time, and this change was significant at the 60 h measurement ($P < 0.01$ compared with baseline values).

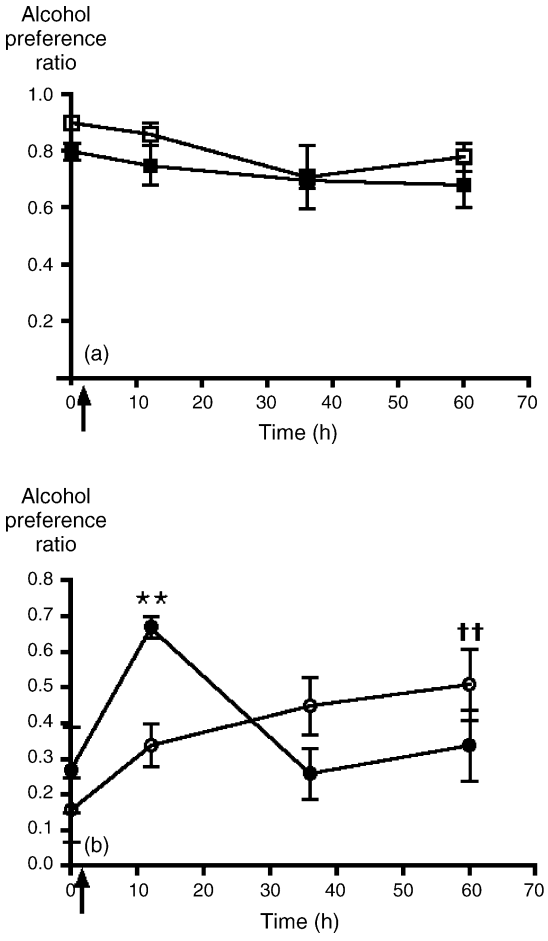

Fig. 4. Effects of intracerebroventricular injection of the CRF antagonist, alpha-helical CRF, on the alcohol preference of high alcohol-preferring (a) and low alcohol-preferring (b) mice. Open squares, circles = vehicle injections, black squares, circles = alpha-helical CRF. Arrows indicate time of administration of the CRF antagonist; $^{\dagger\dagger}P < 0.01$ for comparison with baseline preference; $^{**}P < 0.01$ for comparison between CRF antagonist and vehicle injections.

Table 1
Blood corticosterone concentrations (nM) in low and high preference mice, measured prior to alcohol consumption

Treatment group and time of sample	Blood corticosterone concentration (nM, mean ± S.E.M.)
Low preference, pm	80.7 ± 26.2
High preference, pm	100.4 ± 39.0
Low preference, am	352.9 ± 76.7
High preference, am	306.8 ± 47.7

There were no significant differences between the corticosterone concentrations in low alcohol preference and high alcohol preference at either time point.

Table 2
Blood corticosterone concentrations in low alcohol preference mice after 3 weeks once daily intraperitoneal injections of saline

Treatment group	Blood corticosterone concentration (nM, mean ± S.E.M.)
Handled	391.6 ± 34.8
Saline injection, i.p.	417.9 ± 26.3

3.6. Corticosterone concentrations

The blood concentrations of corticosterone prior to alcohol preference measuring are shown in Table 1. There were no differences in the plasma corticosterone levels of mice who were subsequently demonstrated to be high or low preference, either when the samples were taken during the diurnal surge, just prior to the nocturnal peak in activity, or during the basal levels when the corticosterone remains low during the light phase.

The procedure of taking the blood samples did not affect the distribution of alcohol preference in the two sets of 48 mice, when this was screened for 3 weeks following the sampling (data not shown).

Three weeks, once daily, injections of saline did not alter the blood corticosterone concentrations, when these were measured at the end of the procedure (Table 2).

4. Discussion

Administration of the Type II receptor glucocorticoid antagonist, RU38486, did not alter the alcohol consumption or preference of high alcohol preference mice, at a dose that was effective in preventing the rise in preference of low alcohol preference animals induced by repeated vehicle injections. In the low alcohol preference mice, there was no effect of this antagonist, apart from the prevention of the increase in preference. The Type I receptor antagonist, spironolactone, had no effects on alcohol preference at a dose shown to have behavioural effects in other situations [3,19], and the i.c.v. doses of both antagonists had no effects, again at doses that have behavioural actions [5]. However, the corticosterone synthesis inhibitor, metyrapone, decreased the alcohol consumption of both high and low preference mice, the effects being seen in both instances after 2 days administration of this drug.

The results using RU38486 suggest that the difference between the alcohol intake of low and high alcohol preference mice does not involve Type I or II glucocorticoid receptors. However, the action of metyrapone suggests that glucocorticoid release is involved in the control of alcohol consumption in both groups of mice. This is in partial agreement with results obtained by Fahlke and co-workers in rats. These authors found administration of metyrapone at 50 mg/kg twice daily decreased alcohol consumption only in high alcohol-preferring rats [8]. It is possible that our demonstration of this effect in both high and low alcohol-preferring animals was due to the higher dose of metyrapone used in the present study, but Fahlke and colleagues found adrenalectomy decreased alcohol intake in both high and medium alcohol-preferring animals [9], but not low preferring [7]. This suggests that there is a difference between the involvement of glucocorticoids in control of alcohol consumption in the strains of rats used by Fahlke and co-workers and the mice in the present study. In agreement with the present work, no effects of the Type II receptor corticosterone antagonist, RU38486, on alcohol consumption in high or low preferring rats were seen by Fahlke et al. [10], although a lower dose was used (25 mg/kg twice daily) than in our work (100 mg/kg once daily). The lack of acute effect of corticosterone on the alcohol consumption in our mice is in agreement with the results of Fahlke et al. [10].

Only a small number of studies have been carried out on effects of the other stress hormones in alcohol consumption. ACTH was reported to cause an increase in alcohol drinking in rats [22]. CRF was reported to reduce alcohol consumption of rats and CRF knockout mice demonstrated higher voluntary alcohol consumption than wild-type animals [2,26]. Our results demonstrated that ACTH1–39, and CRF had no acute effects on alcohol preference, at doses reported to cause behavioural changes in other studies [2,13,15]. However, the CRF antagonist transiently raised the alcohol preference of our low preferring animals. This suggests that CRF may be acting to reduce the alcohol preference in these animals, which would be compatible with the results of Bell et al. [2] and Olive et al. [26]. Alpha-helical CRF is not specific for any subtype of CRF receptor [4]. The lack of effect of ACTH, however, suggests that this action of CRF is not via ACTH release, but may be a neuronal action, such as has been demonstrated for CRF in behavioural studies [33]. An anxiogenic action of CRF is thought to be involved in the behavioural signs of alcohol withdrawal, as a selective antagonist of this hormone decreased anxiety-related behaviour during the acute phase of alcohol withdrawal [30]. CRF has also been shown to be involved in reinstatement of operant responding for alcohol [17]. The low alcohol-preferring animals in our C57 breeding line may therefore have greater central activity or concentrations of CRF than the high alcohol-preferring mice; this is currently under investigation. The fact that administration of CRF did not affect the alcohol preference of high preferring mice could have been due to differences in responsivity to CRF; this is also under further investigation.

The measurements of blood corticosterone concentrations showed that there was no direct relationship between the innate differences in alcohol preference of our C57 line of mice and circulating levels of the hormone. This was also the case for

the increased alcohol preference caused by the repeated vehicle injections, despite the evidence from the drug treatment that this effect involves corticosterone (discussed below).

The effects of metyrapone and of RU38486 in preventing the increase in alcohol preference due to repeated vehicle injections clearly indicates that corticosterone is involved in this effect. That the increased preference was a response to the stressful aspects of the procedure was suggested by our previous work that showed that the injection procedure, rather than the administration of fluid, was crucially involved in this slowly developing increase in alcohol preference [24], The increase was prevented by a CCKB antagonist, but diazepam, at an anxiolytic does, had no effect [18]. The slow development of the increased preference, together with the lack of acute effects of corticosterone in low alcohol preference mice, and the lack of prolonged changes in blood corticosterone, suggests that some adaptation to the repeated minor stress is involved, rather than a direct action of the hormone.

In conclusion, the results suggest that the corticosterone is involved in the control of alcohol consumption in both low and high alcohol preference mice, but that the difference in alcohol preference in these animals is not due to differences in corticosterone activity or in circulating levels of this hormone. However, differences in the central activity of CRF, possibly involving neuronal actions, may be involved in this distinction in alcohol preference. The increased preference for alcohol in initially low preference animals caused by repeated vehicle injections appears to involve a release of corticosterone elicited by the injection procedure, that acts on Type II glucocorticoid receptors, but this produces slowly developing neuronal changes that result in the increased alcohol consumption.

Acknowledgement

We thank NIAAA for financial support for these studies.

References

[1] J.K. Belknap, J.C. Crabbe, E.R. Young, Voluntary consumption of alcohol in 15 inbred mouse strains, Psychopharmacology 112 (1993) 503–510.
[2] S.M. Bell, J.G. Reynolds, T.E. Thiele, J. Gan, D.P. Figlewicz, S.C. Woods, Effects of third intracerebroventricular injections of corticotropin releasing factor (CRF) on ethanol drinking and food intake, Psychopharmacology 139 (1998) 128–135.
[3] M.A. Cole, B.A. Kalman, T.W.W. Pace, F. Topczewski, M.J. Lowrey, R.L. Spencer, Selective blockade of the mineralocorticoid receptor impairs hypothalamo-pituitary adrenal axis expression of habituation, J. Neuroendocrinol. 12 (2000) 1034–1042.
[4] F.M. Dautzenberg, G.J. Kilpatrick, R.L. Hauger, J.-L. Moreau, Molecular biology of the CRH receptors—in the mood, Peptides 22 (2001) 753–760.
[5] E.R. De Kloet, Brain corticosteroid receptor balance and homeostatic control, Front Neuroendocrinol. 12 (1991) 95–164.
[6] M. Durschlag, H. Wurbel, M. Stauffecher, D. Von Holst, Repeated blood collection in the laboratory mouse by tail incision—modification of an old technique, Physiol. Behav. 60 (1996) 1565–1568.
[7] C. Fahlke, C.J. Erickson, Effect of adrenalectomy and exposure to corticosterone on alcohol intake in alcohol preferring and alcohol avoiding rat lines, Alcohol Alcohol. 35 (2000) 139–144.
[8] C. Fahlke, J.A. Engel, C.P.J. Eriksson, E. Hard, B. Soderpalm, Involvement of corticosterone in the modulation of ethanol consumption in the rat, Alcohol 3 (1994) 195–202.
[9] C. Fahlke, E. Hard, R. Thomasson, J.A. Engel, S. Hansen, Metyrapone-induced suppression of corticosterone synthesis reduces ethanol consumption in high-preferring rats, Pharmacol. Biochem. Behav. 48 (1994) 977–981.
[10] C. Fahlke, E. Hard, C.P.J. Eriksson, J.A. Engel, S. Hansen, Consequence of long-term exposure to corticosterone or dexamethasone on ethanol consumption in the adrenalectomised rat, and the effect of type I and type II corticosteroid receptor antagonists, Psychopharmacology 117 (1995) 216–224.
[11] T.L. Fidler, V.M. LoLordo, Failure to find postshock increases in alcohol preference, Alcohol Clin. Exp. Res. 20 (1996) 110–121.
[12] N.E. Goeders, G.F. Guerin, Effects of surgical and pharmacological adrenalectomy on the initiation and maintenance of intravenous cocaine self-administration in rats, Brain Res. 722 (1996) 145–152.
[13] A.G. Gunin, V. Emelianov, A.S. Tolmachev, Effect of adrenocorticotrophic hormone on the development of oestrogen-induced changes and hyperplasia formation in the mouse uterus, Reproduction 123 (2002) 601–611.
[14] S.C. Hienrichs, K.T. Britton, G.F. Koob, Both conditioned taste preference and aversion induced by corticotropic-releasing factor, Pharmacol. Biochem. Behav. 40 (1991) 717–721.
[15] J.P. Hinson, D. Renshaw, M. Carrol, S. Kapast, Regulation of rat adrenal vasoactive intestinal peptide content: effects of adrenocorticotropic hormone treatment and changes in dietary sodium intake, J. Neurochem. 13 (2001) 769–773.
[16] M. Jouhaneau-Bowers, J. Le Magnen, ACTH self-administration in rats, Pharmacol. Biochem. Behav. 10 (1979) 325–328.
[17] A.D. Le, B. Quan, W. Juzytch, P.J. Fletcher, N. Joharchi, Y. Shaham, Reinstatement of alcohol seeking by priming injections of alcohol and exposure to stress in rats, Psychopharmacology 135 (1998) 169–174.
[18] H.J. Little, M.J. O'Callaghan, A.R. Butterworth, J. Wilson, J. Cole, W.P. Watson, Low alcohol preference among the "high alcohol preference" C57 strain mice; preference increased by saline injections, Psychopharmacology 147 (1999) 182–189.
[19] D.L. McCullers, P.G. Sullivan, S.W. Scheff, J.P. Herman, Traumatic brain injury regulates adrenocorticosteroid receptor mRNA levels in rat hippocampus, Brain Res. 947 (2002) 41–49.
[20] G.E. McLearn, D.A. Rodgers, Differences in alcohol preference among inbred strains of mice, Quart. J. Stud. Alcohol 20 (1959) 691–695.
[21] F. Menzaghi, S. Rassnick, S. Heinrichs, H. Baldwin, E.M. Pich, F. Weiss, G.F. Koob, The role of corticotropin releasing hormone factor in the anxiogenic effects of ethanol withdrawal, Ann. NY Acad. Sci. 739 (1994) 176–184.
[22] J.F. Nash, R.P. Maickel, Stress-induced consumption of alcohol by rats, Life Sci. 37 (1985) 757–765.
[23] M.J. Ng Cheong Ton, Z. Brown, A. Michalakeas, Z. Amit, Stress induced suppression of maintenance but not of acquisition of ethanol consumption in rats, Pharmacol. Biochem. Behav. 18 (1983) 141–144.
[24] M.J. O'Callaghan, A.P. Croft, H.J. Little, Effects of intraperitoneal injections of saline on the alcohol and sucrose consumption of C57/BL10 strain mice, Psychopharmacology 160 (2002) 206–212.
[25] M.J. O'Callaghan, A. Croft, W.P. Watson, S.P. Brooks, H.J. Little, Low alcohol preference among the 'high alcohol preference' C57 strain of mice; factors affecting such preference, Pharmacol. Biochem. Behav. 72 (2002) 475–481.
[26] M.F. Olive, K.K. Mehmert, H.N. Koenig, R. Camarini, J.A. Kim, M.A. Nannini, C.J. Ou, C.W. Hodge, A role for corticotropin releasing factor (CRF) in ethanol consumption, sensitivity and reward as revealed by CRF-deficient mice, Psychopharmacology 165 (2003) 181–187.
[27] P.V. Piazza, V. DeRoche, J.-M. Deminiere, S. Maccari, N. Le Moal, H. Simon, Corticosterone in the range of stress-induced levels possesses reinforcing properties: implications for sensation-seeking behaviours, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 11738–11742.
[28] P.V. Piazza, M. Marinelli, C. Jodogne, V. Deroche, F. Rouge-Pont, S. Maccari, M. Le Moal, Inhibition of corticosterone syn-

thesis with metyrapone deceases cocaine-induced locomotion and relapse of cocaine self-administration, Brain Res. 658 (1994) 259–264.

[29] T.J. Phillips, J.C. Crabbe, Behavioural studies of genetic differences in alcohol actions, in: J.C. Crabbe, R.A. Harris (Eds.), The Genetic Basis of Alcohol and Drug Action, Plenum Press, New York, 1991, pp. 25–104.

[30] S. Rassnick, S.C. Heinrichs, K.T. Britton, G.F. Koob, Microinjection of a corticotropin releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal, Brain Res. 605 (1993) 25–32.

[31] A.J. Roberts, J.C. Crabbe, L.D. Keith, Genetic differences in hypothalamic-pituitary-adrenal axis responsiveness to acute ethanol and ethanol withdrawal, Brain Res. 579 (1992) 296–302.

[32] G.E. Rockman, A. Hall, J. Hong, G.B. Glavin, Unpredictable cold-immobilization stress effects of voluntary alcohol consumption in rats, Life Sci. 40 (1987) 1245–1251.

[33] H.D. Veldhuis, D. De Weid, Different behavioral actions of corticotropin-releasing factor (CRF), Pharmacol. Biochem. Behav. 21 (1984) 707–713.

[34] J.R. Volpicelli, R.R. Ulm, N. Hopson, The bidirectional effects of shock on alcohol preference in rats, Alcohol Clin. Exp. Res. 14 (1990) 913–916.

[35] W.P. Watson, Calcium channel antagonists in the ethanol withdrawal syndrome and other convulsive states, Ph.D. Thesis, University of Bristol, 1994.

[36] W.P. Watson, H.J. Little, Effects of diltiazem in convulsive states differ from those previously reported for dihydropyridine calcium channel antagonists, Psychopharmacology 114 (1994) 321–328.