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Up-regulation of cortical AMPA receptor binding in the Fawn-Hooded rat following ethanol withdrawal

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Abstract

The present study has employed quantitative receptor autoradiography to compare the binding of (*S*)-[^{3}H]5-fluorowillardiine to (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors in the brains of alcohol-preferring Fawn-Hooded (FH) rats, alcohol non-preferring Wistar–Kyoto (WKY) rats, and FH rats following a 28-day period of 5% ethanol consumption with or without ethanol withdrawal. Significantly higher binding of [^{3}H]5-fluorowillardiine was found in the cingulate cortex (+12%) and claustrum (+13%) in alcohol naïve FH rats compared to WKY rats. Chronic ethanol consumption decreased binding of (*S*)-[^{3}H]5-fluorowillardiine in four cortical regions (frontal, parietal, occipital and temporal cortex), hippocampus and septohippocampal nucleus. In contrast, ethanol withdrawal induced a significant ''rebound'' increase in binding by +22% in frontal and parietal cortex, by +17% in cingulate cortex and +13% in claustrum, and by +14% in the septohippocampal nucleus compared to chronic ethanol-exposed FH rats. The findings suggest that AMPA receptors in frontal cortical regions are sensitive to ethanol and therefore may be implicated in the predisposition of alcohol preference in FH rats.

Keywords: AMPA ((*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate); AMPA receptor; Up-regulation; Ethanol; Alcohol-preference

1. Introduction

Ethanol, a widely abused drug, can produce neuropharmacological effects through interactions with several major central neurotransmitters including γ-aminobutyric acid (GABA), dopamine, serotonin, acetylcholine and glutamate in addition to an ability to disorder neuronal membranes (Chin and Goldstein, 1977; Harris et al., 1984). Electrophysiological studies conducted over the last decade suggest that ethanol can interact with glutamate receptors (Hoffman et al., 1989; Lovinger et al., 1989). The involvement of such interactions in the development of physical dependence and withdrawal seizures in mice after chronic ingestion of ethanol was considered to play a role in ''relapse'' of alcohol intake in rodents (Grant et al., 1990; Littleton, 1995). However, the mechanisms of such glutamate-mediated effects of ethanol on the central nervous system (CNS) still remain to be elucidated.

At least four major subtypes of glutamate receptor have been described. These include the *N*-methyl-D-aspartate (NMDA), kainate, (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and metabotropic receptors (Nakanishi, 1992; Hollmann and Heinemann, 1994; McBain and Mayer, 1994). NMDA and non-NMDA (AMPA-type) receptors are multi-domained receptor–ionophore complexes (Palmer and Lodge, 1993; Mori and Mishina, 1995; Wenzel et al., 1997). Most work associated with ethanol to date has focussed on the ionotropic glutamate receptors, especially NMDA receptors. Electrophysiological and biochemical data demonstrated that acute ethanol administration potently and selectively inhibited the action of agonists at the NMDA receptor in cultured mammalian neuron cells (Hoffman et al., 1989; Lovinger et al., 1989), whereas chronic ethanol intake and withdrawal caused up-regulation of NMDA receptors in mouse hippocampus (Gulya et al., 1991) and enhancement of NMDA receptor function in cortical cells (Chandler et al., 1993; Ahern et al., 1994). Although there are some electrophysiological studies related to the effects of ethanol on AMPA receptors on intact neurons in rodents (Martin et

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al., 1995; Ibbotson et al., 1997), as well as an investigation finding that recombinant AMPA receptors in a human embryonic kidney (HEK) cell line showed high ethanol sensitivity (Lovinger, 1993), the whole picture of the role of AMPA receptors in ethanol preference, abstinence/dependence is still unclear. Considering that ethanol can affect different glutamate receptor subunits (Sucher et al., 1996) and that AMPA receptors mediate fast excitatory neurotransmission with characterized desensitization (Cowen and Beart, 1998; Tsai and Coyle, 1998), further study on the role of the AMPA receptor in alcohol abuse is necessary.

Fawn-Hooded (FH) rats, a strain of rat characterized as high alcohol-preferring (Rezvani et al., 1991) were employed in our study. There are two advantages in using such a genetically predisposed alcohol prone rat. First, comparison of the neurochemical and anatomical differences between FH rats and alcohol non-preferring rats (e.g., Wistar–Kyoto, WKY) will provide some clues about what factors may predispose the animal to crave for ethanol. Secondly, FH rats voluntarily consume large amounts of alcohol and provide a model of human drinking behavior. Based on the ability of ethanol to interact with glutamate receptors, the present study was designed to compare the distribution and density of central AMPA receptors between FH and WKY alcohol-naïve rats, and FH rats following 28 days access to 5% ethanol with or without ethanol withdrawal. To achieve this, the binding of the AMPA receptor agonist (*S*)-[^{3}H]5-fluorowillardiine (Hawkins et al., 1995) was mapped through brain sections of FH and WKY rat using quantitative in vitro receptor autoradiography.

2. Materials and methods

All experiments described herein were performed in accordance with the Prevention of Cruelty to Animals Act 1986 under the guidelines of the Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia.

(*S*)-[^{3}H]5-Fluorowillardiine (36 Ci mmol^{-1}) was obtained from Tocris Cookson (Bristol, UK), L-glutamate was obtained from Sigma (USA). Hyperfilm and tritium microscales were obtained from Amersham International (UK). All other reagents were of either analytical or laboratory grade from various suppliers.

2.1. Ethanol consumption

FH rats (male, 250–340 g) were bred at Central Animal Services, Monash University, the parental stocks came from Dr. Amir Rezvani at the University of North Carolina (USA). Male WKY rats (250–320 g, $n = 4$) were obtained from the Austin Hospital, Melbourne. For fluid consumption monitoring, FH were individually housed in a 12 h light/dark cycle with free access to standard chow. Each cage was equipped with two drinking containers, either both filled with water for FH (naive) group ($n = 5$) or one filled with water and the other filled with 5% ethanol for FH (chronic) ($n = 3$) and FH (withdrawal) groups ($n = 6$). The individual drinking containers were monitored each day for a 28-day period to determine daily consumption of both ethanol and water by the rats. The positions of the two drinking containers were changed randomly to avoid development of place preference. The rats in the chronic ethanol group, FH (chronic), were killed after a 28-day drinking schedule, whereas the rats in the ethanol withdrawal group, FH (withdrawal), were killed after 5% ethanol containers were replaced with water for 24 to 48 h following 28 days access to both ethanol and water. All rats were sacrificed by cervical dislocation and decapitation, the brains were quickly removed and frozen over liquid nitrogen and kept at −80°C until further processed. Alcohol-naïve WKY rats were used as a control, alcohol non-preferring strain (Rezvani et al., 1991).

2.2. Receptor autoradiography

Coronal sections of brain (14 μm) were cut starting at the level of the nucleus accumbens (bregma, 2.2 to 1.6 mm), ventral pallidum (bregma, 0.7 to 0.1 mm) and ventral tegmental area (bregma, −4.5 to −3.9 mm) (Paxinos and Watson, 1986) on a cryostat at −18°C and thaw-mounted onto gelatin/chrome alum coated microscope slides. The slide-mounted sections were stored at −80°C until used in

Table 1
The daily fluid intake and body weight gain among different groups of FH rats
FH (naïve), FH rats given access to two drinking containers both filled with water; FH (chronic), FH rats given access to two drinking containers, one filled with 5% ethanol and the other with water for 28 days; FH (withdrawal), FH rats treated the same way as FH (chronic) group for the same period, but followed by 24 to 48 h withdrawal from 5% ethanol. Data are expressed as mean ± S.E.M.

	FH (naïve) $n = 5$	FH (chronic) $n = 3$	FH (withdrawal) $n = 6$
Daily fluid intake (ml/kg B.W./day)	131.9 ± 26.6	161.1 ± 3.3	167.6 ± 18.3
5% Ethanol (ml/kg B.W./day)		125.5 ± 18.5	110.7 ± 51.4
or (g/kg B.W./day)		5.0 ± 0.7[a]	4.4 ± 2.0[a]
Water (ml/kg B.W./day)	131.9 ± 26.6	34.7 ± 15.2	56.7 ± 40.3
Preference for 5% ethanol (%)		77.9 ± 7.8	65.7 ± 24.8
Body weight gain (g/28 days)	50.0 ± 8.9	43.3 ± 6.2	39.2 ± 12.5

[a] $P < 0.01$, FH (naïve) vs. FH (chronic) or FH (withdrawal) group, using Student's *t*-test.

autoradiography studies. The autoradiography was performed according to the procedure for (*S*)-[^{3}H]5-fluorowillardiine binding to rat brain (Hawkins et al., 1995). Briefly, brain sections were allowed to warm to room temperature before preincubation (30 min, at 4°C) in Tris–HCl (50 mM, pH7.4) containing 100 mM KCl. The sections were then dried under a stream of cool air before further incubation with (*S*)-[^{3}H]5-fluorowillardiine (10 nM) for 40 min at 4°C in the same buffer as used for the preincubation. Non-specific binding was defined in the presence of 1 mM L-glutamate. The slide-mounted sections were then washed in ice-cold buffer (3×2 s) and in ice-cold distilled water (1×2 s), and finally allowed to dry under a gentle stream of cool air. After overnight desiccation, the sections were

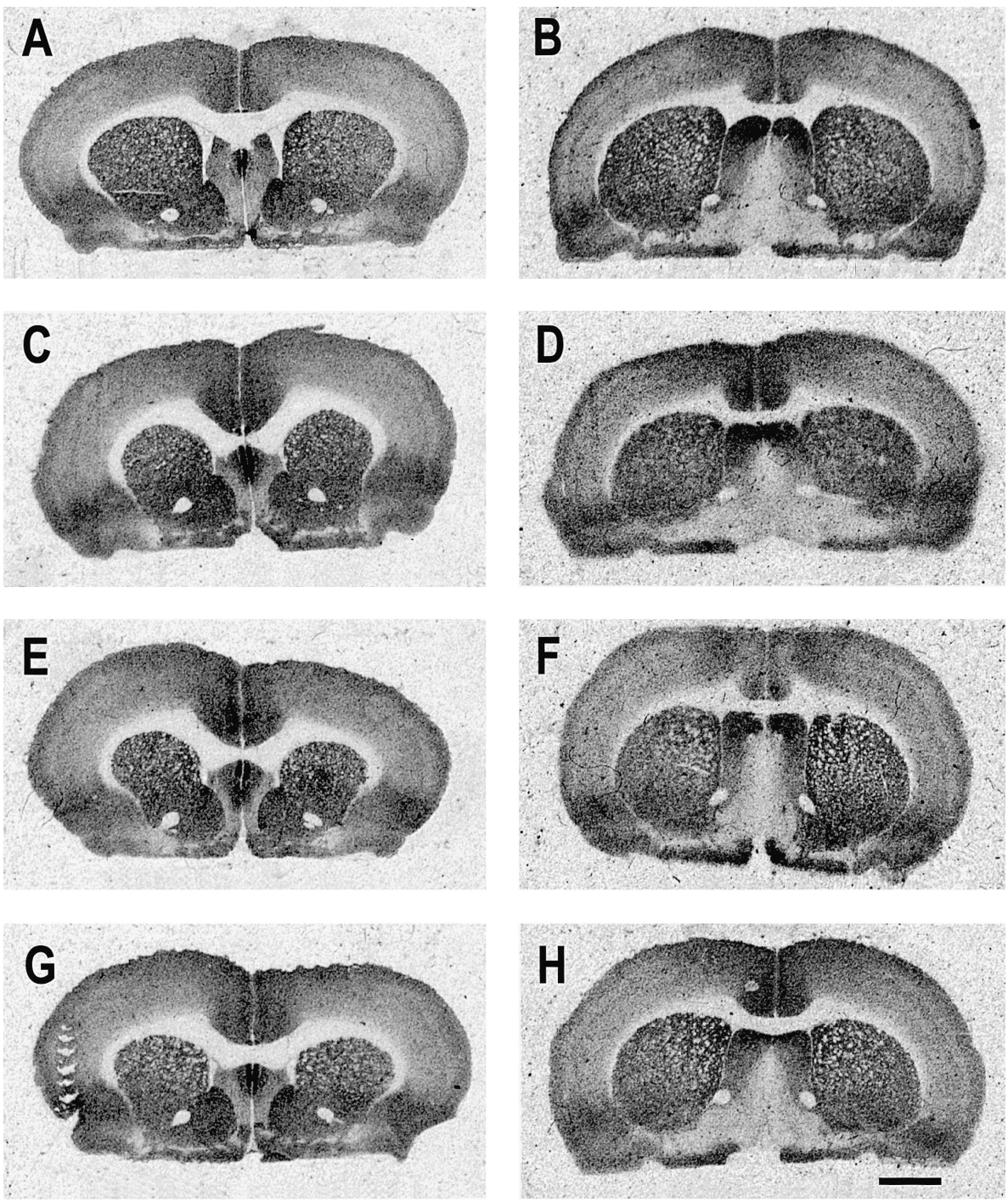

Fig. 1. [^{3}H] Fluorowillardiine (10 nM) binding in alcohol naïve, alcohol non-preferring WKY (naïve) (A–B); alcohol preferring FH (naïve) (C–D); chronic ethanol consumption FH (chronic) (E–F); and ethanol withdrawal FH (withdrawal) (G–H) rat brain. (A), (C), (E), (G), at the level of the nucleus accumbens (NAcc); (B), (D), (F), (H), at the level of the ventral pallidum. Scale, bar = 2 mm.

apposed to [^{3}H]Hyperfilm in the presence of [^{3}H]microscales for 20 days.

2.3. Data collection and analysis

Autoradiographic images on developed films were subsequently quantified, using an MCID M4 image analysis system (Imaging Research, Canada), by comparison of optical density, under constant illumination, of the autoradiograms to the standard microscales. Densitometric analysis of autoradiographic images was performed in all brain slices. Regions such as the hippocampus were analysed without differentiation of molecular layers. In this instance, dentate gyrus, CA1 and CA3 were analysed separately but without dorsal, medial and ventral differentiation. In a similar manner, the core and shell of nucleus accumbens were not differentiated, nor were the various layers through cortical regions. The data are expressed as the means ± S.E.M, which comprised measurements of both sides of each brain nucleus from at least 4 consecutive sections of each rat brain per experimental group ($n = 3$–6 rats per group). Thus, between 12 to 24 slices of brain were analysed for each brain region in each rat group.

2.4. Statistics

The statistical software program, SigmaStat (Jandel) was employed throughout. A Mann–Whitney *U*-test was used to determine differences between strains, while a Kruskal–Wallis non-parametric analysis of variance (ANOVA) was used to determine differences between control and ethanol groups. In the case of multiple tests, a Bonferroni correction was applied. Student's *t*-test was employed to analyse ethanol consumption data. A significance level of $P < 0.05$ was employed throughout.

3. Results

3.1. Drinking behavior

The intake of fluids and body weight gain of three different experimental groups are summarized (Table 1). During 28 days of monitoring, the daily fluid intake and body weight gain of rats was found not to differ among the rat groups. Rats in FH (chronic) and FH (withdrawal) group consumed large amounts of ethanol, with 5.0 ± 0.7 and 4.4 ± 2.0 g/kg/day, respectively, when they were given access to 5% ethanol and water in a free-choice paradigm. The alcohol preference of those rats is also reflected by their daily intake volume of 5% ethanol as a proportion of their daily fluid intake, representing 78% for FH (chronic), 66% for FH (withdrawal) rats. We have previously demonstrated that WKY rats consume a very low quantity of ethanol in a free-choice situation (Chen et al., 1998).

3.2. Binding of (S)-[^{3}H]5-fluorowillardiine in brains of alcohol-preferring naïve FH (naive) and alcohol non-preferring WKY (naïve) rats

The binding of (*S*)-[^{3}H]5-fluorowillardiine in FH (naive) and WKY (naive) rats displayed a similar pattern of regional distribution. The highest binding density was found in the hippocampus, particularly in dentate gyrus and CA1 regions. Dense binding was also found in the septohippocampal nucleus, nucleus accumbens, caudate-putamen, and the frontal cortex, especially in the claustrum and cingulate cortex. Binding was much lower in the thalamus, inferior colliculus and central gray (Fig. 1). The strain comparison of (*S*)-[^{3}H]5-fluorowillardiine binding between alcohol-naïve FH and WKY rats brain is shown in Table 2. Significantly increased binding of (*S*)-[^{3}H]5-fluorowillardiine was found in the cingulate cortex and claustrum in FH (naïve) rat compared to WKY (naive) rats. In contrast, the lateral septum and olfactory tubercle in FH (naïve) rats displayed a reduction of (*S*)-[^{3}H]5-fluorowillardiine binding compared to WKY rats, but statistical significance was only observed in the lateral septum (−12%) of FH (naive) compared to WKY (naive) rats (Table 2).

Table 2
The distribution of (*S*)-[^{3}H]-5-fluorowillardiine binding in FH and WKY rat brain
Abbreviations: Cx (cg, cl, FP, OT), the cingulate cortex, claustrum, frontal and parietal cortex, occipital and temporal cortex; CG, central gray; CPu, caudate–putamen; Hipp (DG, CA1, CA3), dentate gyrus, hippocampal fields of CA1 and CA3 of Ammon's horn; LSI, lateral septum; NAcc, nucleus accumbens; SHi, septohippocampal nucleus; Tu, olfactory tubercle.

Region	FH (naïve) fmol mm^{-2} ($n = 5$)	WKY (naïve) fmol mm^{-2} ($n = 4$)	FH (naïve)/ WKY (naïve) (%)
Cx			
(cg)	4.1 ± 0.15	3.8 ± 0.11[a]	+12
(cl)	4.1 ± 0.08	3.6 ± 0.11[a]	+13
(FP)	2.8 ± 0.08	2.6 ± 0.06	NS
(OT)	3.7 ± 0.10	3.4 ± 0.10	NS
CG	1.2 ± 0.05	1.2 ± 0.02	NS
CPu	3.4 ± 0.12	3.4 ± 0.10	NS
Hipp			
(DG)	7.1 ± 0.15	6.7 ± 0.13	NS
(CA1)	6.7 ± 0.10	6.8 ± 0.15	NS
(CA3)	4.6 ± 0.10	4.4 ± 0.11	NS
LSI	3.6 ± 0.09	4.1 ± 0.06[a]	−12
NAcc	5.6 ± 0.15	5.2 ± 0.15	NS
SHi	6.1 ± 0.22	5.7 ± 0.25	NS
Tu	2.7 ± 0.09	2.8 ± 0.12	NS

[a] $P < 0.05$, comparison between alcohol-naïve FH (naïve) and WKY (naïve) rats; using Mann–Whitney *U* test. The data are from 4 consecutive tissue sections per region per rat ($n = 4$–5 rats in each group) and expressed as mean ± S.E.M.

3.3. Binding of (S)-[^{3}H]5-fluorowillardiine in the brains of control water drinking group, FH (naive) rats, chronic alcohol drinking group, FH (chronic) rats and alcohol withdrawal group, FH (withdrawal) rats

After 28 days chronic alcohol drinking, the binding density of (*S*)-[^{3}H]5-fluorowillardiine tended to decrease in FH (chronic) rat brain, for example, by −25% in the dentate gyrus and hippocampal CA1 region, −27% in hippocampal CA3 region, −13% in the occipital and temporal cortex, and by −14% in the septohippocampal nucleus compared to FH (naive) rats. Chronic ethanol also caused a trend towards decrease in the whole frontal and parietal cortex and nucleus accumbens whereas other regions such as the caudate–putamen remained unchanged. In contrast, alcohol withdrawal induced a significant increase in the binding of (*S*)-[^{3}H]5-fluorowillardiine by +22% the frontal and parietal cortex, by +17% in the cingulate cortex and +13% in the claustrum, and by +14% in the septohippocampal nucleus. However, the nucleus accumbens, and the olfactory tubercle only exhibited a trend towards increase. Compared with the ''rebound'' increase of (*S*)-[^{3}H]5-fluorowillardiine binding in the frontal cortical regions which display high binding above the control level in alcohol-naïve FH rats and the ''recovery'' to control level in the septohippocampal nucleus, the binding in the hippocampus remained essentially unchanged after ethanol withdrawal compared to chronic ethanol treatment (Fig. 2).

4. Discussion

The FH rat, a strain of inbred rat characterized by a genetic serotonin (5-HT) deficiency, exhibits alcohol preference under a two-bottle free-choice paradigm (Rezvani et al., 1990, 1991). Although the deficient 5-HT function in the CNS is a possible causative mechanism, the altered functioning of other neurotransmitter systems, such as γ-aminobutyric acid (GABA)ergic, opioidergic and dopaminergic systems in the brain, particularly in mesocorticolimbic reward system may also contribute in part to the etiology of high alcohol intake in FH rats (Chen et al., 1998; Cowen et al., 1998). The existence of an altered density of AMPA receptors in the mesocorticolimbic regions such as the cingulate cortex and lateral septum compared to alcohol non-preferring rats (WKY) demonstrates that glutamate receptors may also be implicated in such behavioral traits of FH rats.

(*S*)-[^{3}H]-5Fluorowillardiine is an agonist, which can potently and specifically bind to a glutamate receptor subtype, the AMPA receptor (Hawkins et al., 1995). The present data obtained from autoradiographic mapping of (*S*)-[^{3}H]5-fluorowillardiine binding revealed the possible correlation between the propensity of alcohol preference and an altered density of AMPA receptors in specific brain regions. In the frontal cortex, particularly in the cingulate cortex, FH (naive) rats displayed significantly higher binding of (*S*)-[^{3}H]5-fluorowillardiine than WKY rats. In contrast, the density of binding was reduced in the lateral

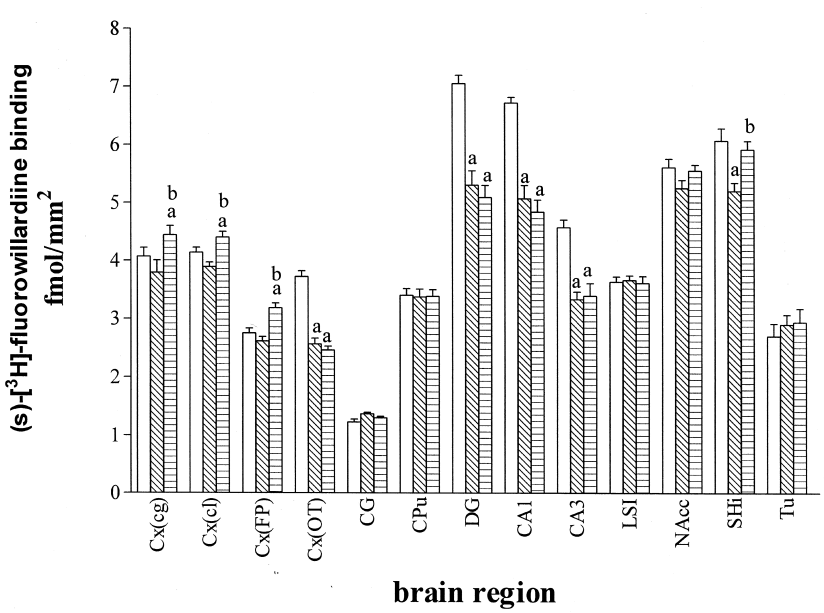

Fig. 2. Quantitative [^{3}H]fluorowillardiine binding in rat brain slices. Control water drinking FH (naive) rats (open column), chronic ethanol consumption, FH (chronic) rats (diagonal lines), and ethanol withdrawal, FH (withdrawal) rats (horizontal lines). Data are expressed as the mean ± S.E.M. of four tissue sections per region from each rat ($n = 3$–6 rats/group). [a] $P < 0.05$ FH (naive) rats vs. FH (chronic) rats or FH (withdrawal); [b] $P < 0.05$ FH (withdrawal) vs. FH (chronic) rats, using Kruskal–Wallis ANOVA test with Dunn's post-hoc test. Abbreviations are as follows: Cx (cg) and Cx (cl), cingulate cortex and claustrum; Cx (FP), the frontal and parietal cortex; Cx (OT), occipital and temporal cortex; CG, central gray; CA1 and CA3, fields of CA1 and CA3 of Ammon's horn; CPu, caudate–putamen; DG, dentate gyrus; LSI, lateral septum; NAcc, nucleus accumbens; SHi, septohippocampal nucleus; Tu, olfactory tubercle.

septum of FH rats compared to WKY rats. Moreover, the similar dense distribution of AMPA binding sites in the caudate-putamen and central gray in both FH (naive) and WKY (naive) rats further substantiates that the differential binding of (*S*)-[^{3}H]5-fluorowillardiine in the rat brains is region-specific. It must however be remembered that the strain differences in (*S*)-[^{3}H]5-fluorowillardiine binding observed may not necessarily be associated with alcohol preference per se.

While glutamatergic neurons are distributed throughout the CNS, they are prominently represented in the cerebral cortex and limbic regions of the brain (Cotman and Monagham, 1987). The forebrain, particularly the cingulate cortex, as a part of mesocorticolimbic reward/reinforcement system, has a dense efferent projection to the core of the reward system, the nucleus accumbens (Koob, 1992), which may play an important role in the formation of stimulation-response habits (Bussey et al., 1996). These projections from the frontal cortex to the nucleus accumbens are glutamatergic and both the frontal cortex and the nucleus accumbens receive dopaminergic projections from the ventral tegmental area (Koob, 1992). Modulation of excitatory output by dopamine in the cortex occurring concurrently with dopamine effects in the nucleus accumbens could lead to a variety of changes in nucleus accumbens function (Groenewegen et al., 1990). Experimental findings indicated that the AMPA/kainate receptor antagonist 6,7-dinitroquinoxaline-2, 3-dione (DNQX) administered intracerebroventricularly (i.c.v.) in rats blocked cocaine-induced conditioned locomotion, illustrating the interaction between glutamatergic and dopaminergic systems for the conditional factors maintaining drug-seeking behavior (Cervo and Samanin, 1996). Of interest, Wan and co-workers' study (Wan et al., 1995) demonstrated that the intra-nucleus accumbens infusion of AMPA significantly reduced prepulse inhibition and the effect was blocked by haloperidol and by 6-hydroxydopamine lesion of the nucleus accumbens, suggesting that AMPA in the nucleus accumbens may facilitate presynaptic dopamine function and the dopamine-glutamate interaction may be a critical regulatory substrate of sensorimotor gating (Wan et al., 1995). The differential density of AMPA binding sites between alcohol-preferring rats and alcohol non-preferring rats found in the present study suggests that the altered AMPA receptors in mesocorticolimbic brain regions (i.e., the cingulate cortex) might contribute towards alcohol preference in alcohol-preferring FH rats.

Chronic alcohol ingestion was previously found to produce increased binding of [^{3}H]dizocilpine to NMDA-receptors in many regions of mouse brain, e.g., cerebral cortex, hippocampus, thalamus and striatum (Gulya et al., 1991). In a postmortem comparison between normal human control brain and alcoholics, Freund and Anderson (1996) observed up-regulated binding sites of the NMDA receptor-gated ion channel, including agonist sites (NMDA-sensitive [^{3}H]glutamate), and antagonist sites ([^{3}H]DL-(*E*)-2-amino-4-propyl-5-phosphono-3-pentenoate (CGP-39653), and a [^{3}H]dizocilpine binding site). However, the AMPA and kainate receptors were found not significantly different between both groups. In an another animal experiment with rats, it was reported that long-term, but not short-term, ethanol exposure increased levels of immunoreactivity of the NMDA-R1 subunit, an obligatory component of NMDA receptors, and the Glu-R1 subunit, a component of many AMPA receptors in the ventral tegmental area (Ortiz et al., 1995). The current results demonstrate the number of AMPA binding sites was decreased in many brain regions following chronic alcohol exposure, but increased after the rats experienced 24 to 48 h withdrawal from alcohol. The increase of AMPA receptors is obvious when comparison is made between the alcohol withdrawal group and the chronic alcohol exposure group. Withdrawal after chronic alcohol exposure induced a significant increase in the binding of (*S*)-[^{3}H]5-fluorowillardiine in cortical regions and a trend toward an increase in the nucleus accumbens and the olfactory tubercle. In contrast, the binding in the hippocampus of ethanol-withdrawal rats was similar to the chronic ethanol-treated rats and without apparent rebound. The regional, discrete alterations of AMPA binding sites after different alcohol challenges (chronic exposure or withdrawal) suggests that complicated mechanisms may be involved in such diversity of changes, especially in the ethanol withdrawal episode. The different distribution of multiple combinations of various subunits and specific sensitivity of different subunits (Breese et al., 1995; Catania et al., 1995; Chandler et al., 1999) may determine their differential responses to alcohol. Such a hypothesis is strengthened by the evidence that recombinant AMPA-type receptors showed high ethanol sensitivity in cortical neurons (Lovinger, 1993) and that the expression of AMPA receptor mRNA subtype GluR-C but not GluR-A or GluR-B was elevated by chronic alcohol treatment (Bruckner et al., 1997). Nevertheless, many other mechanisms may also be implicated in the differential alteration of AMPA receptors binding to two different ethanol manipulations (chronic ethanol intake or ethanol withdrawal). These mechanisms may include the allosteric conformational changes of receptors by direct ethanol action (Carter et al., 1995), or the environment surrounding the neurons or brain regions where AMPA receptors are located. AMPA receptors are more likely to be directly altered by ethanol in the chronic exposure group compared to withdrawal. In the case of ethanol withdrawal, it seems that allosteric conformational change is less likely due to the diminished presence of ethanol. A ''disinhibition'' of AMPA receptors may start from the cessation of ethanol intake. The diversity of alteration of AMPA receptor binding found in the present study, where there was an obvious rebound increase in the cingulate cortex but no change in the hippocampus within 48 h withdrawal of ethanol, indicates that complicated processes are involved. Sensitivity of AMPA receptors to withdrawal may be influenced by a number of factors such

as differential sensitivity of receptor conformations (Gulya et al., 1991), the types and density of neuronal afferents to the region where AMPA receptors are located (Ibbotson et al., 1997), or differential responses to altered hormone levels in different brain areas (Roberts and Keith, 1995), may all contribute to the region-specific alteration of AMPA receptors or to diverse changes observed in rat brain after ethanol withdrawal. Therefore, it is understandable that ethanol withdrawal produced a diversity of c-Fos protein expression in the cerebral cortex and hippocampus (Wilce et al., 1994). Altogether, it is thus plausible to deduce that region-specific alteration of AMPA binding sites induced by alcohol withdrawal may affect the activity of neurons in a specific region or vice versa. Thus, a number of events either primary or secondary may be implicated in the responses of AMPA receptors observed in the present study. It is hoped that future research may shed light on these issues.

Although the detailed mechanisms of how AMPA receptors are involved in the influence of the glutamatergic system on the reward system in alcohol abuse are not well understood, it is worthwhile to define the role of AMPA receptors played in the action of a new clinical anti-craving compound. As a new emerging anti-craving compound for ethanol (Spanagel and Zieglgansberger, 1997; Spanagel et al., 1996), acamprosate is believed to exert its anti-craving effect by reducing the enhanced L-glutamate release in brain during ethanol withdrawal and by reducing glutamate-mediated excitatory postsynaptic potentials (Zeise et al., 1993). Whether the effects of acamprosate also involve an action on AMPA receptors is of interest to be studied in the future.

In conclusion, the autoradiographic results show that alcohol-preferring FH rats (naïve) exhibit altered (*S*)-[^{3}H]5-fluorowillardiine binding in mesocorticolimbic brain regions, such as the cingulate cortex, claustrum and lateral septum in comparison with alcohol non-preferring WKY (naïve) rats. Chronic alcohol treatment induced a decrease in the densities of AMPA receptors in specific brain regions (i.e., the frontal cortex, hippocampus). In contrast, alcohol withdrawal caused a significant increase of the binding sites in the septohippocampal nucleus, the frontal cortex, particularly in cingulate cortex, and only a trend toward increase in the nucleus accumbens and the olfactory tubercle. The differential, region-specific density of AMPA receptors between the alcohol-preferring rats and alcohol non-preferring rats might contribute to the difference of their alcohol preference. The ''rebound'' increased density of AMPA receptors in the frontal cortex after withdrawal suggests an alteration of sensitivity of the glutamate system in this region as an adaptation, which may also be associated with alcohol withdrawal symptoms. Indeed, it must be noted that the alteration of AMPA receptors induced by chronic ethanol and withdrawal was only observed in the alcohol-preferring FH rat under a two-bottle free choice paradigm. Considering that there existed a strain difference of AMPA receptor binding between FH and WKY rats, further studies are needed to clarify that the possible role of AMPA receptors played in general alcohol abstinence/dependence. Therefore, selection of multi-strains or species of animal under various paradigms of alcohol administration (i.e. intoxication of animals by alcoholic diet or by repeated injection of ethanol) will help to pinpoint whether the role played by AMPA receptors is a general phenomenon in the ethanol withdrawal scenario. Additionally, by observing electrophysiological responses of AMPA receptors to application of agonists or antagonists in different ethanol treated tissues will also help us to understand the mechanism by which alteration of AMPA receptor binding is mediated via ethanol challenges.

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