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Effects of naltrexone on the accumulation of L-3,4-dihydroxyphenylalanine and 5-hydroxy-L-tryptophan and on the firing rate induced by acute ethanol administration

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Abstract

In order to characterize the effects of naltrexone, a μ-opioid receptor antagonist, on acute ethanol-induced functional modification of dopaminergic neurons in the nigrastriatal and mesolimbic dopamine systems, the accumulation of L-3,4-dihydroxyphenylalanine (L-DOPA) in the cerebral cortex, dorsal striatum and nucleus accumbens and of 5-hydroxy-L-tryptophan (5-HTP) in the hippocampus was measured in normal rats using the μ-hydroxybenzylhydrazine dihydrochloride (NSD-1015) enzymatic inhibition method. In addition, the firing rates of dopaminergic neurons were recorded in the substantia nigra and ventral tegmental area. Naltrexone resulted in a decrease in the dopaminergic neuronal firing rates activated by ethanol and eventually in a reduction of the dopamine synthesis induced by ethanol in the dorsal striatum and nucleus accumbens, but not in the cerebral cortex. Mesolimbic dopamine neurons were slightly more sensitive to ethanol and naltrexone than were nigrostriatal dopamine neurons. The widespread inhibitory action of naltrexone also decreased the ethanol-induced stimulation of hippocampal serotonin synthesis.

Keywords: Naltrexone; Ethanol; L-DOPA (L-3,4-dihydroxyphenylalanine); 5-HTP (5-hydroxy-L-tryptophan); Firing rate

1. Introduction

Acute ethanol intake induces locomotor activity, motor impairment, hypothermia, and loss of the righting reflex. A number of investigations have established that such deficits arise from the functional modification of central monoaminergic neurons (cf. Nutt and Glue, 1986). Notably, dopamine is an important brain transmitter that mediates a variety of functions such as locomotion and reward-related behaviors through activation of the nigrostriatal system comprising the substantia nigra, dorsal striatum and frontoparietal cortex, and the mesolimbic system including neurons in the ventral tegmental area innervating the ventral striatum, nucleus accumbens and cingulate cortex (Wise and Rompre, 1989; Diana et al., 1992). Moreover, it has been reported that the sensitivity to ethanol-induced locomotor impairment is reduced in dopamine D_2 receptor-deficient mice (Phillips et al., 1998) and on neuroleptic blockade of striatal dopamine D_2 receptors of alcohol-dependent patients (Heinz, 1999).

Pharmacological studies with naltrexone have mostly shown it to influence the activity of the mesolimbic dopamine system (cf. Di Chiara, 1995). The effects of naltrexone on ethanol-induced changes in extracellular dopamine and serotonin in the nucleus accumbens have been investigated using in vivo microdialysis in awake rats (Benjamin et al., 1993). The results suggested that administration of naltrexone against ethanol dose-dependently reverses ethanol-induced increases in extracellular dopamine and in its metabolite, homovanillic acid, but not in serotonin. Ethanol itself has been shown to activate the neural reward systems, in the basis of ex vivo and in vivo measurement of mesolimbic dopamine, serotonin and their metabolites that demonstrated regionally specific ethanol-induced effects on the above monoamines in the nucleus accumbens and the lateral hypothalamus (Khatib et al., 1988; Wozniak et al., 1991).

In contrast, low doses of ethanol did not alter monoamine synthesis and release in the dorsal striatum, the major terminal field of the nigrostriatal neurons (Di

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Chiara and Imperato, 1986) suggesting that the nigrostriatal neurons are of less importance for evaluation of the pharmacological interaction of naltrexone with ethanol-induced monoamine synthesis.

The present study evaluated the hypothesis that naltrexone attenuates the rewarding properties of ethanol by interfering with ethanol-induced stimulation of dopamine activity in dorsal striatum and nucleus accumbens that were under tonic control of μ-opioid receptor system originating from the substantia nigra (nucleus A9; A9) and the ventral tegmental area (nucleus A10; A10), respectively. Hence, the accumulation of L-3,4-dihydroxyphenylalanine (L-DOPA) in the cerebral cortex, the dorsal striatum and the nucleus accumbens, as well as of 5-hydroxy-L-tryptophan (5-HTP) in the hippocampus was measured, using μ-hydroxybenzylhydrazine dihydrochloride (NSD 1015) enzymatic inhibition. Previous studies have shown that metabolite levels, under conditions in which l-aromatic amino acid decarboxylase is inhibited with NSD-1015, can be considered as indicators of monoamine metabolism or release (Blomqvist et al., 1993). Changes in the ethanol-activated firing rate by naltrexone pretreatment were also measured in A9 and A10.

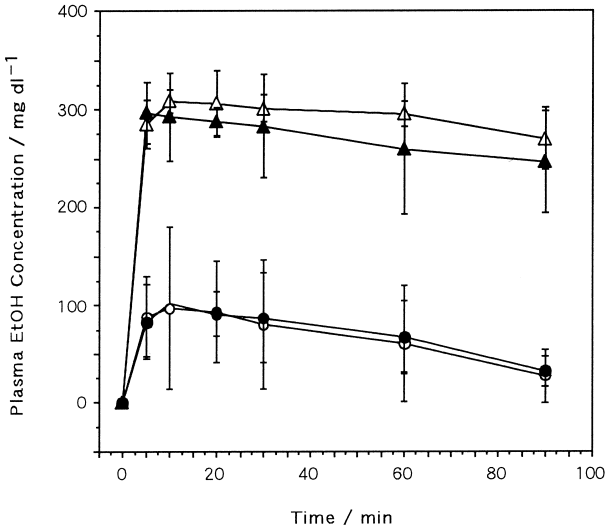

Fig. 1. Plasma ethanol concentrations after i.p. administration of 1.0 (○, ●) and 2.5 g kg^{-1} (△, ▲) ethanol. Open and closed signs indicate pretreatment without and with 30 mg kg^{-1} naltrexone (p.o.), respectively. The ethanol concentration of blood collected from the tip of the tail was determined by the enzymatic method of Jones et al (1970). Each point represents the mean ± S.D. (vertical bar) for seven to nine samples per point. A set of data was not necessarily collected from the same rat.

2. Materials and methods

Male Sprague–Dawley rats (Seac Yoshitomi; Fukuoka, Japan), weighting 250–300 g, were housed in individual cages. All experimental procedures used in this study were approved by the internal animal use committee of Kurume University School of Medicine, Japan.

Overall statistical significance of differences between groups was determined by analysis of variance. Post hoc paired comparisons of individual group differences were made using Dunnett test (two-tailed t statistic). Statistical significance was accepted at the $P < 0.05$ level.

2.1. Monoamine assay

2.1.1. Sample preparation

Ethanol (0.5, 1.0, 2.5, and 5.0 mg kg^{-1}; intraperitoneally (i.p.)) was injected 90 min before decapitation. The time of administration was determined as being sufficient for metabolism of the administered ethanol, based on measurement of plasma ethanol concentration as shown in Fig. 1. The acute effects of ethanol on the synthesis and metabolism of brain dopamine and serotonin were measured 30 min after inhibition of brain aromatic acid decarboxylase with NSD-1015 (Sigma-Aldrich, St. Louis, MO) at 100 mg kg^{-1} i.p. In addition, the effects of naltrexone were evaluated by administration 30 min before ethanol injection. The control groups were treated with vehicle at the respective times. All rats received 3.5 kW microwave exposure, focused on the heads, for 1.8 s with a Microwave-applicator (Muromachi Machine, TMW-6402, Japan). The brain was dissected into the cerebral cortex, the dorsal striatum, the nucleus accumbens, and the hippocampus. The tissue samples obtained from two rats were combined and pooled as one sample. These samples were then stored at −80°C until the monoamine concentrations were assayed with high performance liquid chromatography, using electrochemical detection (HPLC–ECD; EiCOM, ECD-300).

2.1.2. Extraction and separation of L-DOPA and 5-HTP

All samples were weighed and added to an antioxidant (consisting of 0.1 mol dm^{-3} perchloric acid containing 0.2% EDTA and 0.5% sodium disulphite) at four volumes for the cerebral cortex, eight volumes for the dorsal striatum and the nucleus accumbens, and 15 volumes for the hippocampus. Each sample was homogenized using a Polytron homogenizer and then centrifuged at 15 000 × g for 10 min at 0°C. For separation of L-DOPA, 1 ml of each supernatant was passed through a Sephadex G-10 column (ϕ 6 × 34 mm, Amersham Pharmacia Biotech, Tokyo), which had been activated with 20 mmol dm^{-3} ammonia solution and buffered with 0.5 mol dm^{-3} formic acid solution. The column was washed with 0.5 ml of 10 mmol dm^{-3} formic acid solution and L-DOPA was then eluted using 1.8 ml of the same solution into a low actinic test tube containing 100 μl of 0.15 mmol dm^{-3} ascorbic acid. For the separation of 5-HTP, the same columns as for L-DOPA were prepared and 1 ml of each supernatant was applied as described above. 5-HTP was washed successively with 3 ml of 10 mmol dm^{-3} folic acid and 1 ml of 5 mmol dm^{-3} phosphate buffer solution (pH 8.5) and then

eluted with 3 ml of the same phosphate buffer into a low actinic test tube containing 50 μl of 6 mol dm^{-3} acetic acid and 20 μl of 0.5 mmol dm^{-3} ascorbic acid.

2.1.3. Determination of L-DOPA and 5-HTP

A 1-ml aliquot from each elution was used as an internal standard and was added to 50 μl of 1 μg ml^{-1} 3,4-dihydroxybenzylamine (Sigma-Aldrlich) or 50 μl of 4 μg ml^{-1} vanillic acid for measurement of L-DOPA and 5-HTP, respectively. The samples were injected in 20 μl into the HPLC–ECD. Each component was separated in an isocratic reversed-phase system under two different sets of conditions. For analysis of L-DOPA and 5-HTP, the working electrodes were set at 640 and 730 mV, respectively, versus an Ag/AgCl reference electrode. For eluting L-DOPA, the mobile phase in the separation column (Nucleosil 5 C18 ϕ 4.6 × 250 mm, particle size 7 μm, Chemco) contained 8.75 v/v% ethanol, 1.63 mmol dm^{-3} sodium-1-heptanesulfonate, 0.016 mmol dm^{-3} EDTA-2Na, and 105 mmol dm^{-3} K_2HPO_4 in water. The pH was adjusted to 3.0 using 5 mol dm^{-3} NaOH. The flow rate was 1.2 ml min^{-1}. For eluting 5-HTP, the mobile phase in the separation column contained 16.0 v/v% ethanol, 0.46 mmol dm^{-3} sodium-1-octanesulfonate, 0.019 mmol dm^{-3} EDTA-2Na, 37.8 mmol dm^{-3} citric acid and 56.8 mmol dm^{-3} K_2HPO_4 in water. The pH was adjusted to 3.8 with 5 mol dm^{-3} NaOH. The flow rate was 1.2 ml min^{-1}.

2.2. Firing rate measurement

Electrodes were inserted in A9 and A10 according to the Paxinos and Watson (1982) atlas, adapted for anesthetized rats. Electrode penetrations were made in the dorsal–ventral direction from 6.0 to 8.0 mm below the dura through A9 and A10: 2.7–3.0 mm and 2.7–3.0 mm anterior to the lambda suture; 2.0–2.5 mm and 0.6–1.0 mm lateral to the sagittal suture, respectively.

Glass microelectrodes were filled with 3 mol dm^{-3} sodium acetate containing 2% pontamine sky blue and the tips were broken back to approximately 1 mm using a microscope. The in vitro impedance of these electrodes was 8–12 M Ω at D.C. in saline. Electrical signals were amplified using a high impedance amplifier set with a bandpass of 100 Hz–3 kHz, displayed on an oscilloscope, and led into a window discriminator. The dopaminergic neurons were identified using previously established criteria (Bunny et al., 1973; Wang, 1981). Base firing rates were determined for 5 min before the injection of ethanol. Only one cell was studied in each animal. At the end of each experiment, the site of the electrode tip was marked

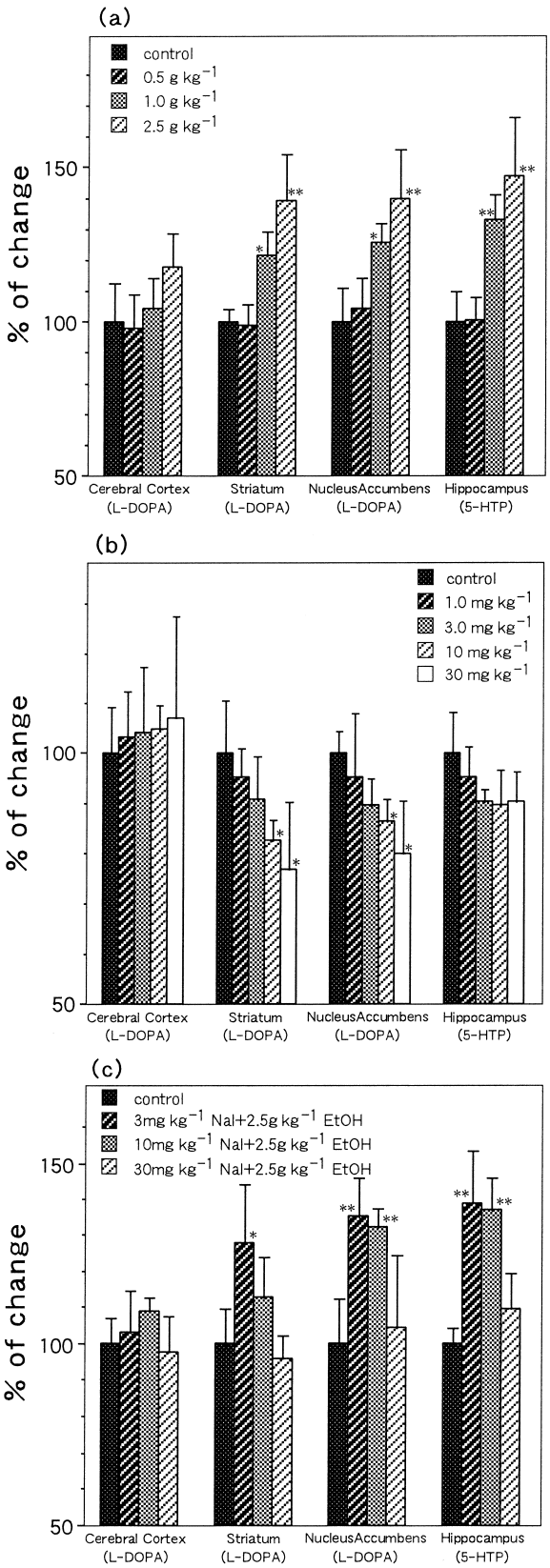

Fig. 2. Effects of ethanol (a) and naltrexone (b) on the accumulation of L-DOPA and 5-HTP, and the effects of naltrexone (Nal) on the increases in L-DOPA and 5-HTP induced by ethanol (EtOH) administration (c). Results are expressed as percentages of saline control. Each column represents the mean ± S.D. (vertical bar) for six to seven samples per column. $^{*}P < 0.05$, $^{**}P < 0.01$ (Student's *t*-test) compared to the control group.

by passing a 25–50 μA cathodal current through the electrode barrel for 10 min in order to deposit a spot of pontamine sky blue. The rats were then perfused transcardially with 10% buffered formalin phosphate. The location of each recording site was then verified histologically.

3. Results

After 1.0 or 2.5 g kg^{-1} of ethanol was i.p. injected into rats that were pretreated p.o. with or without 30 mg kg^{-1} naltrexone, samples of tail blood were taken and the ethanol concentrations were measured. The blood ethanol concentration reached a peak at 10 min followed by a very slow decline. Pretreatment with 30 mg kg^{-1} naltrexone had no effect on plasma ethanol concentration (Fig. 1).

Under control conditions, the mean concentration of L-DOPA in the cerebral cortex, the dorsal striatum and the nucleus accumbens was 0.425 ± 0.0597 ($n = 23$), 1.65 ± 0.230 ($n = 22$) and 1.58 ± 0.364 ($n = 23$) μg g-$protein^{-1}$, respectively, and that of 5-HTP in the hippocampus was 0.340 ± 0.0419 ($n = 24$) μg g-$protein^{-1}$.

We found that 5.0 g kg^{-1} (i.p.) of ethanol was over the LD50 and killed 64% of the rats injected, and therefore, the data from this dose were not included. Doses of 1.0 and 2.5 g kg^{-1} of ethanol significantly increased the accumulation of L-DOPA, by 22% and 39%, in the dorsal striatum, and by 26% and 40%, in the nucleus accumbens, respectively, and significantly increased 5-HTP accumulation by 33% for 1.0 g kg^{-1} of ethanol and 47% for 2.5 g kg^{-1} of ethanol in the hippocampus, as shown in Fig. 2(a). However, dopamine synthesis in the cerebral cortex did not increase significantly following administration of even 2.5 g kg^{-1} of ethanol. On the other hand, doses of 10 and 30 mg kg^{-1} of naltrexone significantly decreased the accumulation of L-DOPA, by 18% and 23%, in the dorsal striatum, and by 14% and 20%, in the nucleus accumbens, respectively (Fig. 2(b)). However, L-DOPA accumulation in the cerebral cortex and 5-HTP accumulation in the hippocampus did not significantly differ from baseline levels after vehicle injection. The i.p. injection of vehicle did not affect the neurochemical changes produced by ethanol but pretreatment with naltrexone resulted in a significant dose-related decrease in L-DOPA accumulation in the dorsal striatum and nucleus accumbens and in 5-HTP accumulation in the hippocampus (Fig. 2(c)). The effect of naltrexone on L-DOPA accumulation in the cerebral cortex did not differ statistically between the basal and ethanol-induced levels.

The i.p. administration of ethanol elevated the firing rates of dopamine neurons in both the A9 and A10 regions, as shown in Fig. 3. In both areas, the onset of increase in firing was delayed for 4–8 min after the ethanol injection and the increase persisted for at least 30 min. No relevant effects were observed after the injection of an equivalent volume of vehicle. The dopamine neuron in A10 was more sensitive to ethanol than that in A9 in all cases, though not statistically significantly so as previously reported (Mereu

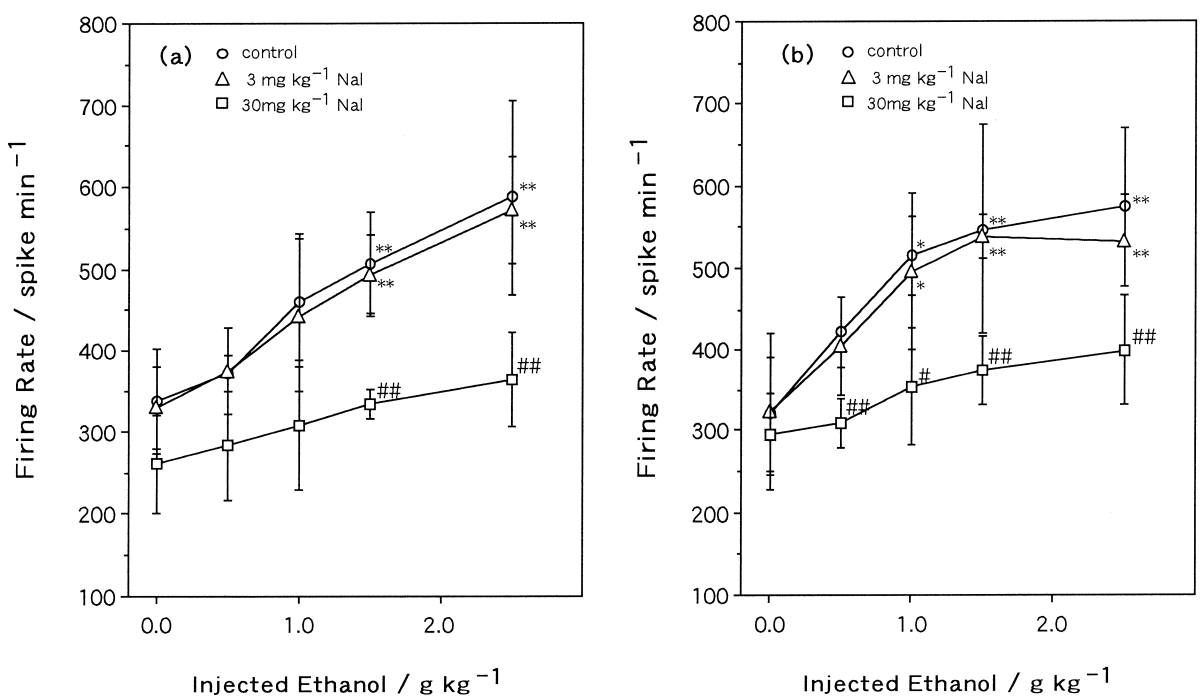

Fig. 3. The effects of 3 and 30 mg kg^{-1} naltrexone on the dose-dependent increase in the firing rate of (a) A9 and (b) A10 dopaminergic neurons induced by ethanol. Each point represents the mean ± S.D. (vertical bar) for eight to eight to 14 cells (one cell per rat). Comparable volumes of saline had no effect on cell activity. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the respective baseline rate. $\#P < 0.05$, $\#\#P < 0.01$ compared to the group not pretreated with naltrexone.

et al., 1984). For example, the amount of ethanol required to increase basal activity by 50% was 1.5 mg kg^{-1} for the A9 neuron but 0.81 mg kg^{-1} for the A10 neuron. A dose of 3 mg kg^{-1} of naltrexone scarcely influenced the firing rates in A9 and A10. On the other hand, with the ethanol doses used in the present study, pretreatment with 30 mg kg^{-1} naltrexone was able to inhibit the A9 and A10 neuronal firing rates by 24–38% and 27–32%, respectively, compared to those in rats not pretreated with naltrexone. Notably, this pretreatment appeared to appreciably depress the A10 dopamine neuron activated by low doses of ethanol.

Mean firing rates in A9 and A10 decreased after pretreatment with 30 mg kg^{-1} of naltrexone, however, these effects were variable and therefore not statistically significant.

4. Discussion

In the present study, the effects of ethanol on the functional state of control dopamine neurons were evaluated by monitoring the accumulation of L-DOPA and 5-HTP using the NSD-1015 enzymatic inhibition method and by recording the firing rates in A9 and A10.

How the blood ethanol concentration was elevated after i.p. ethanol injection in this study had to be determined. A decrease in blood ethanol concentration due to naltrexone was found in acutely ethanol-intoxicated rats, in which naltrexone presumably interacted with the hepatic NAD^+-dependent oxidative metabolism (Badawy and Su, 1985). In the present system, combined treatment with 30 mg kg^{-1} naltrexone and 1.0 and 2.5 g kg^{-1} of ethanol did not alter the blood ethanol concentration significantly, suggesting that naltrexone in this experimental protocol was unrelated to modification of ethanol metabolism or bioavailability factors (Fig. 1).

Ethanol significantly affected the synthesis of brain monoamines. Acute doses of more than 1.0 g kg^{-1} of ethanol produced a reliable and statistically significant increase in L-DOPA accumulation in the dorsal striatum and the nucleus accumbens as shown in Fig. 2(a). Although these results for the nucleus accumbens are consistent with those of an earlier study (Karoum et al., 1976), Blomqvist et al. (1993) have reported that ethanol does not alter NSD 1015-induced DOPA accumulation in the dorsal striatum, suggesting that the nigrostriatal dopamine system is less sensitive to ethanol-induced dopamine neuron activation than is the mesolimbic dopamine system. However, the firing rate determined in the present study demonstrates that the nigrostriatal dopamine system is also directly involved in dopamine production at relatively lower concentrations (1.0 mg kg^{-1} i.p.) of ethanol (Fig. 3). Ethanol failed to affect L-DOPA accumulation in the noradrenaline-rich cerebral cortex, indicating that the noradrenaline synthesis rate is not influenced by ethanol.

Previous observations with microdialysis (Benjamin et al., 1993) have demonstrated that naltrexone does not decrease the basal levels of dopamine in the nucleus accumbens. However, in the present system, L-DOPA accumulation decreased significantly in the dorsal striatum and nucleus accumbens on administration of more than 10 mg kg^{-1} of naltrexone (Fig. 2(b)). Moreover, 30 mg kg^{-1} (p.o.) of naltrexone depressed the firing rates of A9 and A10 under control conditions, although this finding was not statically significant (Fig. 3). Gonzales and Weiss (1998) also recently reported that 0.25 mg kg^{-1} (s.c.) of naltrexone decreases the basal dopamine level in normal rats by approximately 24%.

Low to moderate doses of naltrexone were found to have little effect on the ethanol-induced activity of the A9 and A10 dopamine neurons (data not shown). After pretreatment with 30 mg kg^{-1} naltrexone, ethanol-induced L-DOPA accumulation in the dorsal striatum and nucleus accumbens was completely prevented (Fig. 2(c)). Pretreatment with 30 mg kg^{-1} of naltrexone depressed the firing rate activated by acute ethanol administration. Information about the tonic activity of the mesolimbic reward system prompted experiments in which μ-opioid and its antagonist were applied directly into A10 and the nucleus accumbens (David et al., 1998). The μ-opioid receptor antagonist decreased dopamine release in the nucleus accumbens when applied to A10. This indicates that the basal release of dopamine in the nucleus accumbens is under tonic control of the opioid system: μ-opioid receptor activity originating from A10 increases basal activity of the mesolimbic reward system (Spanagel et al., 1992). These findings suggest that the reversal effects of naltrexone on ethanol-induced activation presumably involve antagonism of μ-opioid receptors in A9 and A10. Other possibilities are that very specific changes in certain opioid receptor subtypes are induced by ethanol (Charness, 1989) or that opioid-like condensation products are formed as a result of a reaction between acetaldehyde and brain DOPA, thereby contributing to the reinforcing properties of ethanol (Cashaw et al., 1987).

We cannot exclude the possibility that nondopaminergic mechanisms coupled to opioid receptors may have contributed to the suppression of acutely ethanol-induced neuronal modification by naltrexone. In fact, naltrexone reduced the increases in hippocampal serotonin synthesis produced by ethanol (Fig. 2(c)). The enhancement of central 5-HT tone, which may exert a modulatory role in behavioral control, reduces the alcohol intake and may diminish certain rewarding stimuli (Sellers et al., 1992; Lau and Frye, 1996).

In conclusion, naltrexone resulted in depression of dopaminergic neuronal firing rates activated by acute ethanol administration and eventually resulted in a reduction of dopamine synthesis induced by ethanol in the terminal dopamine area, the striatum and the nucleus accumbens. Nevertheless, the mesolimbic dopamine neurons

were slightly more sensitive to ethanol and naltrexone than the nigrostriatal dopamine neurons. The widespread inhibitory actions of naltrexone also depressed the ethanol-induced stimulation of hippocampal serotonin synthesis.

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