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The combined effects of chronic ethanol/desipramine treatment on β-adrenoceptor density and coupling efficiency in rat brain [1]

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Abstract

Both ethanol and desipramine influence β-adrenoceptor regulation. We reported previously that ethanol partially counteracted desipramine's effects on β-adrenoceptor. Previous studies utilized β-adrenoceptor radioligands that also bind to 5-HT_{1B} receptors, thus, changes in 5-HT_{1B} receptors could have confounded the results. The effects of chronic ethanol, desipramine and ethanol/desipramine treatment on β-adrenoceptor coupling efficiency to G_s protein in rat brain were examined using ^{125}I-iodocyanopindolol after blocking binding to 5-HT_{1B} receptors. In the frontal cortex, ethanol uncoupled β-adrenoceptor from G_s. Desipramine decreased β-adrenoceptor density, particularly in the high-conformational state, with no effect on coupling. In combined treatment, desipramine prevented ethanol-induced uncoupling. In the hippocampus, desipramine enhanced β-adrenoceptor coupling, but ethanol had no effect. In combination with desipramine, ethanol enhanced desipramine-induced decrease in β-adrenoceptor density in the high-conformational state, but uncoupled β-adrenoceptors, an effect not observed with ethanol alone. These results suggest a complex interplay between ethanol and antidepressants in modulating β-adrenoceptor function.

Keywords: Ethanol; Desipramine; Antidepressant; G_s protein; Coupling; Depression; Alcoholism

1. Introduction

Although a few studies have shown that ethanol downregulates β-adrenoceptor density, the majority of studies have shown that ethanol has no effect on β-adrenoceptor density in rat or mouse brain (Banerjee et al., 1978; Thurman et al., 1980; Kuriyama et al., 1981; Light and Stull, 1982; Hoffman et al., 1987; Rommelspacher and Strauss, 1987; Valverius et al., 1987, 1989b; Bode and Molinoff, 1988a,b; Turkka et al., 1989, 1990; Hunt and Dalton, 1991) or human brain (Valverius et al., 1989a). Downregulatory effects of tricyclic antidepressants on rat brain β-adrenoceptor density, on the other hand, is well-established (Vetulani et al., 1976; Sulser et al., 1978, 1984; Richardson and Hertz, 1983; Biegon, 1986; Honegger et al., 1986; Ordway et al., 1988, 1991; Paul et al., 1988; Sethy et al., 1988; Duncan et al., 1989; Turkka et al., 1989; Nelson et al., 1991; Paetsch and Greenshaw, 1993; Goodnough and Baker, 1994). However, the effects of ethanol and/or desipramine on β-adrenoceptor coupling efficiency to G_s protein and the adenylyl cyclase second messenger system are more intricate.

Chronic ethanol treatment is associated with decreased cyclic adenosine monophosphate (cAMP) responses (Kuriyama and Israel, 1973; Kuriyama, 1977; Askew and Charalampous, 1978; Bode and Molinoff, 1988a; Valverius et al., 1988). Despite ethanol's lack of effect on total β-adrenoceptor density, chronic ethanol treatment shifts β-adrenoceptor density into a low-affinity state, indicating uncoupling of the receptor from G_s protein (Saito et al., 1987; Valverius et al., 1987, 1988, 1989a,b; Saffey et al., 1988). These observations have not been replicated by

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others (Turkka et al., 1990). Chronic ethanol exposure was associated with decreased G_s protein levels and G_s expression in rat brain (Charness et al., 1988; Mochly-Rosen et al., 1988; Nhamburo et al., 1988; Wand and Levine, 1991; Rabin, 1993), and in erythrocytes and brains from alcoholics (Nakamura, 1993; Ozawa et al., 1993, 1994b). Decreased G_s levels may account for the decrease in β-adrenoceptor coupling. However, ethanol-induced decreases in G_s protein levels were not replicated by others, and opposite results were also reported (Rabin, 1993; Williams et al., 1993; Hatta et al., 1994; Iles and Nagy, 1995). Finally, quantitative changes in G protein levels do not appear to mediate ethanol-induced downregulation in adenylyl cyclase activity (Tabakoff et al., 1995).

Virtually all previous studies have shown decreased cAMP responses following tricyclic antidepressant treatment (Pilc et al., 1990; Sulser, 1990). Decreased cAMP responses, in conjunction with decreased β-adrenoceptor density, following tricyclic antidepressant treatment suggest homologous desensitization. There is some evidence to suggest that tricyclic antidepressants modulate β-adrenoceptor coupling efficiency to G_s protein. Two studies have shown that decreased β-adrenoceptor density after tricyclic antidepressant treatment was specific to the transitory G_s-coupled high-affinity state of the receptor (Hancock and Marsh, 1985; Manier et al., 1989). Tricyclic antidepressant treatment decreased G_s and G_i protein levels, but not those of G_o (Lesch et al., 1991). Imipramine decreased G_o protein mRNA, but had no effect on G_s or G_i mRNA (Lason and Przewlocki, 1993). Other studies suggested that tricyclic antidepressants alter the dynamics of β-adrenoceptor–G protein interaction, G protein function, and G_s–adenylyl cyclase interaction (Okada et al., 1986, 1988; Tsuchiya et al., 1988; Yamamoto et al., 1990; Odagaki et al., 1991). Therefore, whether the decrease in cAMP responses is secondary to a β-adrenoceptor uncoupling or to a decrease in G_s protein levels/function is unclear.

Previous β-adrenoceptor binding studies investigating the effects of ethanol on β-adrenoceptor regulation have used [^{3}H]dihydroalprenolol or ^{125}I-iodocyanopindolol exclusively, ligands which also bind to 5-HT_{1B} receptors (Hoyer et al., 1985a,b; Middlemiss, 1986; Edwards and Whitaker-Azmitia, 1987). This has also been the case in virtually all studies investigating the effects of antidepressants on β-adrenoceptors (references cited above). The few studies investigating effects of tricyclic antidepressants on β-adrenoceptors using the highly specific β-adrenoceptor ligand CGP12177 have only investigated changes in total β-adrenoceptor density, but not coupling efficiency (Honegger et al., 1986; Paetsch and Greenshaw, 1993; Goodnough and Baker, 1994).

In a recent investigation (Gurguis et al., 1998), we demonstrated that 35–40% of ^{125}I-iodocyanopindolol binding in the frontal cortex or hippocampus was to 5-HT_{1B} receptors. This binding represented a major component of the site for which isoproterenol has low affinity, since blockade of 5-HT_{1B} receptors has no effect on β-adrenoceptor density in the high-affinity state. We also demonstrated that the percentage of β-adrenoceptors in the high-affinity state, which couples G_s protein upon agonist stimulation, was approximately 95%, in contrast to previous, low estimates of 65% and 39% in the frontal cortex and hippocampus, respectively, when measured in the absence of 5-HT_{1B} receptor blockade. Therefore, results of previous studies on the effects of ethanol and/or tricyclic antidepressants on β-adrenoceptor regulation are confounded by 5-HT_{1B} receptor binding.

Confounding effects of 5-HT_{1B} receptors are even more significant in view of studies showing that chronic ethanol exposure induced upregulation of 5-HT_{1B} receptors (Nevo et al., 1995; Pandey et al., 1996). In addition, 5-HT_{1B} receptors have been implicated in the regulation of ethanol intake and tolerance (McBride et al., 1993, 1997; Crabbe et al., 1995; Risinger et al., 1996; Wang et al., 1996). Therefore, results of studies reporting that concomitant ethanol administration counteracted antidepressant-induced downregulation of β-adrenoceptor density or coupling (Rommelspacher and Strauss, 1987; Turkka et al., 1990) could have been confounded by the effects of ethanol on 5-HT_{1B} receptors. This confounding effect may not be apparent in saturation experiments and may affect changes in the relative distribution of β-adrenoceptors between the high- and low-affinity states in displacement experiments. Finally, inconsistent reports indicate that chronic treatment with tricyclic antidepressants may be associated with decreased 5-HT_{1B} heteroreceptor sensitivity or density (Mizuta and Segawa, 1988; Sleight et al., 1989; Montero et al., 1991; Johanning et al., 1992; Bolanos-Jimenez et al., 1994).

In this study, we investigated the effects of chronic ethanol exposure, treatment with desipramine, and combined ethanol/desipramine administration on β-adrenoceptor regulation in rat frontal cortex and hippocampus after blockade of binding to 5-HT_{1B} receptors. We measured the maximum binding capacity of β-adrenoceptors and the antagonist (^{125}I-iodocyanopindolol) dissociation constant using antagonist saturation experiments. We also investigated effects on the relative distribution of β-adrenoceptors in the high- and low-affinity states, agonist affinity to both states and coupling efficiency to G_s protein using agonist (isoproterenol) displacement experiments.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 250–300 g were used in all experiments. Rats were allowed to adapt for 1 week after shipment before the start of experiments.

Rats were housed four to six per cage in similar chambers, maintained under constant temperature and lighting conditions (12/12 h light/dark cycle), with access to food and water ad libitum. Rats were treated with placebo, ethanol, desipramine or combined ethanol/desipramine for 10 days, as previously detailed (Karanian et al., 1986; Turkka et al., 1989, 1990). Briefly, ethanol exposure was effected by housing rats in air-tight chambers containing ethanol vapor at concentrations of 25 mg/l for 10 days. This method has been shown to produce constant blood ethanol levels of approximately 141.8 ± 12.5 mg/dl (Turkka et al., 1990; Karanian et al., 1986). Ethanol exposed rats displayed behaviors suggestive of severe intoxication or physical dependence, as evidenced by severely decreased alertness, weakness, lethargy, drowsiness and decreased motor activity. Desipramine (10 mg/kg body weight/24 h) was administered intraperitoneally (i.p.). Control and ethanol-exposed rats received the same daily volume of saline injections i.p.

2.2. Tissue membrane preparation

On experiment days, rats were sacrificed at the same time of day (0800–0900 h), 24 h after the last saline or desipramine injection. Ethanol exposed rats were sacrificed immediately after they were taken out of the inhalation chambers to avoid the development of withdrawal symptoms or potential changes in β-adrenoceptor binding parameters that might accompany ethanol withdrawal. The brain was removed and placed on an ice-cold dissecting block. The frontal cortex and hippocampus were dissected and placed in ice-cold homogenization buffer (50 mmol Tris–HCl, 1 mmol EDTA, 5 mmol $MgCl_2$, pH 7.4). Membranes were prepared as previously described (Valverius et al., 1987). Brain tissue was homogenized by a polytron and centrifuged at $600 \times g$ for 10 min at 4°C. The supernatant was recentrifuged at $48{,}000 \times g$ for 20 min and the pellet was resuspended and incubated at 37°C for 30 min. Membranes were then washed and recentrifuged three times at $48{,}000 \times g$ for 20 min at 4°C. Finally, the membrane pellet was resuspended in the homogenization buffer. Final protein concentration was approximately 0.2 mg/ml.

2.3. β-Adrenoceptor binding assay

Fresh membrane preparations were used for binding assays in all experiments. Binding assays were conducted according to the method of Burgisser and Lefkowitz (1984), with modifications (Gurguis et al., 1998). Briefly, ^{125}I-iodocyanopindolol was used as a ligand in the presence of 10 μmol unlabeled serotonin to block binding to 5-HT_{1B} receptors in a total volume of 250 μl. The reaction was started by the addition of 100 μl membrane preparation, and the reaction mixture was incubated at 37°C for 75 min.

Saturation experiments were conducted using seven concentrations of ^{125}I-iodocyanopindolol (5–100 pmol). Non-specific binding was measured in the presence of unlabeled isoproterenol (0.1 mmol) and was approximately 5% of total binding. Displacement experiments of ^{125}I-iodocyanopindolol (25 pmol) binding to β-adrenoceptors were conducted using 18 different concentrations of isoproterenol (1 nmol–1 mmol) to measure β-adrenoceptor density in the high- and low-conformational states and agonist affinity to both states.

The reaction was stopped with 5 ml ice-cold wash buffer (50 mmol Tris–HCl, 15 mmol $MgCl_2$, 100 mmol NaCl, pH 7.5). Membranes were filtered over Whatman GF/B filters using a cell harvester (Brandel M-24) and rinsed three times with 5 ml ice-cold wash buffer. Radioactivity was counted using a gamma counter (Beckman 5500) with 73% counting efficiency. Proteins were quantified using the method of Lowry et al. (1951).

2.4. Chemicals

Desipramine, (−)-isoproterenol, serotonin, ascorbate and bovine serum albumin were obtained from Sigma (St. Louis, MO, USA). ^{125}I-iodocyanopindolol (SA 2200 Ci/mmol) was obtained from DuPont, New England Nuclear (Boston, MA, USA).

2.5. Binding data analysis

Binding data from saturation and displacement experiments were analyzed using LIGAND program (Munson and Rodbard, 1983). For saturation experiments, LIGAND uses a weighted-average, iterative curvilinear fitting technique to deduce the maximum binding capacity (B_{max}) and the antagonist's (^{125}I-iodocyanopindolol) dissociation constant (K_d) from β-adrenoceptors. Non-specific binding was estimated by LIGAND in the final binding parameters as a function of the total radioligand concentration. For displacement experiments, non-linear least-square regression analysis was used to calculate isoproterenol binding measures to β-adrenoceptors. Displacement curves were tested for a two-site model. A two-site model was accepted only if the goodness-of-fit was better for a two-site model than for a one-site model (F-test, $P \leq 0.05$). β-adrenoceptor density in the high- and low-conformational states (R_H and R_L, respectively) and the dissociation constant of isoproterenol from the receptor in the high- and low-conformational states (K_H and K_L, respectively) were derived from two-site models. Agonist-measured total receptor density (R_T, where $R_T = R_H + R_L$), the percentage of receptors in the high-conformational state ($\%R_H$) and the K_L/K_H ratio were calculated. The $\%R_H$ and the K_L/K_H ratio have been correlated with the agonist's intrinsic activity and proposed as measures of coupling efficiency of the agonist-occupied receptor to G_s protein (Davies and

Table 1
Brain β-adrenergic receptor binding parameters in the frontal cortex in control rats and rats treated with alcohol and/or desipramine

Frontal cortex	K_d (pM)	B_{max} (fmol/mg protein)	R_H (fmol/mg protein)	R_L (fmol/mg protein)	R_T (fmol/mg protein)	$\%R_H$	K_H (nM)	K_L (μM)	K_L / K_H
Control rats ($n = 13$)	9.11 ± 0.64	107.71 ± 1.82	112.30 ± 3.31	2.96 ± 0.23	115.26 ± 3.39	97.44 ± 0.18	69.29 ± 3.48	11.02 ± 3.26	169.71 ± 56.39
Alcohol-treated rats ($n = 10$)	9.59 ± 0.28	100.80 ± 4.07	104.72 ± 5.09	8.34 ± 1.94[d]	113.06 ± 4.31	92.42 ± 1.84[d]	53.59 ± 3.88[i]	2.82 ± 0.89	46.82 ± 13.87
Desipramine-treated rats ($n = 12$)	8.98 ± 0.46	75.18 ± 3.00[a,b]	74.36 ± 2.73[a,b]	3.22 ± 0.41[e]	77.57 ± 2.73[a,b]	95.81 ± 0.53[g]	71.25 ± 3.65[j]	10.25 ± 2.21	141.03 ± 32.32
Alcohol + desipramine-treated rats ($n = 11$)	8.48 ± 0.30	57.81 ± 1.26[a,b,c]	66.42 ± 2.74[a,b]	3.02 ± 0.24[f]	69.26 ± 2.84[a,b]	95.66 ± 0.27[h]	54.99 ± 3.88[k,l]	6.33 ± 1.62	105.27 ± 23.44
F-statistic	0.838	76.796	41.924	7.971	50.739	5.701	6.122	2.532	1.844
P-value	NS	0.000	0.000	0.000	0.000	0.002	0.002	0.070	NS

Values are mean ± SEM.
Bonferroni-corrected *P*-values are mentioned below. [a]$P < 0.000$ vs. control rats. [b]$P < 0.000$ vs. alcohol-treated rats. [c]$P < 0.000$ vs. desipramine-treated rats. [d]$P < 0.001$ vs. control rats. [e]$P < 0.002$ vs. alcohol-treated rats. [f]$P < 0.001$ vs. alcohol-treated rats. [g]$P < 0.06$ vs. alcohol-treated rats. [h]$P < 0.09$ vs. alcohol-treated rats. [i]$P < 0.03$ vs. control rats. [j]$P < 0.01$ vs. alcohol-treated rats. [k]$P < 0.04$ vs. control rats. [l]$P < 0.02$ vs. desipramine-treated rats.

Table 2
Brain β-adrenergic receptor binding parameters in the hippocampus in control rats and rats treated with alcohol and/or desipramine

Hippocampus	K_d (pM)	B_{max} (fmol/mg protein)	R_H (fmol/mg protein)	R_L (fmol/mg protein)	R_T (fmol/mg protein)	$\%R_H$	K_H (nM)	K_L (μM)	K_L / K_H
Control rats ($n = 13$)	10.24 ± 0.86	46.64 ± 1.20	47.95 ± 1.21	2.01 ± 0.23	49.96 ± 1.22	95.97 ± 0.46	52.07 ± 3.49	6.45 ± 1.86	124.16 ± 33.13
Alcohol-treated rats ($n = 10$)	10.10 ± 0.50	42.22 ± 1.38[a]	45.93 ± 1.41	1.55 ± 0.17	47.48 ± 1.43	96.73 ± 0.35	51.27 ± 3.64	13.71 ± 5.26	254.46 ± 91.96
Desipramine-treated rats ($n = 12$)	11.82 ± 0.63	26.91 ± 1.06[b,c]	26.08 ± 1.06[b,c]	1.33 ± 0.14	27.41 ± 1.11[b,c]	95.16 ± 0.45	58.48 ± 3.75	25.33 ± 6.06[i]	458.15 ± 122.26[m]
Alcohol + desipramine-treated rats ($n = 11$)	9.86 ± 0.59	20.52 ± 0.50[b,c,d]	20.99 ± 0.89[b,c,e]	4.43 ± 0.93[f,g,h]	25.42 ± 0.80[b,c]	82.93 ± 3.30[b,c,h]	33.42 ± 4.04[h,k,l]	2.70 ± 1.24[j]	31.06 ± 7.30[j]
F-statistic	1.698	131.311	140.783	8.788	125.625	15.745	8.149	6.110	5.791
P-value	NS	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.002

Values are mean ± SEM.
Bonferroni-corrected *P*-values are mentioned below. [a]$P < 0.04$ vs. control rats. [b]$P < 0.000$ vs. control rats. [c]$P < 0.000$ vs. alcohol-treated rats. [d]$P < 0.001$ vs. desipramine-treated rats. [e]$P < 0.02$ vs. desipramine-treated rats. [f]$P < 0.004$ vs. control rats. [g]$P < 0.001$ vs. alcohol-treated. [h]$P < 0.000$ vs. desipramine-treated. [i]$P < 0.009$ vs. control rats. [j]$P < 0.002$ vs. desipramine-treated. [k]$P < 0.005$ vs. control rats. [l]$P < 0.01$ vs. alcohol-treated rats. [m]$P < 0.02$ vs. control rats.

Lefkowitz, 1980, 1981; DeLean et al., 1980, 1982; Kent et al., 1980; Davies et al., 1981).

2.6. Statistical analysis

Data are presented as mean ± standard error of the mean. One-way analysis of variance with Bonferroni correction was used to test for differences among the four treatment groups. Relationships between the same binding parameter in the two brain regions from the same brain were explored using Pearson's product moment analysis.

3. Results

3.1. In the frontal cortex

Ethanol exposure had no effect on B_{max} or R_T. However, ethanol-treated rats had significantly higher R_L than controls, desipramine-treated or ethanol/desipramine-treated rats. Consequently, $\%R_H$ was significantly lower in ethanol-treated rats compared to controls, desipramine-treated and ethanol/desipramine-treated rats. Although ethanol decreased the apparent K_H and K_L, it significantly lowered the K_L/K_H ratio (Welch's $t = 2.116$, $P < 0.05$) compared to controls. Thus, ethanol treatment significantly decreased both $\%R_H$ and the K_L/K_H ratio.

Desipramine-treated rats had significantly lower B_{max} and R_T than controls and ethanol-treated rats. This decrease in β-adrenoceptor density was due primarily to a significant decrease in R_H, since there were no changes in R_L. Unlike ethanol, desipramine had no effects on K_H, K_L, $\%R_H$ or the K_L/K_H ratio.

Ethanol/desipramine treatment was associated with significantly lower B_{max}, R_T, and R_H. Ethanol/desipramine-treated rats had significantly lower K_H than controls and desipramine-treated rats. However, in this combined treatment, desipramine prevented the ethanol-in-

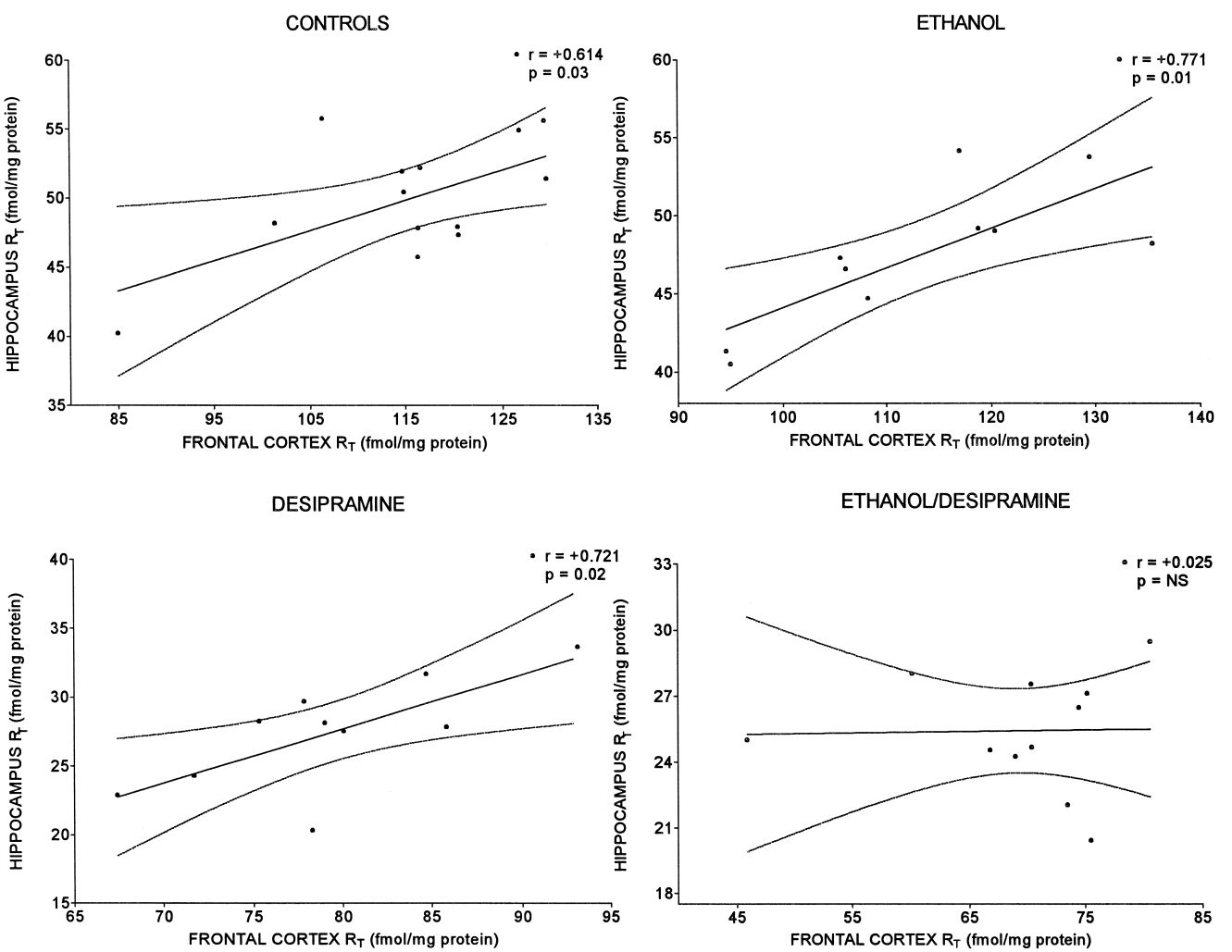

Fig. 1. The relationship between frontal cortex and hippocampus β-adrenergic receptor total density in control, ethanol-treated, desipramine-treated and ethanol/desipramine-treated rats.

duced decrease in $\%R_H$ and the K_L/K_H ratio. Data are summarized in Table 1.

3.2. In the hippocampus

Ethanol treatment had no effect on measures of β-adrenoceptor density or agonist dissociation constants K_H and K_L. Moreover, ethanol exposure had no effect on $\%R_H$ or the K_L/K_H ratio.

Desipramine treatment significantly decreased B_{max} and R_T. The decrease in R_T was again due to a significant decrease in R_H. Desipramine treatment significantly increased K_L, but had no effect on K_H. Consequently, the K_L/K_H ratio was significantly increased.

Combined ethanol/desipramine treatment was associated with decreased measures of β-adrenoceptor density. Ethanol had an additive effect to desipramine, further lowering R_H, but it significantly increased R_L. Ethanol, however, prevented the increase in the K_L/K_H ratio observed in desipramine-treated rats. Furthermore, ethanol/desipramine treatment lowered the K_L/K_H ratio, an effect that was not observed with ethanol treatment alone. Thus, ethanol significantly decreased both $\%R_H$ and the K_L/K_H ratio only in combination with desipramine. Data are summarized in Table 2.

Treatment with either ethanol, desipramine or combined ethanol/desipramine had no effect on K_d in either the frontal cortex or the hippocampus.

Exploring relationships between the same binding parameter in the frontal cortex and hippocampus, the following correlations were observed in Section 3.3 below.

3.3. In control rats ($n = 13$)

R_H in the frontal cortex correlated positively with R_H in the hippocampus ($r = 0.587$, $P = 0.03$). R_T in the frontal cortex was positively correlated with R_T in the hippocampus ($r = 0.614$, $P = 0.03$). K_H in the frontal cortex was positively correlated with K_H in the hippocam-

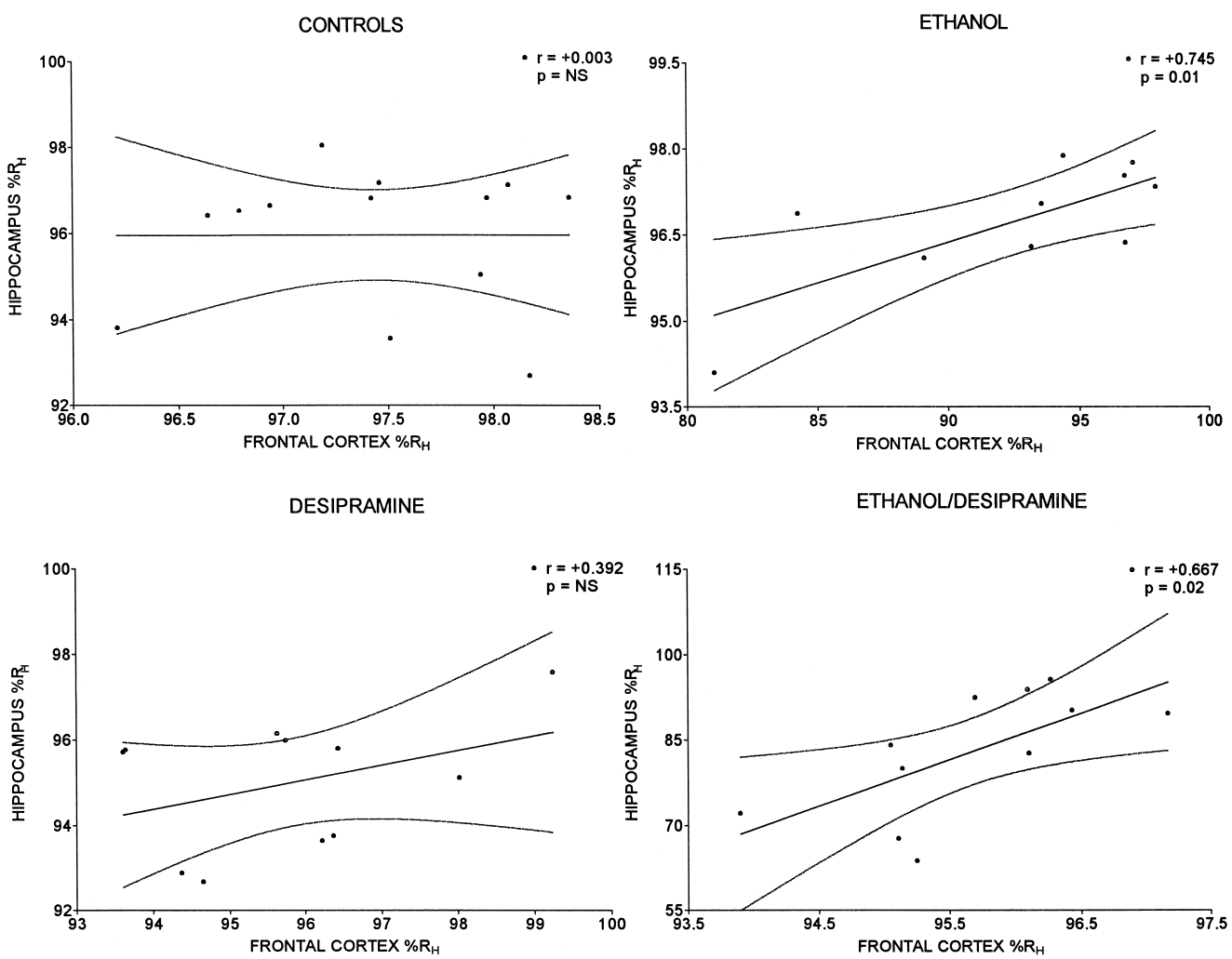

Fig. 2. The relationship between frontal cortex and hippocampus percentage of β-adrenergic receptors in the high-affinity state in control, ethanol-treated, desipramine-treated and ethanol/desipramine-treated rats.

pus ($r = 0.549$, $P = 0.05$). K_d in the frontal cortex correlated positively with K_d in the hippocampus ($r = 0.643$, $P = 0.02$) (see Fig. 1).

3.4. In ethanol-treated rats ($n = 10$)

Percentage R_H in the frontal cortex correlated positively with $\%R_H$ in the hippocampus($r = 0.745$, $P = 0.01$). R_H in the frontal cortex was positively correlated with R_H in the hippocampus ($r = 0.698$, $P = 0.02$). R_L in the frontal cortex correlated positively with R_L in the hippocampus ($r = 0.716$, $P = 0.02$). R_T in the frontal cortex was positively correlated with R_T in the hippocampus ($r = 0.771$, $P = 0.009$). B_{max} in the frontal cortex correlated positively with B_{max} in the hippocampus ($r = 0.678$, $P = 0.03$).

3.5. In desipramine-treated rats ($n = 12$)

B_{max} in the frontal cortex correlated positively with B_{max} in the hippocampus ($r = 0.702$, $P = 0.01$). K_d in the frontal cortex correlated positively with K_d in the hippocampus ($r = 0.584$, $P = 0.05$) (see Fig. 2).

3.6. In ethanol / desipramine-treated rats ($n = 11$)

Percentage R_H in the frontal cortex correlated positively with $\%R_H$ in the hippocampus ($r = 0.667$, $P = 0.02$). K_L/K_H ratio in the frontal cortex was positively correlated with K_L/K_H ratio in the hippocampus ($r = 0.732$, $P = 0.01$). R_L in the frontal cortex was positively correlated with R_L in the hippocampus ($r = 0.710$, $P = 0.01$).

Hence, in control rats β-adrenoceptor density and agonist affinity were correlated in the two brain regions, but there were no relationship between functional coupling measures. Treatment with ethanol, desipramine, or both altered these relationships and induced new relationships that were not observed in controls. This effect was seen particularly in ethanol or ethanol/desipramine treatment, where coupling measures were correlated in both brain regions.

4. Discussion

This study provides, for the first time, a detailed analysis of the effects of ethanol, desipramine and combined ethanol/desipramine on multiple aspects of β-adrenoceptor regulation and coupling to the adenylate cyclase system after excluding the confounding effects of 5-HT_{1B} receptors. The results indicate an intricate interaction between the two drugs on brain β-adrenoceptors that is region-specific. In the frontal cortex and, particularly, in the hippocampus, combined ethanol/desipramine treatment was associated with further decrease in β-adrenoceptors B_{max} and R_T, suggesting that ethanol has an additive effect on desipramine-induced downregulation of β-adrenoceptor density. However, the effects of combined treatment on β-adrenoceptor coupling were different in the two brain regions. In the frontal cortex, desipramine in combination with ethanol prevented ethanol-induced β-adrenoceptor uncoupling, as reflected in the lack of increase in R_L and decrease in both $\%R_H$ and the K_L/K_H ratio observed with ethanol treatment alone. In the hippocampus, combined treatment was associated with significant β-adrenoceptor uncoupling, as reflected in upregulation of R_L, and decreases in $\%R_H$ and the K_L/K_H ratio. This effect was not observed with ethanol treatment alone in the hippocampus. Therefore, combined ethanol/desipramine not only prevented desipramine-induced β-adrenoceptor supercoupling, but further induced uncoupling. Inhibition of hippocampal β-adrenoceptor function may underlie the deleterious clinical effects of ethanol ingestion in patients concurrently receiving antidepressant medications.

In this investigation, desipramine, in combination with ethanol, decreased β-adrenoceptor density in both the frontal cortex and hippocampus. This was not observed in a previous study from our laboratory in which ethanol prevented β-adrenoceptor density downregulation by desipramine in the absence of a 5-HT_{1B} receptor blockade (Rommelspacher and Strauss, 1987; Turkka et al., 1990). Ethanol increases 5-HT_{1B} receptor density (Nevo et al., 1995; Pandey et al., 1996). Therefore, the lack of desipramine-induced β-adrenoceptor density downregulation observed in these studies was due to the upregulation of 5-HT_{1B} density by ethanol, which masked desipramine's effects.

Desipramine decreased total β-adrenoceptor density by 33% and 45% in the frontal cortex and hippocampus, respectively. This downregulatory effect on β-adrenoceptor density was primarily due to decreased R_H, since there was no change in R_L, consistent with previous findings (Hancock and Marsh, 1985; Manier et al., 1989). Decreased β-adrenoceptor density is also consistent with findings of decreased β-adrenoceptor mRNA and cAMP response element-binding protein/cAMP response element-directed gene transcription (Hosoda and Duman, 1993; Schwaninger et al., 1995). Decreased β-adrenoceptor density, particularly in the high-affinity state, may also suggest enhanced PKA activity by an agonist-occupied receptor, resulting in phosphorylation and downregulation of the β-adrenoceptor molecule (Benovic et al., 1985; Sibley et al., 1985; Strasser and Lefkowitz, 1985; Bouvier et al., 1989; Hausdorff et al., 1989). Activation of protein kinase A has also been shown to be necessary for ethanol-induced desensitization of cAMP responses (Rabin et al., 1992). This may explain the additive effects of combined ethanol/desipramine treatment on downregulation of β-adrenoceptors. Finally, activation of protein kinase C has

been implicated in antidepressant-induced downregulation of β-adrenoceptors and may underlie results of the present investigation (Asakura et al., 1989; Manji et al., 1991b).

In a previous report we did not observe downregulation of β-adrenoceptor density by desipramine in the hippocampus in the absence of blocking ^{125}I-iodocyanopindolol binding to 5-HT_{1B} receptors (Turkka et al., 1989). The present findings are inconsistent with these earlier observations, and may be due to the confounding effects of ^{125}I-iodocyanopindolol binding to 5-HT_{1B} receptors. Antidepressants have been shown, although inconsistently, to decrease 5-HT_{1B} receptor density or sensitivity (Mizuta and Segawa, 1988; Sleight et al., 1989; Montero et al., 1991; Johanning et al., 1992; Bolanos-Jimenez et al., 1994). Desipramine had no effect on either measure of β-adrenoceptor coupling or agonist affinity to β-adrenoceptors in either affinity state in the frontal cortex. This suggests that decreased cAMP responses following tricyclic antidepressant treatment are due, in part, to homologous desensitization of β-adrenoceptors. However, given that decreased cAMP responses following antidepressant treatment are disproportionate to decreases in β-adrenoceptor density, modulation of other post-receptor mechanisms of G protein function and G protein–adenylyl cyclase interactions by antidepressants cannot be ruled out (Tsuchiya et al., 1988; Yamamoto et al., 1990).

Desipramine had no effect on β-adrenoceptor coupling in the frontal cortex, but induced supercoupling in the hippocampus, suggesting that tricyclic antidepressant effects on β-adrenoceptor coupling are region-specific. Desipramine's effects on β-adrenoceptor regulation in the hippocampus are complex. While it downregulated β-adrenoceptor density, it induced β-adrenoceptor supercoupling to G_s, as reflected in an increased K_L/K_H ratio. This increase replicates previous findings from our laboratory (Turkka et al., 1989). The increase in the K_L/K_H ratio, concomitant with downregulation of β-adrenoceptor density and cAMP responses following desipramine treatment, was also observed in C6 glioma cell cultures (Manji et al., 1991a). Desipramine induction of β-adrenoceptor supercoupling to G_s protein is consistent with increased [^{3}H]GTP binding and decreased phosphatase activity of the $G_{s\alpha}$ subunit following antidepressant treatment (Yamamoto et al., 1990). However, the contribution of these multiple mechanisms to the ultimate and well-documented decrease in cAMP responses after antidepressant treatment remains unclear and should be explored further in future investigations.

In the frontal cortex, ethanol induced β-adrenoceptor uncoupling, as reflected in the four-fold decrease in the K_L/K_H ratio. In addition, ethanol induced a small shift in the relative distribution of β-adrenoceptor density toward the low-affinity state, as reflected in increased R_L. Consequently, there was a small (5%), albeit significant, decrease in $\%R_H$. Therefore, ethanol-induced β-adrenoceptor uncoupling in the frontal cortex was reflected in both coupling measures. In contrast, ethanol had no effect on β-adrenoceptor coupling or other binding parameters in the hippocampus. Ethanol-induced β-adrenoceptor uncoupling is consistent with studies showing that chronic ethanol exposure was associated with decreased cAMP responses, G_s protein levels or G_s expression (Saito et al., 1987; Charness et al., 1988; Nhamburo et al., 1988; Saffey et al., 1988; Nakamura, 1993; Ozawa et al., 1994a,b). Results of this investigation have not dissected out the effects of ethanol and/or desipramine in β_1- vs. β_2-adrenoceptors. A number of studies, however, have shown that downregulatory effects of antidepressants may be limited to β_1-, not β_2-adrenoceptors, which represent the majority of receptors in the frontal cortex and hippocampus.

Ethanol exposure, desipramine treatment or combined ethanol/desipramine treatment induced relationships between the binding parameters of β-adrenoceptors in both brain regions that were not observed in control rats. For example, estimates of R_L were correlated in both brain regions in ethanol exposed rats, but not in controls. This may be consistent with the upregulatory effects of ethanol on β-adrenoceptor density in the low-conformational state. Desipramine's effects on β-adrenoceptor coupling were region-specific. Consequently, the relationship between agonist affinity to the receptor in the high-conformational state in the two brain regions which was observed in control rats was not seen in desipramine treated rats. Finally, R_H, R_T, K_H and K_d, but not coupling measures, were correlated in the two brain regions in control rats. This suggests that while β-adrenoceptor binding characteristics in the two brain regions are related, β-adrenoceptor function in these two regions are independent under normal conditions. Combined treatment with ethanol and desipramine induced strong correlations in both coupling measures in the two brain regions, suggesting that the two drugs combined alter the functional relationship between brain regions.

The present results show that ethanol, consistent with the majority of studies cited above, had no effect on total β-adrenoceptor density in the frontal cortex or the hippocampus. In the hippocampus, ethanol had only minimal (9%) downregulatory effects on antagonist-measured β-adrenoceptor density; there were no changes in other measures of β-adrenoceptor density. Ethanol's effects on β-adrenoceptor coupling appeared to be region-specific, inducing β-adrenoceptor uncoupling only in the frontal cortex. Ethanol also minimally decreased $\%R_H$, but there was no complete rightward shift in agonist displacement curves to a one-site model. This replicates previous findings from our laboratory (Turkka et al., 1990), but is inconsistent with studies showing a complete rightward shift in agonist displacement curves and a conversion of β-adrenoceptors into a low-affinity state (Valverius et al., 1987). Future studies may investigate the combined effects of these drugs on post-receptor signal transduction mechanisms, particularly on changes in G protein function triggered by

the agonist-occupied receptor and G protein–adenylyl cyclase interactions.

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