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BRIEF COMMUNICATION

Housing Conditions Alter $GABA_A$ Receptor of Alcohol-Preferring and -Nonpreferring Rats

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THIELEN, R. J., W. J. McBRIDE, L. LUMENG AND T.-K. LI. *Housing conditions alter $GABA_A$ receptor of alcohol-preferring and -nonpreferring rats.* PHARMACOL BIOCHEM BEHAV **46**(3) 723–727, 1993.—The effects of housing conditions on some functional properties of the $GABA_A$ benzodiazepine (BZD) receptor in the cerebral cortex were examined in the selectively bred alcohol-preferring (P) and -nonpreferring (NP) lines of rats. Compared to rats housed in pairs (P with P and NP with NP), P and NP rats housed individually had 44% ($p < 0.005$) and 32% ($p < 0.01$) lower values, respectively, for GABA-stimulated $^{36}Cl^-$ influx into cortical microsacs. The maximal effect (V_{max}) of flunitrazepam (FNZ) to enhance GABA-stimulated $^{36}Cl^-$ uptake was 44% higher in individually housed P rats than pair-housed P rats ($p < 0.05$) and 51% higher than individually housed NP rats ($p < 0.05$). There was no difference between single and pair-housed NP rats for V_{max} values of FNZ enhancement of GABA-stimulated $^{36}Cl^-$ influx. The results show housing conditions can alter some of the functional properties of the $GABA_A$/BZD receptor in the P and NP lines of rats. The differential effect of housing conditions on FNZ enhancement of $^{36}Cl^-$ influx, observed between the lines, may be a result of higher levels of anxiety being produced by brief isolation in the P rat.

Alcohol-preferring P rats Alcohol-nonpreferring NP rats Housing conditions Cl^- influx
$GABA_A$ benzodiazepine receptor

MANY studies have shown changes in $GABA_A$/benzodiazepine (BZD) receptor characteristics in animals exposed to stressful stimuli [for review, see (6)], but the results have been inconsistent. Depending upon brain region, age, and ligand used, social isolation has been reported to decrease (11,20) or result in no change (11,21) of BZD binding. Morinan et al. (21) found no differences in [^{3}H]flunitrazepam (FNZ)- and GABA-stimulated [^{3}H]FNZ binding to membrane preparations of frontal cortex, hippocampus, amygdala, and cerebellum in male Sprague-Dawley rats that were isolated for 22 days compared to group-housed rats. However, Miachon et al. (20), using quantitative autoradiography, demonstrated lower affinity for [^{3}]FNZ binding sites in the cerebellum of male Wistar rats individually housed compared to group-housed rats. Insel et al. (11), using quantitative autoradiography, found in vivo [^{3}H]Ro 15-1788 (flumazenil) binding was also decreased in the cortex, hippocampus, and superior and inferior colliculi of separated 10-day-old rats compared to unseparated rat pups; however, there were no apparent differences in the in vivo binding of [^{3}H]flumazenil between the separated and unseparated rat pups in the olfactory bulb, striatum, or cerebellum. Thus far, the effects of different housing conditions on the functional characteristics of the $GABA_A$/BZD receptor complex have not been examined.

The alcohol-preferring (P) and -nonpreferring (NP) lines of rats have been selectively bred for divergent alcohol-seeking behavior (15,17). These lines of rats have also been shown to differ in their sensitivity to intoxicating doses of ethanol (18). Lines of mice, selectively bred for differences in initial sensitivity to the acute intoxicating effects of ethanol (19), have been shown to differ in $GABA_A$/BZD function, as measure by $^{36}Cl^-$ influx into cortical microsacs (1). Studies were initiated, therefore, to examine $GABA_A$/BZD receptor function by measuring $^{36}Cl^-$ influx into cortical microsacs. While compar-

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ing $GABA_A$/BZD receptor function in cortical microsacs from pair-housed P and NP rats, lower GABA-stimulated $^{36}Cl^-$ influx values were observed in animals killed and assayed 24–48 h after their cage-mate. These initial observations were extended to examine the effect of short-term housing conditions (pair-housed vs. individually housed) on GABA-stimulated $^{36}Cl^-$ influx and FNZ enhancement of GABA-stimulated $^{36}Cl^-$ influx into cortical microsacs from P and NP rats.

METHOD

Animals

Alcohol-naive, adult (70–90 days), male P and NP rats from the S33–35 generations (15,17) were used in this study. Animals were housed in a temperature-controlled room, maintained on a 12L : 12D cycle (lights on at 0400 h), with food and water available ad lib. The animals were separated into four groups based on line and housing condition. Animals in the pair-housed groups were housed two (P with P, NP with NP) to a tub (standard plastic animal containers) for the duration of the experiment. Animals in the individually housed groups were initially housed in pairs and were then individually housed 1–2 days before being killed. All rats were handled daily and habituated to the guillotine for at least 1 week prior to each experiment. The animals were killed by decapitation.

Chemicals

GABA, flunitrazepam, picrotoxin, and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO). To solubilize FNZ, it was necessary to initially dissolve FNZ in DMSO followed by dilution in buffer so that the final concentration of DMSO was less then 1% (v/v). $^{36}Cl^-$ ($Na^{36}Cl$, 8–14 mCi/g Cl) was acquired from ICN (Irvine, CA).

Microsac Preparation

Microsacs were prepared by the method of Harris and Allen (9). Briefly, the cerebral cortex was carefully dissected on ice and homogenized (10–12 strokes) using a glass-Teflon homogenizer (Thomas, size C) in 4.5 ml of ice-cold buffer (145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM D-glucose, and 10 mM 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES) adjusted to pH 7.5 with Tris base). The homogenate was centrifuged at 900 × *g* for 15 min. The supernatant was decanted and the pellet resuspended in 8 ml of ice-cold buffer. The resulting suspension was then centrifuged at 900 × *g* for 15 min. The final pellet was resuspended in 7 ml of ice-cold buffer to give a protein concentration of approximately 5–7 mg protein/ml. Protein concentration was determined by the method of Lowry et al. (16).

$^{36}Cl^-$ Influx

The microsac suspension (200 μl) was preincubated for 2 min at 34°C in a shaking waterbath (10). A 200-μl aliquot, containing $^{36}Cl^-$ (1.6 μCi/ml) and increasing concentrations of the test agents, was added to the microsac suspensions to initiate uptake. Uptake was terminated 3 s later by the addition of 4 ml of ice-cold buffer containing 100 μM picrotoxin followed by rapid filtration under vacuum (254 mmHg) through glass microfibre filters (Whatman GF/C) using a Hoeffer manifold (model FH 224V, San Francisco, CA). After removing the towers, the filters were washed with 8 ml of ice-cold buffer containing 100 μM picrotoxin. The filters were transferred to scintillation vials containing 10 ml of scintillation cocktail (CytoScint, ICN, Irvine, CA) and the amount of $^{36}Cl^-$ taken up was determined by liquid scintillation spectrophotometric methods. The amount of $^{36}Cl^-$ bound to the filters in the absence of membranes was subtracted from all values. GABA-dependent influx was defined as the amount of $^{36}Cl^-$ taken up when GABA was present in the medium minus the amount of $^{36}Cl^-$ taken up when GABA was absent (GABA-independent uptake). FNZ-enhanced $^{36}Cl^-$ influx was defined as the amount of $^{36}Cl^-$ taken up in the presence of FNZ and 5 μM GABA minus 5 μM GABA-dependent uptake when FNZ was absent.

Statistical Analysis

All data are expressed as the means ± SEM. EC_{50} and V_{max} values were determined using the nonlinear regression algorithm of Sigmaplot, version 5.0. The data were analyzed by analysis of variance (ANOVA) followed by post hoc Newman-Keuls test when multiple comparisons were carried out.

RESULTS

There were no differences between any of the groups in GABA-independent $^{36}Cl^-$ influx (P pair-housed, 25.2 ± 0.9; P individually housed, 26.7 ± 1.1; NP pair-housed, 26.2 ± 1.1; NP individually housed, 25.4 ± 0.9 nmol Cl^-/mg protein/3 s). A comparison of the effects of housing conditions on 5 μM GABA-stimulated $^{36}Cl^-$ influx into cortical microsacs obtained from P and NP rats is shown in Fig. 1. ANOVA of 5 μM GABA-stimulated $^{36}Cl^-$ influx revealed a significant effect of housing condition, $F(1, 16) = 31.83$, $p < 0.001$. The effect of 5 μM GABA on $^{36}Cl^-$ influx was 44% lower in the P individually housed group ($p < 0.005$) and 32% lower in the NP individually housed group ($p < 0.01$) compared with the P and NP pair-housed animals, respectively. There were no significant differences between P and NP rats that were pair-housed and between P and NP that were individually housed.

A comparison of the effects of housing conditions on FNZ enhancement of 5 μM GABA-stimulated $^{36}Cl^-$ influx into cortical microsacs obtained from P and NP rats is shown in Fig. 2. ANOVA of FNZ enhancement of 5 μM GABA-stimulated $^{36}Cl^-$ influx revealed a significant effect of FNZ concentration, $F(4, 64) = 72.30$, $p < 0.001$, and a significant effect

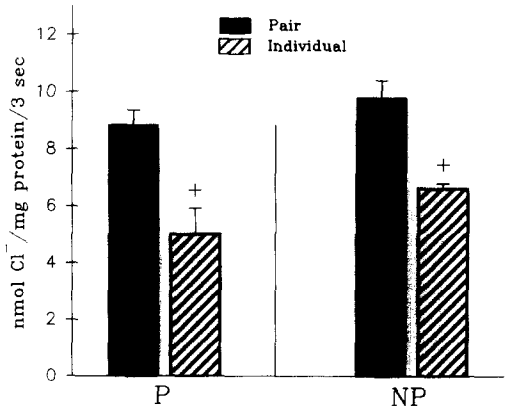

FIG. 1. Comparison of 5 μM GABA-stimulated $^{36}Cl^-$ influx into cortical microsacs prepared from individually and pair-housed P and NP rats. The data represent Cl^- influx (mean ± SEM) ($n = 5$ each line, each housing condition). $^+p < 0.01$ vs. pair-housed.

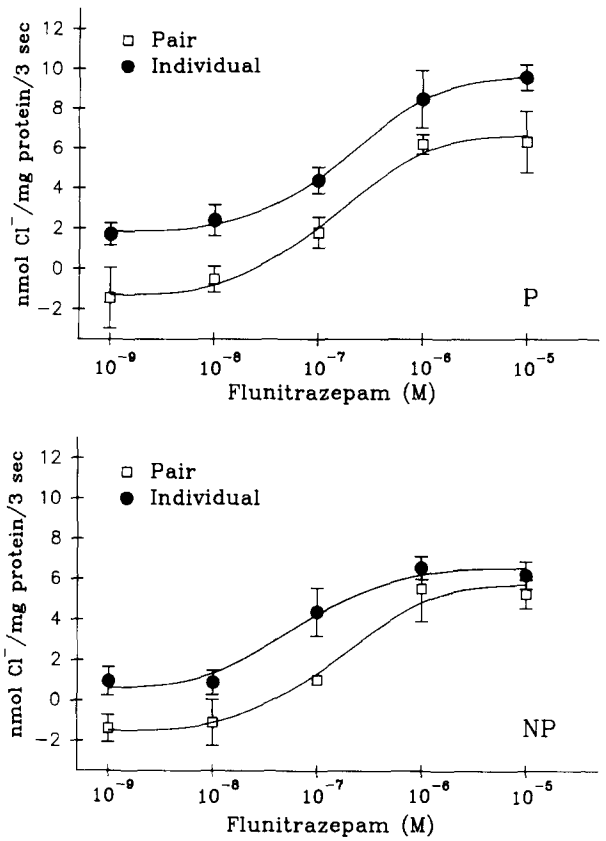

FIG. 2. Comparison of FNZ enhancement of 5 μM GABA-stimulated $^{36}Cl^-$ influx into cortical microsacs prepared from individually and pair-housed P (top panel) and NP (bottom panel) rats. The data represent FNZ-enhanced $^{36}Cl^-$ influx (mean ± SEM) (n = 5 each line, each housing condition). FNZ-enhanced $^{36}Cl^-$ influx was defined as the amount of $^{36}Cl^-$ taken up in the presence of FNZ and 5 μM GABA minus 5 μM GABA-dependent $^{36}Cl^-$ uptake when FNZ was absent.

of housing condition, $F(1, 16) = 14.32$, $p < 0.005$. Separate ANOVA were performed on each line to determine if housing condition altered FNZ enhancement of 5 μM GABA-stimulated $^{36}Cl^-$ influx in only one or both lines. FNZ, concentration-dependently, enhanced the effect of 5 μM GABA on $^{36}Cl^-$ influx in all groups [$F(4, 32) = 36.38$, $p < 0.001$; $F(4, 32) = 37.01$, $p < 0.001$, P and NP lines, respectively]. Housing conditions altered FNZ enhancement of GABA-stimulated $^{36}Cl^-$ influx in the P line, $F(1, 8) = 10.12$, $p < 0.05$, and in the NP line, $F(1, 8) = 4.70$, $p = 0.06$. The effect of housing conditions on FNZ enhancement of GABA-stimulated $^{36}Cl^-$ influx in the P line was a result of an increase in the efficacy of FNZ to enhance GABA-stimulated $^{36}Cl^-$ influx in the individually housed P group compared to the pair-housed P group (Table 1). No difference was found for V_{max} values between pair and individually housed NP rats (Table 1). Housing condition had no effect on the EC_{50} value for FNZ enhancement of GABA-stimulated $^{36}Cl^-$ influx in either line (Table 1).

DISCUSSION

This study shows that there are functional differences in cortical GABA$_A$/BZD receptors as a result of housing conditions in P and NP rats. Housing animals individually results

TABLE 1

EC_{50} AND V_{max} VALUES FOR FLUNITRAZEPAM ENHANCEMENT OF GABA-STIMULATED $^{36}Cl^-$ INFLUX INTO CORTICAL MICROSACS PREPARED FROM PAIR-HOUSED AND INDIVIDUALLY HOUSED P AND NP RATS

	EC_{50} (μM)	V_{max} (nmol Cl^-/mg protein/3 s)
P rats		
Pair	0.27 ± 0.13	7.0 ± 1.0
Individual	0.52 ± 0.27	10.1 ± 0.4*†
NP rats		
Pair	0.15 ± 0.04	5.7 ± 1.0
Individual	0.08 ± 0.02	6.7 ± 0.6

Data are mean ± SEM (n = 5 each line, each housing condition). ANOVA of EC_{50} values revealed no significant differences. ANOVA of V_{max} values revealed a significant effect of line, $F(1, 16) = 8.70$, $p < 0.01$, and housing condition, $F(1, 16) = 6.43$, $p < 0.05$. *p < 0.05 compared with P pair-housed.

†$p < 0.05$ compared with NP individually housed.

in a lower GABA-stimulated $^{36}Cl^-$ flux into cortical microsacs from both lines of rats (Fig. 1). In addition, this form of brief social isolation results in an increase in the efficacy of FNZ to enhance GABA-stimulated $^{36}Cl^-$ influx in microsacs prepared from the cerebral cortex of P, but not NP, rats (Fig. 2, Table 1).

Results from other studies have shown a decrease in GABA$_A$ agonist-stimulated $^{36}Cl^-$ uptake into cortical membrane preparations after rats were exposed to foot shock and inescapable tail shock (5,7). GABA-stimulated $^{36}Cl^-$ influx was also reduced in rats not previously habituated to handling before killing compared with handling habituated rats (5). In addition, it was found that mice confined to the open end of an elevated plus-maze had lower GABA-stimulated influx of $^{36}Cl^-$ into cortical microsacs compared with controls (3). On the other hand, an increase in muscimol-stimulated $^{36}Cl^-$ uptake was observed in cortical and hippocampal synaptoneurosomes after rats were subjected to 10 min of ambient temperature forced swimming (23). Our results, demonstrating a lower GABA-stimulated response in the individually housed vs. the pair-housed rats, agree with the those findings that demonstrated a decrease in agonist stimulation of $^{36}Cl^-$ influx at the GABA$_A$/BZD receptor after the animals were exposed to stress (3,5,7), suggesting that a brief period of individual housing is stressful to both lines.

Previous studies have found decreases (11,20) or no change (11,21) in BZD binding in the cortex after social isolation. Similar results have been reported for a variety of stress paradigms [for review see (6)]. Results from studies with long-sleep (LS) and short-sleep (SS) mice, which were selectively bred for differences in acute sensitivity to the hypnotic effects of ethanol (19), showed that, after confinement to the open arm of an elevated plus-maze, [^{3}H]FNZ binding was increased, decreased, or unchanged, depending on the line and brain area examined (2,3). In the cerebral cortex, confinement to the open arms of an elevated plus-maze resulted in a highly significant decrease in GABA-stimulated $^{36}Cl^-$ influx in both lines and an increase in GABA-enhanced [^{3}H]FNZ binding in the SS, but not the LS mice. These results are in agreement with the present findings for the P and NP lines when measuring $^{36}Cl^-$ influx in response to GABA and FNZ in cortical microsacs. Taken together, the results of both studies support

the hypothesis that a genetic component is involved in stress-induced changes of the $GABA_A$/BZD receptor complex (3). Neuroactive steroid levels have been shown to be altered after acute and chronic stress in other animals (22), and it was suggested that neuroactive steroids might be involved in these responses in LS and SS mice (3,4). However, there is little genetic variation between these lines of mice in the response of $GABA_A$/BZD receptors to neuroactive steroids (4). Therefore, Bowers and Wehner (4) concluded that the differences observed between the LS and SS lines in [^{3}H]FNZ binding, after being confined to the open end of an elevated plus-maze, are not the result of different sensitivities of the $GABA_A$/BZD receptor to neuroactive steroids.

The altered response to GABA and FNZ seen in cortical microsacs prepared from P and NP rats after brief social isolation might result from alterations in $GABA_A$/BZD receptor subunit expression. Kang et al. (12) have shown that some $GABA_A$ receptor subunit mRNAs are elevated in the cerebral cortex of CFW mice 4 h after social stress and remain elevated for at least 72 h. In *Xenopus* oocytes expressing $GABA_A$/BZD receptor subunit mRNAs, alteration of receptor subunit combinations has given results resembling those seen in P rats after brief social isolation. A decreased response to GABA with an increase in FNZ efficacy has been demonstrated in oocytes expressing the bovine α_3, β_1, and γ_{2L} subunits compared to oocytes expressing the α_1, β_1, and γ_{2L} subunits (26). Sigel et al. (24) found that oocytes injected with mRNAs encoding the α_5, β_2, and γ_2 subunits had decreased response to GABA but an increased responsiveness to diazepam compared to oocytes injected with mRNAs encoding the α_5, β_1, and γ_2 subunits. Other possibilities, however, such as alterations in $GABA_A$/BZD receptor phosphorylation (13,14), cannot be ruled out.

It is possible that the difference in FNZ enhancement of $^{36}Cl^-$ flux between the P and NP lines may result from differences in the degree of stress that this form of brief social isolation may cause in the lines. In a recent review, Fernández-Teruel et al. (8) suggested that differences in emotionality, or baseline anxiety, and the aversiveness of the stimuli may result in differences in the balance between putative endogenous BZD agonists and inverse agonists. This would lead to different alterations in $GABA_A$/BZD receptors depending on whether the balance favored agonists or inverse agonists. These investigators (8) suggest that this may account for some of the divergent results that have been obtained in studies examining neuropharmacological changes at the $GABA_A$/BZD receptor in response to stressful stimuli. A recent study (25) showed that, compared with the NP line, P rats exhibited greater foot shock-induced suppression of operant responding in an approach-avoidance conflict test; spent less time in the open arms of an elevated plus-maze; and had a longer interval before stepping down from a platform to a grid floor where a foot shock had been previously received in a passive avoidance test. It was concluded that these tests suggested a greater degree of anxiety in the P compared with the NP line (25). Therefore, it is possible that brief isolation may generate a higher state of anxiety in the P than the NP rat, and this may account for the increased efficacy of FNZ to enhance GABA-stimulated $^{36}Cl^-$ influx observed for individually housed P, but not NP, rats.

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