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Diminished Stress Responses in the Alcohol-Sensitive ANT Rat Line

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TUOMINEN, K. AND E. R. KORPI. *Diminished stress responses in the alcohol-sensitive ANT rat line.* PHARMACOL BIOCHEM BEHAV **40**(2) 409–415, 1991.—During behavioral tests of alcohol sensitivity, rapid alcohol-opposing reactions may constitute an important mechanism in reducing the acute performance-impairing actions of alcohol. The alcohol-sensitive ANT (alcohol nontolerant) rats achieve lower plasma corticosterone concentrations after a tilting plane test of alcohol sensitivity (2 g ethanol/kg, IP) than the alcohol-insensitive AT (alcohol tolerant) rats, suggesting a dampening of activated stress mechanisms in the ANT rats. We have extended the comparison of these rat lines by examining central and peripheral stress responses to an acute 10-min swimming stress without ethanol administration. After the stress, plasma and adrenal corticosterone concentrations, adrenal dopamine concentrations, binding of [^{3}H]Ro 5-4864 to adrenal membranes, and hypothalamic norepinephrine turnover were lower in the ANT than AT rats. Habituation to daily handling did not affect the stress effects or the differences between the rat lines. These results suggest that the alcohol-sensitive ANT rats have a diminished reaction to general stress, even in the absence of ethanol. This may impair their capacity to overcome the sedative and motor-impairing effects of moderate ethanol doses.

Genetic model Alcohol sensitivity Handling habituation Corticosterone Swim stress Monoamines Chloride flux Peripheral-type benzodiazepine receptors

ALCOHOL intoxication is a significant action of alcohol, and uncovering the mechanisms of initial sensitivity might help us better understand alcohol poisoning and alcoholism and develop better treatments for them. In recent years, much progress in understanding the mechanisms of initial alcohol sensitivity has been achieved by using various rodent line pairs produced through selective breeding for different components of acute alcohol actions (3,4). The sedative and motor-impairing effects of alcohol have been studied primarily in alcohol-sensitive, long-sleep LS and alcohol-insensitive, short-sleep SS mouse lines selected for differences in sensitivity to the hypnotic actions of high doses of ethanol (19), and in alcohol-sensitive ANT (Alcohol Nontolerant) and alcohol-insensitive AT (Alcohol Tolerant) rat lines selected for differences in motor impairment after moderately sedative doses of ethanol (7). The possible neurochemical determinants of innately differing sensitivity have been extensively studied; in particular, the role of central inhibitory mechanisms using gamma-aminobutyric acid (GABA) as the chemical messenger between neurons has been studied in both rat lines. In the LS line, alcohol significantly potentiates the agonist-stimulated chloride ion flux through $GABA_A$ receptor-associated ionophores in the brain membrane vesicles, whereas no such stimulation occurs in the SS line (1). On the other hand, the ANT rats show a similar modest alcohol potentiation of muscimol-induced chloride ion flux as the AT rats do (27), suggesting that the mechanisms of initial alcohol sensitivity to acute motor-impairing effects differ from those to hypnotic effects in these models, at least at the neurochemical level.

The SS and LS lines differ in their stress responses to alcohol challenges so that the alcohol-sensitive LS rats have greater plasma corticosterone concentrations than the SS rats (13). We recently found that, following administration of 2 g alcohol/kg body weight, the alcohol-sensitive ANT rats had lower corticosterone concentrations than the AT rats after a tilting plane test procedure (15). In the present study, we wanted to ascertain whether stress responses of several mechanisms in both the central nervous system and peripheral organs to short swimming stress are greater in the AT than ANT rats. Most of the results support the hypothesis that the ANT rats have less intense stress reactions even in the absence of alcohol.

METHOD

Animals, Treatments, and Tissue Samples

Male adult AT and ANT rats from the generation F_{35} were maintained in stainless steel wire mesh cages in groups of 4 to 6 animals. The rats had free access to tap water and R3 rodent pellet food (Ewos AB, Södertälje, Sweden). The animals were kept in a 12/12-h light/dark cycle (lights on at 6:00 a.m.) at an ambient temperature of 22 ± 2°C and a relative humidity of 55 ± 5%.

At the age of 4.5 months (400–470 g), half of the animals from both lines received daily handling by the same person who later performed the behavioral tests and decapitated the animals. Each day the animals were transferred from the maintenance room to the experimental room in plastic cages (Macrolon® III) containing aspen chips (FinTapvei, Kaavi, Finland) and there the

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TABLE 1

PLASMA AND ADRENAL CORTICOSTERONE AND PLASMA TESTOSTERONE CONCENTRATIONS IN SWIM-STRESSED AT AND ANT RATS

	AT Rats		ANT Rats	
	Nonstressed	Stressed	Nonstressed	Stressed
Plasma				
Corticosterone (μM)	0.36 ± 0.09	0.96 ± 0.07†	0.14 ± 0.03	0.79 ± 0.03†
Testosterone (nM)	4.3 ± 0.6	8.4 ± 1.5	3.7 ± 0.6	11.1 ± 3.0*
Adrenal Glands				
Corticosterone (nmol/pair)	1.21 ± 0.49	5.9 ± 0.7†	0.83 ± 0.28	4.1 ± 0.3†
Weight (mg/pair)	62 ± 2	59 ± 2	53 ± 1‡	50 ± 1‡

Results are means ± SEM (n = 10). One-way ANOVA between all groups was significant for all measurements: plasma corticosterone, $F(3,36) = 40.2$, $p<0.001$; plasma testosterone, $F(3,36) = 3.99$, $p = 0.015$; adrenal corticosterone, $F(3,36) = 21.5$, $p<0.001$; adrenal weight, $F(3,36) = 11.2$, $p<0.001$. Tukey's test: *$p<0.05$ and †$p<0.001$ for the differences from the nonstressed rats of the same line; ‡$p<0.01$ for the differences between the corresponding ANT and AT values.

animals were habituated to normal handling (lifted under the belly and kept on the arm) and weighing, they were allowed to explore the tilting plane apparatus, and finally their necks were placed in the guillotine for a few seconds (26). The unhandled animals were only touched during the behavioral tests.

The behavioral tests performed on all animals included the elevated plus-maze test of anxiety (21,25), two weeks after the start of the habituation; the tilting plane test of ethanol-induced motor-impairment [(11); 2 g ethanol/kg, IP, 30 min after the injection], three weeks after the start of the habituation period; and the sleep-time test of ethanol-induced hypnosis [(8); 3.5 g ethanol/kg, IP], six weeks after the start of habituation. The AT and ANT rats differed significantly for each of these tests, but the habituation to handling did not influence these differences in behavior or apparent sensitivity to ethanol between the lines. These results have been reported in detail elsewhere (15).

The handling procedure was continued for two to three more weeks, after which the animals were subjected to quick weighing and 10 min of swimming at 25°C, followed by decapitation with a guillotine. Nonstressed control animals (handled and unhandled from both rat lines) were weighed and immediately decapitated. This was done each day at 9:00 a.m. with only four rats from one rat line; each rat was from a different cage in order to achieve as low stress levels as possible for the nonstressed rats. Thus the AT and ANT rats were used on alternate days. The body weights of the ANT rats were slightly greater than those of the AT rats [ANT: 475 ± 7 g (mean ± SEM, n = 20), AT: 448 ± 12 g; Student's t-test, $p = 0.056$].

The brains, including the pons-medulla regions but not the olfactory bulbs, were quickly removed from the skulls and weighed. Hypothalamus was dissected on a Petri dish on ice as a 2-mm deep block from the ventral surface by following its borders. Hippocampi without the septal nuclei were dissected from the inner folds of the hemispheres opened sagittally after cutting the corpus gallosum. Striata were then bluntly separated from the cerebral cortex and thalamus. The hypothalami, hippocampi, and striata were frozen on solid carbon dioxide, and stored in a freezer at −70°C for the monoamine determinations. The cerebral cortices were separated from the thalamus and olfactory cortex, weighed, and homogenized to be freshly used for the chloride ion flux assay. The brain weights of the ANT rats were greater than those of the AT rats (ANT: 2.25 ± 0.02 g, AT: 2.05 ± 0.02 g; Student's t-test, $p<0.0001$), but the brain weights relative to the body weights were similar in both lines.

Blood samples from trunk blood were collected into heparinized tubes, and the plasma was separated. Plasma samples were stored frozen at −70°C for the corticosterone and testosterone determinations.

Adrenal glands, kidneys, and testes were dissected, frozen immediately on solid carbon dioxide, and stored at −70°C for the determination of [^{3}H]Ro 5-4864 binding and of catecholamine and corticosterone concentrations in the adrenal glands.

Analytical Procedures

Plasma corticosterone concentrations were determined by radioimmunoassay with an ^{125}I corticosterone kit for rats (ICN Biomedicals, Inc., Costa Mesa, CA). The radioactivities were measured with an LKB Wallac 1282 Compugamma Universal Gamma Counter.

Adrenal corticosterone levels were determined in the following manner. Adrenal glands were homogenized in 80 volumes of ice-cold 0.25 M sucrose solution. Aliquots were diluted with corticosterone extraction buffer, extracted with ethyl acetate, evaporated to dryness under a flow of nitrogen, and taken up into corticosterone sample dilution buffer. Finally, adrenal corticosterone concentrations were determined with the same method as for plasma corticosterone.

Plasma testosterone concentrations were determined by radioimmunoassay with the Extraction Testosterone ^{125}I RIA-Kit (Farmos Diagnostica, Oulunsalo, Finland).

Brain samples were weighed and sonicated in ice-cold 0.1 M perchloric acid supplemented with 0.05% (w/v) disodium edetate and 0.1% sodium metabisulphite, and 2 μM dihydroxybenzylamine and N-methyl-5-HT as internal standards. The samples were then centrifuged and directly injected into the chromatographic system. Monoamines (dopamine, DA; norepinephrine, NE; serotonin, 5-HT), their precursors (tyrosine, TYR; tryptophan, TRP), and metabolites (3,4-dihydroxyphenylacetic acid, DOPAC; homovanillic acid, HVA; 3-methoxy-4-hydroxyphenylethyl glycol, MHPG; 5-hydroxyindoleacetic acid, 5-HIAA) were determined with ion-pair reversed phase HPLC with coulometric detection as described in detail elsewhere (14). MHPG was analyzed after acid hydrolysis of the MHPG sulphate-conjugate. Adrenal gland homogenates, prepared as described above for the corticosterone assay, were quickly diluted 1:1 with a similar

TABLE 2

BINDING OF [^{3}H]Ro 5-4864 TO MEMBRANES FROM VARIOUS TISSUES OF THE AT AND ANT RATS AS INFLUENCED BY SWIMMING STRESS

	AT Rats		ANT Rats	
Tissue	Nonstressed	Stressed	Nonstressed	Stressed
Adrenal gland	59 ± 4	73 ± 3*	50 ± 2	53 ± 4†
Testis	5.3 ± 1.0	4.8 ± 0.2	5.5 ± 0.9	6.6 ± 1.0
Kidney	7.1 ± 0.4	7.3 ± 0.3	7.3 ± 0.4	7.7 ± 0.5

Results are means ± SEM (n = 10) expressed in pmol/mg protein. ANOVA between the groups was significant in the adrenal samples, $F(3,36) = 9.26$, $p<0.001$. Tukey's test: *$p<0.05$ for the difference from the nonstressed group of the same rat line; †$p<0.001$ for the difference between the corresponding ANT and AT samples.

perchloric acid solution as the brain samples (containing double concentrations of all solutes), centrifuged, and measured for DA, NE, and epinephrine (E) using the same shortened step-gradient elution program as for MHPG (14). The final concentrations were derived from the peak height ratios of the analytes to the internal standards in the samples and prepared standard solutions (all standards from Sigma Chemical Co., St. Louis, MO).

Muscimol-stimulation of $^{36}Cl^-$ flux into cerebral cortical homogenates was carried out as a modification (16) of the method of Harris and Allan (10). Briefly, cerebral cortices without the olfactory tubercles were homogenized manually in 10 volumes of ice-cold assay medium (in mM: NaCl 145, KCl 5, $MgSO_4$ 1, $CaCl_2$ 1, D-glucose 10, HEPES 10; pH was adjusted to 7.4 with Tris-base) using a Kontess glass-glass homogenizer and 5 up-and-down strokes. After diluting the homogenates to 30 volumes, they were allowed to pass by gravity through a nylon cloth with a mesh size of 60 μm. The homogenates were centrifuged at $930 \times g_{av}$ for 15 min at 2–4°C on a Sorvall RC5C centrifuge with a SS-34 rotor. The pellets were washed once by resuspension in 30 volumes of assay medium and repeated centrifugation, and finally resuspended in 10 volumes of the assay medium. Duplicate aliquots of 250 μl containing about 1 mg protein (18,22) were pipetted into borosilicate glass tubes in an ice-water bath. Then, the tubes were transferred to a shaking water bath at 30°C for 2 min, after which the samples were rapidly vortexed with an equal volume of 0.4 μCi of $^{36}Cl^-$ (630 μCi/ml, New England Nuclear), containing different concentrations (0–100 μM) of muscimol (Sigma). The flux was stopped after 3 s by quenching the samples with 4 ml of ice-cold assay buffer, followed by filtration onto Whatman GF/C glass fiber filters using a single Hoefer filtration unit at a pressure of 25 cm of Hg. The filters were washed twice with 4 ml of the medium, dried, and counted for radioactivity in Optiphase "HiSafe" II scintillation cocktail using an LKB Wallac 1210 Ultrobeta scintillation counter.

The binding of [^{3}H]Ro 5-4864, a ligand for the peripheral-type benzodiazepine receptors, was determined in adrenal, renal, and testicular membranes prepared in Tris-HCl buffer (pH 7.5) following the method of Syapin and Alkana (24) with minor modifications. Triplicate tissue samples (80 μg of protein in re-

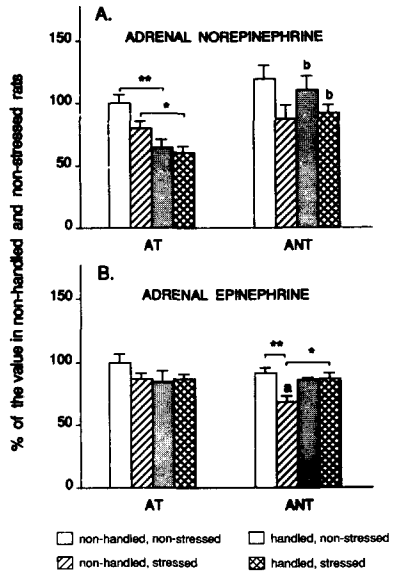

FIG. 1. Adrenal NE and E concentrations in AT and ANT rats as influenced by handling habituation and swim stress. Columns are means + SEM for 5 animals per group. In the nonhandled, nonstressed AT rats, the concentrations of NE and E were 26 ± 3 (mean ± SEM) and 200 ± 13 nmol/pair of adrenal glands, respectively. In the NE determinations, ANOVA between groups was significant, $F(7,32) = 6.29$, $p<0.001$. In the E determinations, ANOVA gave $F(7,32) = 2.76$, $p = 0.023$. Tukey's test: $^b p<0.01$ for the difference from the corresponding AT value.

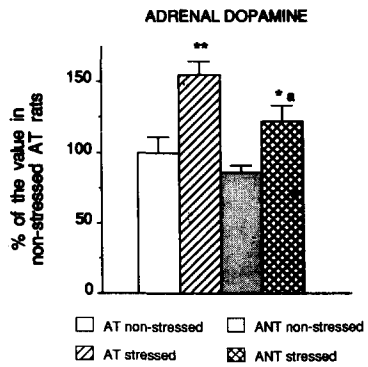

FIG. 2. Adrenal DA concentrations in stressed and nonstressed AT and ANT rats. Columns are means + SEM of ten rats in each group. The DA concentration in nonstressed AT rats was 1.3 ± 0.2 nmol/pair of adrenal glands. ANOVA between groups was significant, $F(3,36) = 9.98$, $p<0.001$. Tukey's test: **$p<0.01$ for the difference from the nonstressed value of the same line.

TABLE 3

CONCENTRATIONS OF MONOAMINES AND THEIR PRECURSOR AMINO ACIDS IN VARIOUS BRAIN REGIONS OF SWIM-STRESSED AND CONTROL AT AND ANT RATS

Brain Region/ Compound	AT Rats		ANT Rats		
	Nonstressed	Stressed	Nonstressed	Stressed	ANOVA
Hypothalamus					
TYR	77 ± 4	83 ± 8	76 ± 5	78 ± 6	—
DA	2.9 ± 0.2	3.5 ± 0.2	3.2 ± 0.2	3.3 ± 0.2	—
NE	13.1 ± 0.5	13.3 ± 0.7	13.0 ± 0.3	12.2 ± 0.5	—
TRP	15.5 ± 0.8	15.9 ± 0.6	15.7 ± 0.7	16.1 ± 0.6	—
5-HT	7.1 ± 0.3	7.9 ± 0.4	7.2 ± 0.3	8.1 ± 0.2	S*
Hippocampus					
TYR	90 ± 5	103 ± 5‡	83 ± 3	83 ± 3	L†
DA	0.10 ± 0.01	0.12 ± 0.02	0.07 ± 0.02	0.08 ± 0.01	L*
NE	2.9 ± 0.2	3.0 ± 0.2	3.0 ± 0.1	3.0 ± 0.1	—
TRP	23.7 ± 0.7	26.7 ± 1.2	25.1 ± 1.5	25.1 ± 0.5	—
5-HT	3.4 ± 0.1	3.6 ± 0.1	3.7 ± 0.1	3.9 ± 0.1	L*
Striatum					
TYR	94 ± 14	97 ± 12	95 ± 4	110 ± 6	—
DA	64 ± 11	62 ± 5	54 ± 4	60 ± 5	—
NE	0.35 ± 0.05	0.28 ± 0.04	0.33 ± 0.06	0.28 ± 0.03	—
TRP	16.5 ± 2.2	18.5 ± 3.5	20.0 ± 1.8	20.2 ± 2.1	—
5-HT	3.3 ± 0.3	4.0 ± 0.4	3.6 ± 0.2	4.0 ± 0.3	—

Results are given as means ± SEM (n = 10) in μmol/kg tissue wet weight. Three-way ANOVA: rat line effect L; stress effect S; *$p<0.05$, †$p<0.01$; no significant handling effects. ANOVA between groups: hippocampal tyrosine (TYR), $F(3,36)=5.37$, $p=0.004$. Tukey's test: ‡$p<0.01$ for the significance of the difference from the corresponding ANT value.

nal and testicular samples and 30 μg in adrenal samples in a final volume of 1 ml) were incubated for 90 min in an ice-water bath in the presence of 10 nM [N-methyl-^{3}H]Ro 5-4864 (specific radioactivity: 85.8 μCi/mmol, New England Nuclear) for the renal and testicular samples and 20 nM for the adrenal samples. The nonspecific binding was assayed in the presence of 5 μM unlabelled Ro 5-4864 (Fluka Chemie AG, Buchs, Switzerland). The bound ligand was separated from the free ligand by filtration onto Whatman GF/B glass fiber filter sheets under reduced pressure using a Brandel M-48R filtration unit. The filters were washed twice with 4 ml of ice-cold assay buffer, dried in the air, and measured for radioactivity as described above for the chloride flux samples.

Statistics

The results were subjected to three-way analysis of variance to estimate the significance of any rat line, stress and handling effects using SAS procedures (see SAS User's Guide: Statistics, version 5; SAS Institute, Inc.). The significances of differences between the four groups (or eight groups in case of adrenal norepinephrine and epinephrine) were assessed using ANOVA followed by Tukey's HSD test using Systat programs (Systat, Inc.). Finally, since the initial hypothesis predicted greater responses in the AT than ANT rats, the values of the stressed animals were compared between the lines using one-tailed Student's *t*-test. All statistical analyses were done on raw data. For illustrative purposes the results in the figures are given as percentages of the nonstressed AT values with percentual SEMs calculated directly for each raw value.

RESULTS

In most determinations, rat line and stress significantly affected the results, while handling produced significant effects only in the case of adrenal NE and E levels. Therefore, the handling effects have been omitted from further statistical analyses of other measurements than adrenal NE and E. Although there were no significant rat line × stress interactions in any of the measurements, the initial hypothesis of greater stress-related values in the AT rats was tested by comparing the values of the stressed AT and ANT animals.

In the plasma corticosterone concentrations, ANOVA revealed significant rat line, $F(1,32)=10.70$, $p=0.0026$, and stress, $F(1,32)=109.17$, $p<0.0001$, effects (Table 1). In both rat lines, stress greatly elevated corticosterone concentration, but the concentration after the stress was significantly lower in the ANT than AT rats (Student's *t*-test, $p<0.01$). Testosterone concentration was also influenced by stress in both rat lines, $F(1,32)=10.71$, $p=0.0026$, but its concentration was not different between the lines. There was a five-fold increase in adrenal corticosterone content from stress in the AT and ANT rats, $F(1,32)=58.56$, $p=0.0001$, with the ANT rats achieving significantly ($p<0.01$) lower levels than the AT rats after stress. The adrenal glands of the AT rats were heavier than those of the ANT rats.

The binding of [^{3}H]Ro 5-4864 to adrenal peripheral-type benzodiazepine receptors was affected by rat line, $F(1,32)=17.58$, $p=0.0002$, and stress, $F(1,32)=5.72$, $p=0.0228$ (Table 2). The stress increased the binding significantly only in the AT animals, whereas the binding in the ANT rats lower than that in the AT rats and not affected by the stress at all. These differences do not reflect the difference in the size of the adrenals since the results were expressed per mg protein of adrenal membranes. The binding of Ro 5-4864 was similar and unaffected by stress in the testicular and renal membranes of both rat lines.

The adrenal norepinephrine levels were significantly affected by rat line, $F(1,32)=19.51$, $p<0.0001$, stress, $F(1,32)=10.25$, $p=0.0031$, and handling, $F(1,32)=6.69$, $p=0.0145$ (Fig. 1A). These effects were relatively minor, but, interestingly, the NE

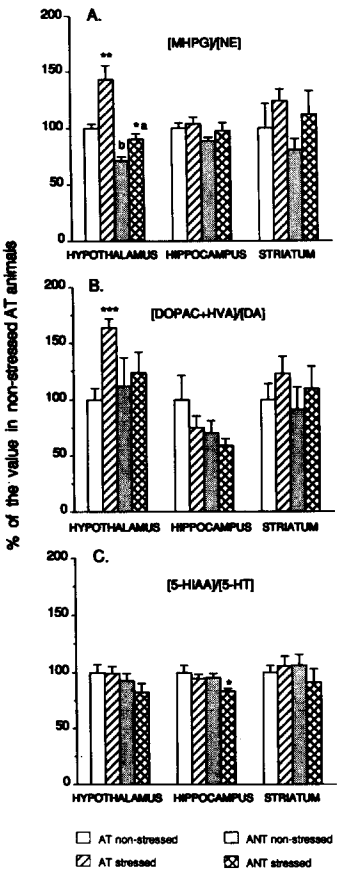

FIG. 3. Ratios of monoamine metabolites to their parent amines in various regions of the stressed and nonstressed AT and ANT rats. (A) The ratios of MHPG and NE. Columns are means+SEM for 10 animals in each group. The MHPG/NE ratios for the nonstressed AT rats were 0.091 ± 0.003 (mean $\pm$ SEM), 0.27 ± 0.01, and 1.81 ± 0.37 in the hypothalamic, hippocampal, and striatal samples, respectively. In the hypothalamic samples, ANOVA between groups was significant, $F(3,36) = 17.0$, $p<0.001$. (B) The ratios of DOPAC + HVA and DA. The ratios for the nonstressed AT rats were 0.25 ± 0.03, 3.0 ± 0.6, and 0.45 ± 0.06 in the hypothalamic, hippocampal, and striatal samples, respectively. In the hypothalamic samples, ANOVA was significant, $F(3,36) = 3.95$, $p=0.0156$. (C) The ratios of 5-HIAA and 5-HT. The ratios for the nonstressed AT rats were 0.49 ± 0.03, 0.59 ± 0.03, and 1.2 ± 0.07 in the hypothalamic, hippocampal, and striatal samples, respectively. ANOVA between groups did not reach significance in any of the brain regions. Tukey's test: $*p \leq 0.05$, $**p<0.01$ for the difference from the nonstressed ratios of the same line; $^{a}p<0.01$, $^{b}p<0.0001$ for the difference from the corresponding AT ratios.

level of the handled, nonstressed rats was significantly lower in the AT than ANT line [rat line $\times$ handling interaction, $F(1,32) = 4.72$, $p=0.0374$]. The adrenal epinephrine concentration was slightly affected by the stress, $F(1,32) = 5.02$, $p=0.0321$, and a stress $\times$ handling interaction, $F(1,32) = 6.81$, $p=0.0137$ (Fig. 1B). There were no significant rat line effects in the E levels.

The adrenal dopamine levels were low compared to the NE and E levels and affected by rat line, $F(1,32) = 6.29$, $p=0.0174$, and stress, $F(1,32) = 23.73$, $p<0.0001$ [see (23)] (Fig. 2), but not by the handling experience. The DA level increased significantly in the AT rats only, with their levels being significantly ($p<0.01$) higher than those of the ANT rats after the stress.

There were only minor rat line and stress effects between the rat lines in the concentrations of monoamines and their precursors in the hypothalamus, hippocampus, and striatum (Table 3). Swimming stress only affected the hypothalamic serotonin concentration, but even in this case the effect did not reach significance in either one of the rat lines.

The ratios of the amine metabolites and the parent amines should better illustrate the functional state of the monoaminergic system (Fig. 3). Indeed, clear stress, $F(1,32) = 16.93$, $p=0.0003$, and rat line, $F(1,32) = 29.28$, $p<0.0001$, effects were observed in the hypothalamic norepinephrine system (Fig. 3A). No significant rat line and stress effects were obtained in the hippocampal and striatal samples. The hypothalamic ratios of MHPG and NE were lower in the ANT than AT rats, both in the control and stressed groups. Only in the AT rats did the swimming stress significantly increase the hypothalamic ratio. The hypothalamic dopaminergic system was significantly affected by stress, $F(1,31) = 5.49$, $p=0.0257$ (Fig. 3B). Stress activated the dopaminergic system only in the AT rats. There were no rat line or stress effects in the hippocampal and striatal samples. There were no significant rat line and stress effects in ratios of 5-HIAA and 5-HT in any brain region (Fig. 3C).

Muscimol stimulation of the $^{36}Cl^{-}$ flux in cerebrocortical homogenates was significantly affected by rat line [ANOVA for repeated samples: $F(1,36) = 12.42$, $p=0.0012$, and stress, $F(1,36) = 4.54$, $p=0.04$] (Fig. 4). Muscimol stimulation was lower in the ANT than AT rats at 20 and 100 μM concentrations, but the stress-induced reductions in the stimulation were not significant in either rat line. There were no significant differences in the baseline fluxes [AT nonstressed, 10.7 ± 0.4 (mean $\pm$ SEM in nmol/mg protein in 3 s, $n=10$); AT stressed, 11.2 ± 0.3; ANT nonstressed, 11.0 ± 0.5; ANT stressed, 11.5 ± 0.4.]

DISCUSSION

It is well-known that, while alcohol can reduce stress-related biochemical changes (5), it can also activate stress mechanisms in nonstressed animals (6). Thus it seems plausible that ethanol-induced activation of stress mechanisms can be part of the process that opposes the unwanted sedative and performance-impairing actions of alcohol. It has been shown, using the tilting plane test, that physical (swimming) and chemical (D-amphetamine) stressors can attentuate the motor-impairing effects of alcohol (17,29). Therefore, if the capacity of an animal to activate stress mechanisms is damped, the animal would be more sensitive to the performance-impairing actions of alcohol during a behavioral assessment of alcohol sensitivity. This seems to be the case with the alcohol-sensitive ANT rats produced by selective breeding for high sensitivity to the motor-impairing actions of moderate alcohol doses.

In the absence of any alcohol challenge, the ANT rats showed a decreased capacity to react to short-term acute swimming stress: in these rats, the 10-min swim produced a smaller accumulation of corticosterone in plasma and adrenal glands and smaller increases in adrenal dopamine concentration, peripheral-type benzodiazepine receptor binding, and apparent turnover of hypothalamic norepinephrine than in the AT rats selectively bred for low alcohol sensitivity. Although the baseline values of these measures were generally lower in the ANT than the AT rats, the changes induced by the stress were also smaller in the ANT than AT rats. Moreover, the average weight of the adrenal glands was significantly lower in the ANT than the AT rats, in spite of their slightly greater body weights. In the cerebral cortical homogenates of the ANT rats, the muscimol-stimulation of the chloride flux as a measure of $GABA_A$ receptor function was lower than in those of the AT rats. These results indicate that the ANT rats do have smaller stress responses than the AT rats, which is in

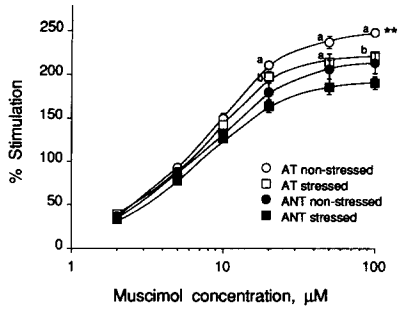

FIG. 4. Muscimol-stimulated $^{36}Cl^-$ flux in cerebrocortical homogenates from swim-stressed AT and ANT rats. The points are means with SEM bars for 10 animals in each group. ANOVA between groups was significant at muscimol concentrations 20–100 μM ($p<0.003$). Tukey's test: $^{a}p<0.05$ and $^{b}p<0.01$ for the differences from the corresponding values of the ANT line.

keeping with the lower plasma corticosterone concentrations seen in ANT versus AT rats after a tilting plane test with alcohol administration (15) and after a 45-min immobilization stress with and without alcohol administration (12). In these two earlier studies, no line difference was found in the nonstressed control animals.

The stress responses were qualitatively similar in both rat lines, so that no rat line × stress interactions were observed. On the other hand, in several measurements, only the AT rats reacted to stress significantly. For instance, the peripheral-type benzodiazepine receptor binding was enhanced only in the AT rats. There is evidence that the peripheral benzodiazepine receptors regulate steroid synthesis at the mitochondrial level (20). The concentration of the ligand, [^{3}H]Ro 5-4864, used was greater than the K_D of the binding to various tissues and, therefore, should have mostly reflected the density of the binding sites (15). There were no stress or rat line differences in the binding to testicular or renal membranes. Plasma testosterone was elevated by the present short-term stress via an unknown mechanism, but resulted in no line difference. The lack of a stress-induced increase in the adrenal [^{3}H]Ro 5-4864 binding in the ANT rats suggests that not all the mechanisms associated with stress responses are activated in these rats. This interpretation is in keeping with previous comparisons of the ANT rats to AT and Mixed rats (a heterogenous starting population of the ANT and AT rat lines) with respect to drug sensitivities and cerebellar $GABA_A$ receptor binding site characteristics (11,28), as we found that it is the ANT line that has deviated from the original population and the AT line.

Habituation to daily handling did not affect the present results, except in the cases of adrenal epinephrine and norepinephrine responses. Neither does it affect the alcohol sensitivities of these same rats in tests of tilting plane performance and sleep time, or their nonintoxicated behavior in the elevated plus-maze test (15). These behaviors are not affected by handling habituation for several weeks in nonselected Wistar rats either (26). These results suggest that handling habituation produces behavioral and physiological changes that are not strong enough to alter the behavior or neurochemistry in relatively stressful novel test situations in the conditions used.

It is noteworthy that the alcohol-sensitive LS mice have greater stress responses to ethanol than the alcohol-insensitive SS mice (13,30), but that just before regaining the righting reflex after a high ethanol dose, the adrenal glands of the SS mice show activation while those of the LS mice show depression (9). Thus it appears that the AT/ANT and SS/LS genetic models of alcohol intoxication are different in their stress adaptations during acute alcohol treatment. This is to be expected since alerting mechanisms could understandably overcome the moderate sedation of the AT/ANT model more efficiently than the deep hypnosis of the SS/LS model.

In conclusion, the present results give evidence that the alcohol-sensitive ANT rats have an impaired capacity to react to stressful stimuli without alcohol administration. If this impairment leads to limited alcohol-opposing adaptation, these animals may show enhanced sensitivity to ethanol and to other sedative drugs. Further studies are needed to establish the biological basis of the impaired stress reactions in the ANTs, their smaller adrenal glands perhaps being one contributory factor.

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