Pharmacology Biochemistry and Behavior, Vol. 41, pp. 795–799, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Hormone Responses to Sedative Drugs and Cold Exposure in Two Rat Lines with High and Low Alcohol Sensitivity

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Received 27 August 1991

KORPI, E. R., K. TUOMINEN AND P. T. MÄNNISTÖ. *Hormone responses to sedative drugs and cold exposure in two rat lines with high and low alcohol sensitivity.* PHARMACOL BIOCHEM BEHAV 41(4) 795–799, 1992.—Two rat lines bred for differences in motor impairment in the tilting plane test after a moderate dose of ethanol were compared for peripheral hormone responses. The alcohol-sensitive ANT rats had significantly lower plasma corticosterone concentrations than the alcohol-insensitive AT rats 30 min after an IP saline injection. Ethanol (2 g/kg, IP) and lorazepam (3 mg/kg, IP) injections increased the corticosterone concentration in ANT rats. Sodium barbital (160 mg/kg, IP) did not produce any increase in these rats; instead, it prevented any increase caused by a tilting plane test procedure 10 min before decapitation. Three trials on the tilting plane significantly elevated the corticosterone concentration in saline-treated ANT rats, but produced no additional increase in drug-treated ANT rats. In AT rats, drug injections caused no significant corticosterone increase but the tilting plane test procedure after barbital (lorazepam) treatment(s) elevated the corticosterone concentration. Cold exposure (+4°C for 30 min) of the drug-naive animals elevated their concentrations of serum and adrenal corticosterone, thyrotropin, and growth hormone, but not of prolactin and luteinizing hormone. The increase in serum corticosterone was greater in AT than ANT rats, whereas the increase in serum thyrotropin was slightly greater in ANT rats. No differences between the rat lines were found in the growth hormone, prolactin, and luteinizing hormone levels. The results confirm and extend our earlier findings of the inability of ANT rats to produce additional stress responses to behavioral challenges when being intoxicated by sedative drugs, which may at least partly account for their increased sensitivity to sedative drugs.

Genetic model Alcohol sensitivity Barbital Lorazepam Cold exposure Corticosterone
Thyrotropin Prolactin Growth hormone Luteinizing hormone

BETTER understanding of the mechanisms of alcohol intoxication would be important for planning preventive or treatment strategies for alcohol-related accidents and other disorders. Our recent study on the alcohol-sensitive ANT and alcohol-insensitive AT rat lines produced by selective outbreeding for high and low acute sensitivity to the motor-impairing effects of a moderate IP dose of ethanol (2 g/kg) (3) suggested that general stress mechanisms might be differently activated in these rat lines in response to behavioral and alcohol challenge (9,14), with ANT rats exhibiting weaker activation of peripheral and central stress mechanisms than AT rats. This difference could at least partly explain the differential sensitivity to motor-impairing effects of ethanol and other sedative GABAergic drugs (5), provided stress responses are accepted as a measure of mechanisms opposing intoxication (10,15). It is to be noted that the activation of stress mechanisms does not apparently play any major alcohol-opposing role in the loss of righting reflex after high alcohol doses (4,8), but that it correlates positively with the ability to regain the righting reflex (16).

In the present study, we measured plasma corticosterone responses to ethanol and GABAergic drugs proper in ANT and AT rats. We also determined serum and adrenal hormone responses to cold exposure in ethanol-naive ANT and AT rats to estimate the specificity of the difference in stress activation between the rat lines.

METHOD

Animals

Male, adult AT and ANT rats from the F_{38} and F_{40} generations were maintained in stainless steel wire mesh cages in groups of four to six animals. Rats had free access to tapwater and R3 rodent pellet feed (Ewos AB, Södertälje, Sweden).

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Animals were kept under a 12 L: 12 D cycle (lights on at 6:00 a.m.) at an ambient temperature of 22 ± 2°C and a relative humidity of 55 ± 5% unless otherwise stated.

Responses to Drugs

The following arrangement was used to measure the corticosterone responses to sedative drugs in 64 AT and 64 ANT rats of the F_{40} generation. At 7:30 a.m., during 4 consecutive days, 16 animals from both lines were transferred in groups of 4 in Macrolon® III cages with aspen chips from their maintenance room to the experimental room. The experiment was started at 9:00 a.m. by injecting rats IP with saline (12 ml/kg), ethanol [2 g/kg, as a 12% (w/v) solution in saline], lorazepam (3 mg/kg, diluted with saline to 0.24 mg/ml; Temesta®, Wyeth-Huhtamäki, Turku, Finland), or sodium barbital (160 mg/kg, dissolved in saline 10 mg/ml; 5,5-diethylbarbituric acid, Merck, Darmstadt, Germany) at 2-min intervals. Eight rats in a row (two cages, one from each line) received the same drug, and pairs of rats (one from each line) were alternately allocated either to the tilting plane test (TPT) group or the untouched "no test" group. In this way, 32 rats were used daily, the order of injection of the drugs always being different on each of the 4 experimental days. Twenty minutes after the end of the injections, half the animals (TPT group) were given three trials on a tilting plane and the sliding angles recorded (1), while the other half (no test group) were kept in their cages untouched. Thirty minutes after the end of injections, animals were decapitated with a guillotine and trunk blood was collected in heparinized tubes. For corticosterone determination, plasma was separated from the blood samples and stored frozen at −70°C. In the case of ethanol-treated animals, aliquots of heparinized whole blood were diluted with distilled H_2O and measured for ethanol concentration using head-space gas chromatography (2). Body weights of the AT (464 ± 6 g; mean ± SEM, n = 64) and ANT (452 ± 4 g) rats were similar.

Responses to Cold Exposure

Twenty ANT and 20 AT rats of the F_{38} generation were used to study the effect of cold exposure on the serum and adrenal hormone concentrations. Rats were habituated in individual Macrolon III cages in a silent room with lights on from 7 a.m. to 7 p.m. at a constant temperature of 30–32°C for 7 days (11). They were transferred to a cold room (+4°C) for 30 min, decapitated, and the trunk blood was collected. Habituated control animals were decapitated similarly. The experiments were carried out between 1 and 3 p.m.

TABLE 1

EFFECTS OF SEDATIVE DRUGS AND TPT ON PLASMA CORTICOSTERONE CONCENTRATION IN AT AND ANT RATS

	AT Rats		ANT Rats	
Drug	No Test	TPT	No Test	TPT
Saline (control)	354 ± 49	420 ± 34	238 ± 25*	331 ± 16†
Ethanol	516 ± 19	523 ± 23	371 ± 16*‡	380 ± 27*
Lorazepam	361 ± 59	501 ± 32	418 ± 23‡	355 ± 23*
Barbital	361 ± 29	466 ± 32†	265 ± 26	244 ± 27*‡

Results are means ± SEM (n = 8). Concentrations are given in ng/ml. TPT, tilting plane test. Tukey's HSD test significance for difference from the corresponding value of the AT rat line, $*p < 0.05$. for difference from the saline (control) value within rat lines, $\dagger p < 0.05$; for difference from the corresponding no test value within each rat line, $\ddagger p < 0.05$.

TABLE 2

EFFECT OF SEDATIVE DRUGS ON THE SLIDING ANGLE ON THE TILTING PLANE IN AT AND ANT RATS

Drug	AT Rats	ANT Rats
Saline (control)	66 ± 3	62 ± 2
Ethanol	44 ± 1*	41 ± 1*
Lorazepam	60 ± 3	45 ± 2*†
Barbital	55 ± 2*	49 ± 3*

Results (sliding angles in degrees) are means ± SEM of eight rats in each group. For difference from the saline (control) value within rat lines, $*p < 0.05$; Tukey's HSD test significance for difference between rat lines, $\dagger p < 0.05$.

Radioimmunoassays

Plasma, serum, and adrenal corticosterone concentrations were determined with a ^{125}I corticosterone kit for rats (ICN Biomedicals, Inc., Costa Mesa, CA) as described in detail by Tuominen and Korpi (13). Serum thyrotropin (TSH), prolactin, luteinizing hormone (LH), and growth hormone concentrations were determined from duplicate samples (0.1 ml) by specific radioimmunoassays. Rat TSH, prolactin, LH, and growth hormone kits were gifts from the National Institutes of Health (NIH). TSH results are expressed in ng/ml of the NIAMDD-TSH-RP-2 standard, which has a biological potency of 35 USP bovine units of TSH/mg in the McKenzie assay. Prolactin results are expressed in ng/ml of the NIAMDD-prolactin-RP-2 standard, which has a biological potency of 30 IU/mg in the pigeon local crop sac assay of Nicoll. Growth hormone results are expressed in ng/ml of the NIAMDD-rGH-RP-2 standard, which has a biological potency of 2.0 IU (bovine units)/mg. LH results are expressed in pg/ml of the NIAMDD-rLH-RP-2 standard. All samples were run in one assay. The intraassay coefficient of variation was <15%.

Statistics

Results were subjected to analyses of variance (ANOVA's) using SAS procedures (see SAS user's guide: Statistics, version 5, SAS Institute, Inc., Cary, NC) to estimate the effects of rat line, drug, and physical treatment. The mean values of the groups were compared using Student's t-test (comparison of two means) or ANOVA followed by posthoc Tukey's HSD test using Systat programs (Systat, Inc.). The statistical comparison of serum growth hormone concentrations was done after logarithmic ($\log_{10}$) transformation of the data.

RESULTS

Responses to Drugs

A three-way ANOVA revealed significant effects of rat line, $F(1,112) = 53$, $p < 0.001$, drug, $F(3,56) = 13$, $p < 0.001$, and tilting plane test, $F(1,112) = 7.48$, $p < 0.01$, in the plasma corticosterone data (Table 1), accompanied by sig-

nificant rat line × drug, $F(3,112) = 2.76$, $p < 0.05$, rat line × test, $F(1,112) = 5.85$, $p < 0.05$, and rat line × drug × test, $F(3,112) = 3.12$, $p < 0.05$, interactions. The corticosterone concentrations after saline administration were about 30% higher in AT than ANT rats. Similar line differences were found also after ethanol and sodium barbital injections, whereas after lorazepam administration the corticosterone concentrations in the ANT's were at least as high as those in the AT's. In the ANT rats, ethanol and lorazepam, but not barbital, increased the plasma corticosterone concentration, whereas in AT rats tendency to an increase was found only after ethanol.

The plasma corticosterone concentrations of the ANT's were lower than those of the AT's even after the TPT procedure, undertaken 10 min before decapitation (Table 1), although in the saline-treated ANT's the TPT procedure significantly elevated the corticosterone concentration. Interestingly, TPT had no effect in the drug-treated ANT's whereas in the AT's TPT significantly elevated the corticosterone concentration in the barbital-treated rats and tended to increase it in the lorazepam-treated ones ($p = 0.056$). The highest corticosterone concentrations were observed in AT rats after ethanol treatment irrespective of TPT.

Two-way ANOVA revealed significant rat line, $F(1,56) = 22$, $p < .0.0001$, and drug, $F(3,56) = 37$, $p < 0.0001$, effects in the performance on the tilting plane (Table 2). A significant rat line × drug interaction was also found, $F(3,56) = 3.79$, $p < 0.02$. All drugs produced significant impairment in the TPT in ANT's, whereas the performance of AT's was not impaired by the lorazepam treatment. Blood ethanol concentrations 30 min after ethanol administration were similar in both rat lines and in both the no test and TPT groups [AT's 48 ± 2 (mean ± SEM, $n = 8$) and 50 ± 2 and ANT's 53 ± 1 and 46 ± 2 mM for the no test and TPT groups, respectively].

Responses to Cold Exposure

The baseline concentrations of serum TSH, prolactin, growth hormone, and corticosterone and adrenal corticosterone were similar in the AT and ANT rats habituated to 30°C for 7 days (Fig. 1). LH levels were 339 ± 47 and 385 ± 53 pg/ml in AT and ANT rats, respectively.

Serum TSH ($p < 0.001$) and growth hormone ($p < 0.05$) levels were significantly elevated by cold exposure in both AT and ANT rats (Fig. 1A,C). TSH levels were higher in ANT than AT rats after cold exposure ($p < 0.05$), whereas no such difference was noted in serum growth hormone, prolactin (Fig. 1B,C), or LH (not shown) levels. Exposure of habituated animals to ±4°C greatly elevated the serum and adrenal corticosterone concentrations in both rat lines (Fig. 1D,E). The

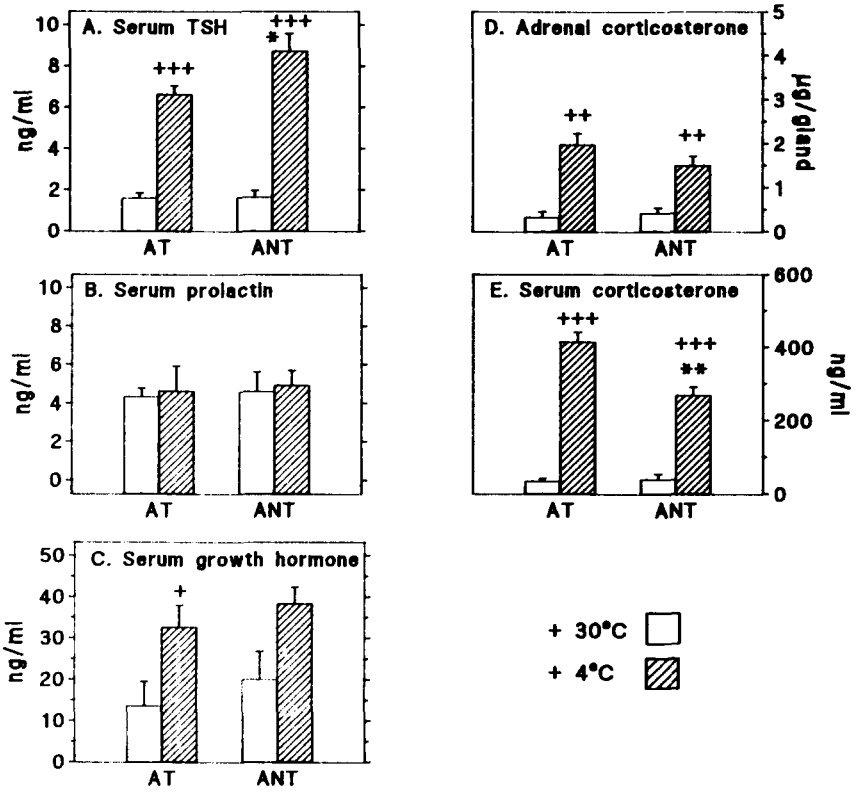

FIG. 1. (A) serum thyrotropin (TSH), (B) prolactin, and (C) growth hormone levels, and (D) adrenal and (E) serum corticosterone concentrations in drug-naive AT and ANT rats kept at 30°C for 7 days (open bars) and in those subsequently exposed to +4°C for 30 min (filled bars). Mean ± SEM of 8 (+30°C) and 12 (+4°C) rats. Student's t-test: $^{+}p < 0.05$, $^{++}p < 0.01$, and $^{+++}p < 0.001$ compared with corresponding values at +30°C (effect of cold exposure); $*p < 0.05$ and $**p < 0.01$ compared with corresponding values in AT rats (line difference).

serum (but not adrenal) concentrations were higher in AT than ANT rats ($p < 0.01$).

DISCUSSION

Both physical (swimming) and chemical (*d*-amphetamine) stressors have been shown to attenuate the motor-impairing effects of moderate ethanol doses (10,15). This is in obvious agreement with the finding of greater response of peripheral and central stress mechanisms to swimming in the alcohol-insensitive AT than the alcohol-sensitive ANT rats (9,14). As a measure of the activation of stress mechanisms, the elevation of plasma corticosterone has been shown to be greater in AT than ANT rats also after the TPT procedure following ethanol administration (9).

The present study extends these findings to show that ethanol injection without other behavioral manipulation is a strong activator of stress mechanisms in AT rats. Two other more specifically GABAergic drugs, lorazepam and sodium barbital, also activated stress mechanisms, but the corticosterone levels in these rat groups were further elevated by three trials on the tilting plane. This seems to offer an explanation for the lack of effect of habituation to the testing situation on the ethanol-induced corticosterone elevation found also in normal Wistar rats (13).

The stress mechanisms of the alcohol-sensitive ANT rats are apparently hyporesponsive already after the minor stress of transferring animals from their maintenance rooms to the experimental room followed by saline injection. Their stress mechanisms were fully activated by drug injections and no further activation occurred in the TPT procedure. A behavioral correlate for these rat line differences was found on the TPT, where ANT rats struggled much less than AT rats to avoid sliding off the rough plane. This was reflected by the differences in the sliding angles (Table 2). Overall, the angles were lower than usual probably because animals did not have any practice on the tilting plane before the three trials.

Cold exposure, an established stimulus of TSH secretion through enhanced hypothalamic thyrotropin-releasing hormone (TRH) activity (11,12), greatly increased TSH levels in both rat lines. Growth hormone levels appeared to react to cold, although to a lesser extent than those of TSH. Serum corticosterone levels were also greatly elevated by cold exposure. The increase in adrenal corticosterone levels suggests enhanced corticosterone synthesis in response to cold exposure. It is known that even prolactin is transiently elevated by this stimulus but that the levels are normalized within 20–30 min in spite of continuing cold exposure [(6), Männistö, unpublished observation]. The present results suggest that 1) cold is not a selective stimulus of TSH secretion alone and 2) the term cold stress is justified.

In habituated, nonstressed animals, the basal levels of serum corticosterone, TSH, prolactin, LH, and growth hormone were similar in ANT and AT rat lines. However, there were some interesting differences between the two lines in their hormonal responses to brief cold exposure. TSH was elevated severalfold in both rat lines, suggesting similar secretion mechanisms. Unexpectedly, the TSH response was somewhat stronger in ANT than AT rats. Again in this novel situation for the rats, the serum corticosterone levels were elevated more in the alcohol-insensitive AT than alcohol-sensitive ANT rats. Adrenal corticosterone increase was similar in both lines.

This study shows that fairly specific differences in adrenal hormone secretion exist between stressed drug-naive and intoxicated AT and ANT rats. ANT rats exhibit lower blood corticosterone levels than AT rats in response to immobilization, a TPT with and without alcohol administration, swimming, transfer to an experimental room (7,9,14), and cold exposure, which suggests that the selective breeding procedure produced difference(s) between ANT and AT rat lines in rather general stress activation mechanisms. Further investigations are required to establish the genetic, molecular, and physiological mechanisms responsible for the difference(s).

ACKNOWLEDGEMENTS

The authors acknowledge the expert technical assistance of Pirkko Johansson and Vuokko Pahlsten and the secretarial contribution of Sari Salo. Serum LH levels were kindly analyzed by Dr. Maija Laakso (Department of Physiology, University of Helsinki).

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