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Neuroendocrine and Beta-Adrenoceptor Response to Chronic Ethanol and Aggression in Rats[1]

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PETERSON, J. T., L. A. POHORECKY AND M. W. HAMM. *Neuroendocrine and beta-adrenoceptor response to chronic ethanol and aggression in rats.* PHARMACOL BIOCHEM BEHAV **34**(2) 247–253, 1989.—Male rats were administered either ethanol (6–8 g/kg/day) or dextrin-maltose, an isocaloric equivalent, for two weeks prior to a 24-hour resident-intruder test. After the first 20 minutes of the aggression test residents showed a greater increase in norepinephrine than intruders (216% vs. 97%), while intruders showed a greater increase in epinephrine (394% vs. 51%) and corticosterone (338% vs. 129%) than residents. Ethanol administration increased the initial epinephrine response of intruders almost two-fold compared to dextrin-maltose intruders. After 24 hours of aggression testing plasma norepinephrine was still elevated in residents (92%) and intruders (71%), however, only intruders continued to show an elevation in plasma corticosterone (98%) and epinephrine (107%). Using a cumulative dose-response technique, the dose of isoproterenol required to produce 50% of the maximal heart rate response (ED_{50}) increased in intruders by 108% following aggression testing with ethanol intruders showing significantly smaller mean change. The increase in ED_{50} was related to drug type, behavior, and plasma corticosterone and epinephrine levels. Rats treated with ethanol had a greater beta-adrenoceptor density than control rats. However, no relationship was found between receptor density and the other measures in this study.

Myocardium Beta-adrenoceptors Down regulation Epinephrine Norepinephrine Corticosterone Aggression Ethanol

AGONIST-INDUCED down-regulation of beta-adrenoceptors has been studied in a variety of cell lines (19, 20, 22, 23, 43, 44) and animals (10). Desensitization has been reported to be a two-step process in astrocytoma cell lines (22,44). It is not clear whether down-regulation of beta-adrenoceptors occurs spontaneously in animals. Hermsmeyer and Robinson have shown that there is a loss of beta-adrenoceptor in chick myocardial cells which suggests that the myocardial beta-adrenoceptor system undergoes developmental changes (20). In addition, Kebabian *et al.* have shown that beta-adrenoceptors within the pineal gland exhibit circadian fluctuations (24). Thus, the beta-adrenoceptor system within different cell types and organs apparently exhibit dynamic changes.

The goal of the experiment reported here was to determine whether a naturalistic stressor, aggression, can induce down-regulation of the myocardial beta-adrenoceptor system of rats. Aggression was expected to elevate peripheral circulating catecholamine (CA) levels, and that this increase in CAs would be related to the degree of down-regulation. A resident-intruder test was employed to generate aggression, and it involved placing a strange rat (intruder) into another rat's home cage (resident). The resident-intruder test was employed because it has been reported to reliably produce high levels of stereotypical aggression in rodents (3, 15, 29). In addition, half the subjects were treated with ethanol (EtOH) for a two-week period prior to aggression testing to study the effect of EtOH on aggression and its consequences. The rationale for studying the effect of EtOH on aggression in this

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study was that EtOH has been reported to act as an anxiolytic agent reducing several of the biochemical correlates of stress (38–40). This stress-reducing property of EtOH would be expected to decrease the neuroendocrine response to aggression, and, therefore, any subsequent changes in the myocardial beta-adrenoceptor system.

METHOD

Subjects

Forty-nine pairs of male, Long-Evans rats from Blue Spruce Farms (Altamont, NY) were used in this experiment. Resident rats weighed 524.2 ± 8.8 g and were 8–10 months old; intruders weighed 364.6 ± 5.3 g and were 3 months old at the start of the study. Both resident and intruder rats were used within 1 month of their shipment. The size differential between resident and intruder animals was created to predispose residents to display offensive aggression. All rats were maintained on a 12-hr light/dark cycle, and had ad lib access to food and water throughout the experimental period. Resident rats were housed in large cages (18 × 18 × 15 inches—width × length × height) with a one-inch layer of pine shavings covering the floor of the cage, and a Plexiglas door to facilitate the observation of aggression.

All experimental rats were implanted with gastric and jugular catheters under anesthesia using sterile surgical techniques prior to experimentation. Jugular catheters were flushed daily with 0.2 ml of 50 U/ml heparin-saline solution to maintain patency. Because of problems with maintaining the patency of catheters during the 3-week experimentation period, 15 resident-intruder pairs did not complete testing, and are not included in the results.

A separate group of 52 Long-Evans rats were implanted with jugular catheters. Blood from these rats was used to immediately replace blood withdrawn from resident and intruder rats to prevent a hypovolemic response in the experimental rats. Donor rats were matched to only one experimental rat at one time. The blood withdrawal procedure is described in more detail in the Blood Ethanol, and Plasma Catecholamine, and Corticosterone Determination section.

These experiments were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals.*

Procedures

Surgical preparation. Rats were anesthetized with sodium pentobarbital (35 mg/kg, IP). Following the induction of anesthesia, the neck, abdomen, and an area behind the scapulae were shaved and scrubbed. A sterile silastic catheter was implanted in the right jugular vein and passed subcutaneously to the area behind the back, and the neck wound closed. A small 1-cm incision was made in the abdomen, the stomach was exteriorized, and a polyethylene catheter was implanted through the muscular portion of the stomach wall and sutured into place. The gastric catheter was passed subcutaneously to the back, and exteriorized by the jugular catheter. Following surgery, rats were placed under a heat lamp until they recovered.

Drug administration. The drug administration phase started six days following surgical preparation. Residents and intruders were divided evenly into two drug groups, one group received an initial dose of 4.0 g/kg/day of EtOH, and the other received an isocaloric dose of dextrin-maltose (DM) via the gastric catheter. EtOH was administered as a 20% v/v solution in water, and the DM was administered as a 32% solution in water. The daily drug dose was divided into three fractions administered every eight hours with the first daily dose given between 8 and 9 a.m. The daily dose was increased by 0.5 g/kg every other day until the residents received 6.0 g/kg/day and the intruders received 8.0 g/kg/day. The final daily dosage received by residents and intruders represents the highest dose either of these groups could tolerate without losing body weight in our laboratory (unpublished data).

Blood ethanol, plasma catecholamine, and corticosterone determination. A 3-ml blood sample was taken to measure blood ethanol, plasma norepinephrine (NE), plasma epinephrine (EPI), and plasma corticosterone (CORT) for each test. Blood samples were taken via the jugular catheter at the same time each day 2 days prior to the start of drug administration (control), and approximately 1 hour following the morning injection on days 1 (acute EtOH), 13 (chronic EtOH), 14 (20 minutes into aggression testing), and 15 (24 hours into aggression testing) of the drug administration period. From each member of a resident-intruder (R-I) pair, 3.0 ml of blood was withdrawn, and the blood was immediately put on ice. Residents and intruders were then immediately reinfused with blood from their matched donors to prevent hypovolemic response. (The donor rats were Blue Spruce rats and were used as only donors. Donor rats were matched to only one rat at a time. Our work showed that the exchange of blood between male rats was well tolerated, and there was no noticeable immune reaction in the 150 rats used during the actual study.) Fifty μl of whole blood was used for the determination of blood EtOH levels using a head space gas chromatographic procedure, as previously described (37). Following centrifugation, the amount of plasma required for the assay of CAs (1.0 ml) and CORT (15 μl) were stored at −30°C until their subsequent assay.

Plasma CAs were analyzed using a high pressure liquid chromatographic (HPLC) procedure with electrochemical detection described by Patel *et al.* (34). This procedure involved adding as internal standard, dihydroxybenzylamine (DHBA), to each 1-ml sample as well as running a set of CA standards containing NE, EPI, DHBA, and dopamine (DA). An amount of 0.5 ml of 0.1 M perchloric acid (PCA) and 0.1 mM ethylenediaminetetraacetic acid (EDTA) was added to test tubes containing either sample or standard. Test tubes were then centrifuged at 2500 RPM for 15 minutes at 4°C. CAs in the supernatant were extracted with alumina after adjustment of the sample pH (8.55–8.60) with a 1.0 M tris-HCl buffer (pH 8.6). After repeated washing of the alumina with water CAs were eluted with 100 μl of PCA, and a 50 μl sample was assayed. The HPLC system used an amperometric detector (model LC-4B Detector Bioanalytic Systems, West Lafayette, IN) and microsorb reverse phase short C-18 column. The voltage of the working electrode was set at +0.75 V vs. Ag/AgCl reference electrode. The limit of detection for the three CAs by this assay was approximately 2–5 pg.

A radioimmunoassay developed by Gwosdow-Cohen *et al.* (18) and Kumar *et al.* (27) was used to determine the concentration of CORT. The antibodies for CORT were obtained from Dr. G. D. Niswender (Department of Psychology, Colorado State University). The intraassay variability was 3.7% and the interassay variability was 7.0%. The antiserum cross reacted to a small extent with deoxycorticosterone (7.1%) and 11-β-hydroxyprogesterone (7.0%), and to a negligible extent with gonadal steroids (0.05%). Plasma (15 μl) was thawed for the assay and were run with a duplicate set of standards (10–1000 pg). CORT was extracted from plasma samples with 0.5 ml of 95% EtOH. Approximately 10,000 cpm of ^{3}H-corticosterone was added to all test tubes and incubated overnight at 4°C. Radioactivity of the samples and standards was counted following incubation, and sample CORT concentrations were determined by a logit-log analysis based on the values of the standards, total counts, and nonspecific binding.

Aggression testing. On the morning of day 14 of the drug regime the intruder rat was placed in the front right-hand corner of the resident rat's home cage. The first 20 minutes of the resident-intruder test was videotaped, and the behavior of both the resident and intruder were later measured using a blind score procedure for 4 offensive and 4 defensive aggressive behaviors [described in detail elsewhere (36)]. At the end of the first twenty minutes of the

resident-intruder test the resident and intruder were removed from the resident's home cage, and placed in individual Plexiglas holding cages. Using a connected extension catheter, blood was withdrawn from outside the animal's cage, and both rats were returned to the resident's home cage for another 24 hours. On the second day the presence or absence of aggressive behavior was scored for all rats over a 2-hour period by visual observation. EtOH administration continued until the end of the experiment for both residents and intruders.

Cumulative dose-response testing. Determinations of the responsivity of the myocardial beta-adrenoceptor system was made using a computer-based pharmacological assay which has been described in detail elsewhere (35). In brief, the dose-response procedure involves the repeated (cumulative) administration of isoproterenol (ISO), a beta-adrenoceptor agonist, via the jugular catheter to determine the dose required to produce 50% of the maximal response (or ED_{50}), in this case an increase in heart rate. On experimental days 4, 5, and 21, rats were anesthetized with a 4% solution of sodium thiamylal (30 mg/kg) via their jugular catheters. A computer system monitored heart rate, and determined the administration of ISO injections which began 10 minutes following the induction of anesthesia. An increasing series of log doses (1–30,000 ng/kg) of ISO were administered via the jugular catheter in a constant volume of 10 μl. The ED_{50} for each session was calculated after testing using a nonlinear line analysis. Following testing subjects were allowed to recover from anesthesia for 1 hour under heat lamp before being returned to their home cage. The ED_{50} determined on experimental days 4 and 5 were averaged and used as control (PRE) while the ED_{50} obtained on experimental day 21 represents the combined effect of drug treatment and aggression testing (POST).

Radioligand binding assay. All rats were sacrificed with an overdose of sodium pentobarbital (100 mg/kg, IV). The heart was removed and placed on ice. The left ventricular free wall was removed and placed on ice. The left ventricular free wall was dissected, placed in 20 mM Tris-HCl (pH 7.5), and frozen at −70°C before being assayed. Beta-adrenergic receptors were quantitated according to the procedure of Arrons and Molinoff (2) except that ^{125}I-pindolol (NEN, Boston, MA) was used. On the day of the assay total binding was determined in a final volume of 250 μl of ^{125}I-pindolol (20–280 pM). Total nonspecific binding was determined in the absence and presence of 100 μM of ISO.

Statistical Analysis

All data are presented as mean ± standard error of the mean. Split-plot analysis of variance, correlational analysis, Wilcoxon tests, and unpaired *t*-tests were performed using the SAS statistical package (Cary, NC). The level of significance was set at $p<0.05$.

RESULTS

Figure 1 shows the plasma CORT response to a 2 g/kg challenge dose of EtOH just prior to (Acute) and after 13 days (Chronic) of EtOH administration. Both residents and intruders given EtOH exhibited an elevation in plasma CORT to the Acute dose ($p<0.0001$), but not to the Chronic dose of EtOH. The 2 g/kg challenge dose of EtOH had no effect on plasma EPI or NE. The blood ethanol level for EtOH intruders and residents is shown in Table 1. Residents had a significantly higher blood EtOH level than intruders after acute ($p<0.01$) and chronic EtOH treatment ($p<0.02$).

Table 1 also shows the blood EtOH level measured for residents and intruders during aggression testing. The blood EtOH level following the first 20 minutes of the resident-intruder test was lower than on the other tests because the dose of EtOH administered 1 hour prior to the resident-intruder test was lowered to 1.25 g/kg of EtOH to avoid producing sedation which would have interfered with behavioral measurements. The blood EtOH levels between resident and intruder rats following 20 minutes and 24 hours of aggression were not significantly different.

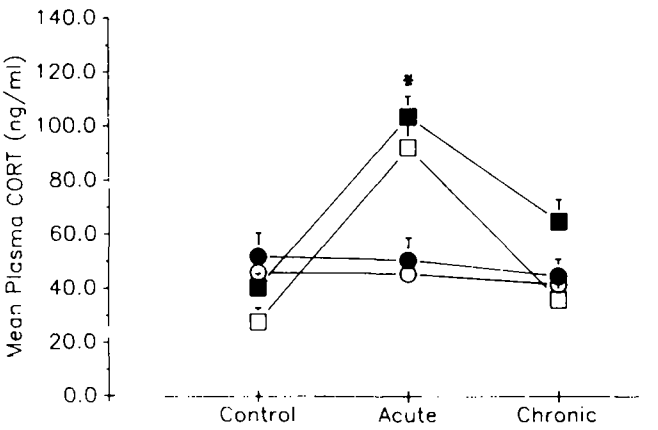

FIG. 1. Mean plasma corticosterone in ng/ml for resident and intruder rats by drug type after control, acute and chronic ethanol treatment. (DM-R ●, EtOH-R ■, DM-I ○, EtOH-I □.) *$p<0.05$, compared to control.

The resident-intruder test was successful in producing aggression by the resident rat within the first 20 minutes in 23 out of the 34 dyadic pairs tested. Intruders showed a greater increase in plasma CORT than residents throughout the aggression test as shown in Table 2. At 24 hours into the aggression test the level of plasma CORT had returned to basal levels for residents, but was still elevated in intruders. In addition, intruders had a higher mean plasma CORT level compared to residents 24 hours into the aggression test ($p<0.001$). Table 3 shows that the EPI response paralleled that of CORT; intruders exhibited the greatest increase in plasma EPI throughout the aggression test, and EPI levels were still elevated 24 hours into the test for intruders, but not residents. In addition, intruders had a higher mean plasma EPI level compared to residents 24 hours into the aggression test ($p<0.001$). Residents showed a greater increase in plasma NE than intruders immediately following the first 20 minutes of the aggression test as shown in Table 4. In addition, plasma NE levels remained elevated above baseline levels in both residents and intruders 24 hours into the aggression test.

Table 5a shows the ED_{50} to the chronotropic effect of ISO before drug administration and after 24 hours of aggression testing. Intruders had a higher ED_{50} following aggression testing

TABLE 1

MEAN BLOOD ETHANOL LEVELS (mg%) FOR RESIDENT AND INTRUDER RATS AFTER THE ACUTE AND CHRONIC ETHANOL TREATMENT AND AFTER 20 MINUTES AND 24 HOURS OF AGGRESSION

	Residents	Intruders
Acute Ethanol	214.0 ± 5.3	184.2 ± 8.1*
Chronic Ethanol	176.9 ± 8.0	148.7 ± 7.6*
20-Minute Aggression Testing	114.1 ± 11.5	112.3 ± 11.4
24-Hour Aggression Testing	182.9 ± 14.3	163.6 ± 9.7

*$p<0.05$, compared to Residents.

TABLE 2

EFFECT OF AGGRESSION ON MEAN PLASMA CORTICOSTERONE (ng/ml) LEVELS AFTER 20 MINUTES AND 24 HOURS OF THE RESIDENT-INTRUDER TEST

	Resident	Intruder
Control	54.4 ± 5.4	38.4 ± 5.2
20 Minutes	124.4 ± 7.2*	168.2 ± 8.2*†
24 Hours	59.5 ± 6.7	75.9 ± 8.6*†

*$p<0.05$ compared to Control.
†$p<0.05$, compared to Resident.

compared to their predrug control measurement, or to the mean ED_{50} of residents following 24 hours of aggression testing. There was no significant difference between the predrug control and postaggression ED_{50} for resident rats.

Table 5b shows the results of the radioligand binding assay performed on left ventricular homogenates taken at the end of the aggression test. Neither subject type, nor aggression had an effect

TABLE 3

EFFECT OF AGGRESSION ON MEAN PLASMA EPINEPHRINE (pg/ml) LEVELS AFTER 20 MINUTES AND 24 HOURS OF THE RESIDENT-INTRUDER TEST

	Resident	Intruder
Control	792 ± 60	533 ± 59†
20 Minutes	1199 ± 79*	2637 ± 190*†
24 Hours	706 ± 74	1102 ± 106*†

*$p<0.05$, compared to Control.
†$p<0.05$, compared to Resident.

TABLE 4

EFFECT OF AGGRESSION ON MEAN PLASMA NOREPINEPHRINE (pg/ml) LEVELS AFTER 20 MINUTES AND 24 HOURS OF THE RESIDENT-INTRUDER TEST

	Resident	Intruder
Control	1093 ± 87	1080 ± 74
20 Minutes	3945 ± 284*	2073 ± 228*†
24 Hours	2098 ± 189*	1841 ± 115*

*$p<0.05$, compared to Control.
†$p<0.05$, compared to Resident.

TABLE 5a

ED_{50} (ng/kg) TO THE CHRONOTROPIC EFFECT OF ISO BEFORE DRUG ADMINISTRATION (PRE) AND AFTER 24 HOURS OF AGGRESSION TESTING (POST)

	Resident	Intruder
PRE	65 ± 5	75 ± 5
POST	88 ± 23	155 ± 21*†

*$p<0.05$, compared to Control.
†$p<0.05$, compared to Resident.

TABLE 5b

^{125}I-PINDOLOL BINDING DATA USING LEFT VENTRICULAR HOMOGENATES TAKEN AT THE END OF AGGRESSION TESTING

	EtOH Rats	Dextrin-Maltose Rats
B_{max} (fmol/mg)	7.49 ± 1.42	3.69 ± 0.36*
K_d (pM)	4.15 ± 0.72	3.74 ± 0.98

*$p<0.01$, compared to EtOH-treated rats.

on the density of affinity of left ventricular beta-adrenoceptors. However, a greater left ventricular beta-adrenoceptor density was found in EtOH-treated rats compared to DM-treated rats. There was no difference found in the left ventricular beta-adrenoceptor affinity between EtOH-treated and DM-treated rats.

The behavior exhibited by residents and intruders 24 hours into the aggression test had a significant influence on hormone levels and ED_{50}. Table 6 shows the change in the plasma hormone levels and ED_{50} from preaggression levels as a function of behavior for residents and intruders 24 hours into aggression testing. Intruders which exhibited defensive aggressive behaviors, such as freezing or upright postures, showed a greater increase in plasma CORT ($p<0.0001$), plasma EPI ($p<0.02$), plasma NE ($p<0.001$), and ED_{50} ($p<0.01$), compared to other intruders which did not show defensive aggressive behaviors. In addition, the nine EtOH intruders which showed defensive aggressive behavior exhibited a smaller increase in ED_{50} (66 ± 20 ng/kg) compared to the nine defensive DM intruders (185 ± 58 ng/kg) ($p<0.05$). Increases in the ED_{50} of intruders was significantly related to increases in EPI ($r = .428$, $p<0.02$) and CORT ($r = .372$, $p<0.05$). Table 6 also shows that intruders had significantly higher levels of plasma CORT ($p<0.01$) and plasma EPI ($p<0.01$), and a lower ED_{50} ($p<0.01$) compared to resident rats. Finally, Table 6 shows that residents which exhibited aggressive behavior had lower plasma EPI ($p<0.01$), and higher levels of plasma NE ($p<0.001$) compared to nonaggressive residents. Although residents as a group did not show an increase in ED_{50} there was a subset of residents which showed a greater than 25 ng/kg increase in ED_{50}. The occurrence of residents showing a greater than 25 ng/kg change in ED_{50} was significantly related to drug type and aggression status (Wilcoxon test, $\chi^2 = 11.81$, $p<0.01$) with 6 out of 9 aggressive EtOH residents and 3 out of 12 nonaggressive DM residents falling into this category.

DISCUSSION

The results from this experiment show that both resident and intruder rats exhibited an activation of the sympathoadrenal medullary and pituitary-adrenocortical systems during aggression testing, and that the myocardial beta-adrenoceptor system showed a functional down-regulation in intruders and some residents.

The higher blood EtOH level exhibited by the EtOH residents following the acute and chronic doses of EtOH could have several possible explanations. First, the rate of metabolism of EtOH is known to differ with age (7,33). The intruders were younger than the resident rats. Since we measured blood EtOH level at a single time point, the peak EtOH levels may have actually been the same in both groups of animals, but the rate of metabolism of EtOH may have been faster in the younger rats. Another explanation for the apparent difference in blood EtOH levels between the resident and intruder rats is that the older rats tend to have less body water and more body fat. Therefore, the older residents might have had less volume per total body weight with which to distribute EtOH.

TABLE 6

THE EFFECT OF BEHAVIOR AND SUBJECT TYPE ON THE CHANGE IN PLASMA CORT, EPI, AND NE LEVELS AND ED_{50} AT 24 HOURS INTO THE AGGRESSION TEST[1]

	Intruders		Residents	
	Nondefensive	Defensive	Nonaggressive	Aggressive
CORT (ng/ml)	3.8 ± 7.3	68.8 ± 7.0 * †	12.9 ± 5.5 †	−6.1 ± 6.8
EPI (pg/ml)	146 ± 55	410 ± 65 * †	51 ± 54 *	−156 ± 58 *
NE (pg/ml)	498 ± 204	968 ± 192 * †	181 ± 136 *	901 ± 137 *
ED_{50} (ng/kg)	20 ± 31	130 ± 33 * †	−2 ± 18	14 ± 30
N	19	15	20	14

[1]Change in each parameter measured as difference from day prior to aggression testing.
*$p<0.05$, compared to same subject type exhibiting different behavior.
†$p<0.05$, compared to other subject type.

Plasma CORT was elevated for both residents and intruders following the first 20 minutes and 24 hours of aggression. The aggression testing acted as a stressor for both residents and intruders even though residents displayed virtually all the offensive aggression. After 24 hours of aggression testing the plasma CORT response of residents showed adaptation while intruders continued to show an elevation of plasma CORT. The plasma CORT data is supported by our own and by previous studies which have found that both residents and intruders show body weight loss (a traditional measure of stress) following aggression testing with intruders exhibiting the greatest loss (3, 15, 29, 36).

The plasma CA response following acute and chronic aggression was controlled by subject type. Acute aggression in residents produced a greater increase in plasma NE output while intruders showed a greater increase in plasma EPI. One interpretation of the greater NE response in residents is that it reflects the greater activity of this group which engaged in mobile offensive aggressive behaviors compared to the static defensive aggressive behaviors which intruders engaged in. Exercise has been shown to increase plasma NE more than plasma EPI (10). The greater plasma EPI exhibited by intruders compared to residents after 20 minutes of aggression may indicate that this group underwent greater stress than residents. Dimsdale and Moss (19) also found that humans climbing several flights of stairs produced a greater increase in plasma NE while public speaking produced a greater increase in plasma EPI. The greater increase in plasma EPI produced by public speaking may be the result of greater emotional stress. Data from this study also supports the hypothesis that plasma EPI provides a better measure of stress than plasma NE. Intruders continued to display an increase in plasma EPI levels after 24 hours of the resident-intruder test while plasma EPI levels for residents had already returned to baseline. Both residents and intruders showed a comparable elevation in plasma NE levels.

Aggression-induced increases in ED_{50} were influenced by three factors: 1) subject type, 2) drug type, and 3) behavior. Intruders which exhibited defensive aggressive behavior showed a greater increase in ED_{50} compared to intruders which did not exhibit defensive aggressive behavior. The increase in ED_{50} of intruders displaying defensive aggressive behavior occurred regardless of the behavior of the resident. This result and the significant correlation between increases in ED_{50} and plasma EPI suggest that the intruder's interpretation of their environment influenced the functional down-regulation of the myocardial beta-adrenoceptor system. Drug type also influenced the down-regulation of both resident and intruder rats. Chronic EtOH treatment blunted the increase in ED_{50} of intruders which displayed defensive aggression. However, the opposite effect was evident in resident rats treated with chronic EtOH which displayed offensive aggression. Neither plasma EPI, nor plasma NE levels were significantly different between chronic EtOH-treated and control intruders. At least two interpretations of the effect of chronic EtOH on the increase in ED_{50} of intruders is possible: 1) measurement of plasma CAs does not accurately reflect the CA concentration myocardial myocytes are exposed to, or 2) EtOH acted directly on the myocardial beta-adrenoceptor system or at the membrane level. Single point measurements may not accurately reflect dynamic levels of plasma CAs in that the metabolic half-life of plasma CAs in rats has been measured to be approximately 58 seconds (45,46). Alternately, EtOH has been shown to decrease myocardial damage produced by the administration of pharmacological doses of adrenergic agonists (16, 30, 31). Unlike the effect of chronic EtOH treatment in intruders which blunted an increase in ED_{50}, residents which displayed offensive aggression exhibit an increase in ED_{50} which other residents did not. Both EtOH-treated and control residents after 24 hours of aggression testing showed a continued elevation in plasma NE but not plasma EPI levels which indicates that NE release from the myocardial sympathetic nervous system produced the down-regulation in aggressive EtOH residents. The plasma NE data, however, do not clarify at what level chronic EtOH treatment increased the ED_{50} of aggressive EtOH residents. Although aggressive EtOH residents had a higher mean level of plasma NE than other groups this difference was not

significant, nor did a correlation analysis of plasma NE vs. ED_{50} increase for residents showing a significant correlation ($r = -.089$, $p > 0.60$).

Because the arterial-venous difference of CAs in the myocardium was not measured in this study, the activity of the sympathetic nervous system serving the myocardium is not clear. The efferent sympathetic nervous system has been shown not to be a mass-action system, rather this system exhibits differences in simultaneous activity (25, 26, 32, 47), and has a complex neuroanatomical organization (1, 9, 21). The fact that the sympathetic nervous system does not act as an all-or-none system and that plasma NE levels reflects only a 3% contribution from the myocardium (11–13) does not permit a conclusion as to whether elevated release of NE from the myocardial sympathetics in chronic EtOH-treated animals accounted for the increase ED_{50}, or whether the increase in ED_{50} of aggressive EtOH residents was due to a direct effect of EtOH on the myocardium. It may seem contradictory to expect a direct effect of EtOH in intruders to reduce increases in ED_{50} while causing an increase in the ED_{50} of residents, however, residents were older than intruders, and developmental differences have been noted in the myocardial beta-adrenoceptor system of rats and other species (6, 8, 17, 23, 24, 41). In addition, if NE is the mechanism responsible for the functional down-regulation in aggressive EtOH residents, then the greater increases in NE levels shown by residents compared to intruders in the 20 minutes of the resident-intruder test may also explain the differences chronic EtOH treatment in resident and intruder rats.

While the functional response of the myocardial beta-adrenoceptor system was diminished to adrenergic stimulation, beta-adrenoceptor system was diminished to adrenergic stimulation, beta-adrenoceptor density and affinity were unchanged. The lack of an effect of aggression on beta-adrenoceptor binding indicates that aggression may have altered the membrane signal transduction by changing the control of cAMP production. The nature of such an alteration in membrane signal transduction is not clear. In vitro studies have shown that agonist-induced desensitization produces a rapid uncoupling between beta-adrenoceptors and adenylate-cyclase which is later followed by receptor down regulation (19, 43, 44). The uncoupling between beta-adrenoceptors and adenylate-cyclase was rapidly (~15 minutes) reversible following the removal of the agonist from the cell medium, while there was no recovery of receptors (43). It is not clear how rapid the recovery of the myocardial beta-adrenoceptor system is in vivo to this type of disruption in signal transduction. Functional cardiomyopathy and a disruption of membrane signal transduction has been found in both ventricles of dogs with experimentally-induced right heart failure while only the right ventricle showed a decrease in beta-adrenoceptor density. Deficits in membrane signal transduction have also been found to precede functional cardiomyopathy (10, 14, 28, 42), and changes in beta-adrenoceptor density (4,42). The evidence cited above and the fact that chronotropic measures of down-regulation parallel inotropic measures imply that the uncoupling of receptor activation from cAMP production may lead to further down-regulation and eventual loss of receptors with continued elevations in peripheral catecholaminergic output. It is interesting to speculate whether the decrease in receptor density and the loss of adaptability in the myocardial beta-adrenoceptor system that has been reported to occur in older animals (6, 8, 17, 23, 24, 41) is due to the cumulative effect of periodic outbursts from the sympathoadrenal medullary system.

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