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Voluntary Alcohol and Cocaine Consumption in "Low" and "High" Stress Plasma Catecholamine Responding Rats

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TAYLOR, J., N. HARRIS AND W. H. VOGEL. *Voluntary alcohol and cocaine consumption in "low" and "high" stress plasma catecholamine responding rats.* PHARMACOL BIOCHEM BEHAV 37(2) 359–363, 1990.—Alcohol (ethanol) and cocaine preference in a free-choice, two-bottle situation was measured in two groups of male and female "low" and "high" plasma catecholamine stress responding rats. Alcohol intake of a 5% solution (percent or mg/kg) showed markedly different but individually consistent intake among animals. "High" plasma catecholamine stress responders consumed more ethanol than did "low" responders. A similar finding was made when animals consumed a 10% solution; fluid intake fell but total ethanol intake remained the same. "High" responders drank more than did "low" responders. After a period of 4 weeks of water only, animals were reexposed to 5% ethanol and a significant positive correlation was seen in the drinking habits of the animals. Afterwards, exposure to a 0.02% cocaine solution resulted in cocaine intake which varied among animals, but was consistent for an individual rat and did not correlate with alcohol consumption. In general, ethanol and cocaine consumption correlated positively with the plasma catecholamine stress response. No significant differences in drinking habits were observed between the sexes. Thus, alcohol preference is a relatively stable characteristic of an animal, is higher in "high" as compared to "low" plasma catecholamine stress responders and does not correlate with voluntary cocaine consumption.

Alcohol Ethanol Cocaine Stress Voluntary consumption Plasma catecholamines

VOLUNTARY alcohol (ethanol) consumption of rats is highly variable. Exposure of rats to a water bottle as well as to a water:ethanol bottle in a free choice situation reveals that some animals prefer ethanol, some animals avoid ethanol and some animals drink from both bottles. The selective breeding of ethanol preferring and nonpreferring rats has resulted in two distinct lines indicative of a genetic basis of alcohol preference or avoidance (5, 6, 8). It has been further claimed that voluntary ethanol consumption increases during or after stressful experiences, although results are still contradictory (3, 9, 11–14).

In our laboratory, we are breeding rats selectively for their plasma catecholamine (epinephrine = E and norepinephrine = NE) stress responses to immobilization in order to obtain "low" and "high" stress responding lines. In order to see whether "low" or "high" responders would differ in their voluntary ethanol consumption, we exposed some of these animals to a free-choice, 2-bottle water and ethanol:water situation. Furthermore, we tested alcohol consumption of individual animals at 2 different alcohol concentrations. After a period of water only, ethanol intake was studied again to determine if the drinking habit of an animal would be consistent over time. Finally, we studied these animals in a free choice water and cocaine:water drinking situation to see whether preference or avoidance for ethanol and cocaine would coexist in the same rats.

METHOD

Subjects

Rats used were male and female rats of the N/Nih (Hansen) strain which were selected from the 2nd generation of our selectively bred "high" or "low" plasma catecholamine stress responders. After weaning, they had been group-housed and allowed to mature. At approximately 3.5 months of age, rats were prepared with a jugular catheter using standard procedures. One blood sample was then obtained prior to and at 5, 15 and 30 min during stress. Plasma levels of NE and E were determined by radioenzymatic analysis (Cat-A-Kit®) and the area under the stress-curve was calculated. Thereafter, animals were selectively mated. After mating, the rats were kept in individual cages.The rats were always housed in light-, temperature- and humidity-

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TABLE 1

PLASMA CATECHOLAMINE STRESS RESPONSES OF SELECTED RATS OF THE 2ND GENERATION OF "HIGH" AND "LOW" STRESS RESPONDERS

	E	NE
High responders, male	56.1 ± 5.4	41.5 ± 3.4
Low responders, male	41.8 ± 9.1*	33.8 ± 2.4*
High responders, female	155.0 ± 12.2†	64.2 ± 5.3†
Low responders, female	91.6 ± 7.1*†	42.7 ± 3.1*†

Values represent area under the stress curve (ng/ml × 30 min) and are means ± SEM. Number of animals is 7–10 per group.

*$p<0.05$, comparing high and low responders.

†$p<0.05$, comparing female and male rats.

controlled rooms. Water and pelleted Purina rat chow were available ad lib.

Experimental Procedure

At approximately 8 months of age (i.e., about 4 months after completion of their breeding responsibilities), rats were weighed and housed in hanging cages. On the front of each cage were attached a feeder and, on each side, a pair of water bottles. Bottles were alternated daily to balance any side preferences of the rats.

One of the drinking bottles was designated "bottle 1" and this bottle always contained water. The other bottle was designated "bottle 2." Bottle 2 was filled with different solutions according to treatment period. During treatment period 1, bottle 2 contained water. During treatment periods 2, 3, 4, and 5, bottle 2 contained 5% ethanol, 10% ethanol, 5% ethanol, and 0.02% cocaine hydrochloride, respectively. During the rest period between treatment 3 and 4 and 4 and 5, bottle 2 contained water. The duration of treatment periods 1, 2, 3, 4, and 5 were 8, 8, 5, 8, and 4 days, respectively. Treatment periods 1, 2 and 3 progressed without interruption. After the treatments, the rats were allowed only water for 31 days before beginning treatment 4. Similarly, rats received an 18-day water-only interval between treatments 4 and 5. On each study day between the hours of 12 p.m. and 2 p.m., all bottles were removed from the cages and their weights were measured and recorded. Bottles were rinsed, refilled with the appropriate solution, protected from light, and reattached to their cages. Bottles attached to empty cages allowed for measurement of evaporation which we found to be negligible.

Data Analysis

For comparison of subject groups and comparison of treatment periods, differences between means were assessed for significance by one- or two-way analyses of variance with repeated measures for the drinking studies followed by the Newman-Keuls post hoc test. Correlations were determined by linear regression analysis and expressed as correlation coefficient (r) values; the probability corresponding to each r value was obtained by calculating double-tailed t-values with (N − 2) degrees of freedom.

RESULTS

Table 1 shows the total catecholamine stress responses of the animals at the age of about 3.5 months. As can be seen, groups differ in their plasma catecholamine stress responses and female animals show higher stress values than do the male rats.

Table 2 shows the means for total fluid intake by the animals. Body weights of the male and female animals at the beginning of this study were 493 ± 11 g and 273 ± 7 g, respectively. Total fluid consumption was consistently and significantly greater in female rats as compared to male rats. Fluid intake for each group did not change markedly throughout the different drinking conditions.

TABLE 2

TOTAL FLUID CONSUMPTION

	Treatment				
	1	2	3	4	5
	Water	EtOH 5%	EtOH 10%	EtOH 5%	Cocaine 0.02%
Male low	71	70	75	72	75
(n = 8)	±3	±9	±7	±3	±4
Male high	71	87	88	68	68
(n = 10)	±6	±4	±6	±4	±4
Female low	130*	130*	139*	146*	124*
(n = 7)	±5	±8	±10	±11	±7
Female high	118*	135*	130*	127*	116*
(n = 10)	±3	±7	±8	±9	±10
All low	99	98	105	107	98
(15)	±8	±10	±10	±11	±7
All high	94	111	109	98	91
(20)	±6	±7	±7	±8	±8

Values represent ml/kg and are means ± s.e.m. Bottle 1 contained water at all times. Bottle 2 contained different solutions according to treatment: 1, water (8 days); 2, 5% ethanol (8 days); 3, 10% ethanol (5 days); 4, repeat of treatment 2; 5, 0.02% cocaine HCl (4 days). Thirty-one days water only between treatments 3 and 4 and 18 day water only between treatments 4 and 5.

*$p<0.05$, comparison female vs. male.

Table 3 shows the voluntary consumption of ethanol or cocaine as a percent of total fluid intake. As can be seen from the water-only choice (treatment 1), no preference for a particular bottle position is apparent within groups. Water is almost equally consumed from each bottle. Alcohol as a 5% solution is consumed significantly more by high as compared to low responding rats. As the concentration of ethanol is increased to 10%, consumption drops but high responding rats still drink significantly more ethanol than do low responding animals. After the rest period, exposure to a 5% ethanol solution shows similar values as found during first exposure with all high responders consuming significantly more ethanol than do all low responders. For all treatment periods, preference or avoidance was highly consistent for individual animals over time. No significant sex difference was apparent although females tend to consume less alcohol than do males. Exposure to a 0.02% cocaine solution also revealed the same difference; high responders consumed more cocaine and this difference is statistically significant.

Table 4 shows the consumption of alcohol or cocaine on a weight basis. High responding animals drink significantly more of the 5% and 10% alcohol than do the low responding rats. Consumption based on body weight is roughly the same for the 5% or 10% alcohol solution. After the rest period, the amount of alcohol consumed is again higher in the high responding rats. No significant sex differences are apparent but females tend to consume more alcohol per body weight. A similar finding is made for cocaine; high responding rats consume more cocaine than do low responding rats.

TABLE 3

CONSUMPTION OF ETHANOL OR COCAINE AS A PERCENTAGE OF FLUID INTAKE

	Treatment				
	1 Water	2 EtOH 5%	3 EtOH 10%	4 EtOH 5%	5 Cocaine 0.02%
Male low (n=8)	44.7 ±5.1	47.6 ±13.1	18.0 ±9.8	47.6 ±12.7	32.9 ±6.4
Male high (n=10)	49.9 ±4.5	78.1 ±9.2	41.9 ±11.1	77.1 ±11.6	54.4 ±7.4
Female low (n=7)	45.8 ±4.9	33.8 ±11.8	18.3 ±9.0	37.1 ±14.1	35.0 ±5.6
Female high (n=10)	56.5 ±4.8	59.7 ±12.3	33.0 ±8.6	71.8 ±10.6	49.3 ±8.1
All low (15)	45.2 ±3.4	41.2 ±8.8	18.2 ±6.1	42.5 ±9.2	33.8 ±4.2
All high (20)	53.2 ±3.3	68.9* ±7.8	37.4* ±6.9	75.5* ±7.7	52.0* ±5.3

Values represent alcohol or cocaine as a percent of total consumption and means ± s.e.m. Experimental design as described in Table 2. Recordings are from bottle 2.

*$p<0.05$, comparison low vs. high.

TABLE 4

CONSUMPTION OF ETHANOL OR COCAINE AS AN AMOUNT PER BODY WEIGHT

	Treatment				
	1 Water	2 EtOH 5%	3 EtOH 10%	4 EtOH 5%	5 Cocaine 0.02%
Male low (n=8)	1.3 ±0.5	1.5 ±0.3	0.9 ±0.4	1.3 ±0.4	4.8 ±0.9
Male high (n=10)	1.4 ±0.2	2.7 ±0.4	2.6 ±0.6	2.1 ±0.3	7.4 ±1.1
Female low (n=7)	2.3 ±0.3	1.9 ±0.7	2.0 ±1.0	2.3 ±0.9	8.8 ±1.6
Female high (n=10)	2.6 ±0.2	3.3 ±0.7	3.3 ±0.7	3.8 ±0.7	10.1 ±1.1
All low (15)	1.8 ±0.2	1.7 ±0.4	1.5 ±0.5	1.8 ±0.5	6.6 ±1.0
All high (20)	2.0 ±0.2	3.0* ±0.4	3.0* ±0.5	2.9* ±0.4	8.7* ±0.8

Values represent amounts (alcohol, g/kg; cocaine, mg/kg) consumed and are means ± s.e.m. Experimental design as described in Table 2. Recordings are from bottle 2.

*$p<0.05$, comparison low vs. high.

Alcohol consumption was relatively consistent for a given rat. Table 5 shows an example of three rats and their relatively stable intake of ethanol during the first ethanol exposure period. Rat 1 represents a high, rat 2 a low and rat 3 an intermediate alcohol consumer. Similar results were obtained for the other periods as well.

Since the same animals were used for the entire study, correlations can be obtained among different treatment results. The most significant correlations were found between week 2 and subsequent weeks as well as catecholamine stress responses (Table 6). As can be seen, a high correlation exists between weeks 2 and 3; high consumers of the 5% ethanol solution also consume higher amounts of the 10% solution and vice versa. After the rest period, reintroduction of a 5% ethanol solution caused the previously high drinkers to be high drinkers again and vice versa. Interestingly, no correlation exists between alcohol or cocaine preference or avoidance; high or low alcohol drinkers are not necessarily the same high or low cocaine consumers. As expected, positive correlations were found between alcohol preference and the total plasma catecholamine stress responses; the higher the stress catecholamine response the higher was alcohol consumption.

DISCUSSION

Rats in this study were obtained from the 2nd generation of our breeding project which selectively breeds high and low plasma catecholamine stress responding rats, although early in the breeding process, animals could be separated statistically into distinct biological groups. Previously, these animals underwent surgery and immobilization and were then bred. About 4 months after mating or pregnancy, animals were used for this experiment. Thus, animals were not experimentally naive but had been subjected to surgery and stress and had experienced mating and rearing of offspring. However, there was a 4-month rest period between the experiments which should have reduced or abolished prior experiences. While these conditions are not scientifically "clean," they are actually more realistic and perhaps represent real-life situations more closely where stress experiences and pregnancies are common to most animals or humans.

The stress plasma catecholamine response was measured about 3 months before this experiment. While the absolute values most probably have increased over time, the distinction between low and high responding rats is still valid at the time of the alcohol drinking study. We have shown that the plasma catecholamine stress response increases during aging but is a characteristic of a given animal and that the rank order of the stress response within a group of rats does not change over a period of up to one year (16).

Rats of the N/Nih strain consume alcohol on a voluntary basis but alcohol preference shows marked variations among animals. This is in agreement with findings from other laboratories (5). In addition, our study shows a number of new observations regarding the correlation of a stress response with ethanol consumption, the stability of the drinking habit of rats in regard to different concentrations of alcohol and over time. Furthermore, our study compares alcohol with cocaine consumption.

In our experiment, animals were not adapted to alcohol first but were started immediately with a choice. Under these conditions, rats separated quickly within the first 2 days into preferring and avoiding animals with a number of animals falling in between these extremes. This behavioral pattern was quite consistent for the rest of the first exposure period to alcohol as well as for all subsequent treatment periods. When a 10% alcohol solution was offered, a high positive correlation was found for both treatments. The same rats which preferred or avoided the 5% solution also preferred or avoided the 10% solution. Intake of the 10% solution was lower than intake of the 5%; however, a comparison of alcohol consumed on a body weight basis shows that almost the

TABLE 5

CONSUMPTION OF 5% ETHANOL AS AN AMOUNT OF BODY WEIGHT IN 3 MALE RATS DURING TREATMENT 2

Days	Rat 1	Rat 2 (g/kg)	Rat 3
1	2.9	0.5	1.5
2	3.1	0.4	1.5
3	1.7	0.6	2.4
4	3.3	0.3	1.5
5	3.5	0.9	1.8
6	2.9	0.1	2.0
7	3.2	0.2	1.4
8	2.5	0.5	1.8

Values are individual ethanol intake data.

TABLE 6

CORRELATION VALUES AMONG TREATMENTS AND PLASMA CATECHOLAMINE STRESS RESPONSES

Treatment 2 vs.	r	*t*	*p*
Treatment 3	.5682	3.9663	<0.01
Treatment 4	.6487	4.8962	<0.01
Treatment 5	.1380	0.7885	>0.05
AUC Epinephrine	.6051	4.0925	<0.01
AUC Norepinephrine	.4412	2.7808	<0.01

Correlations were derived from percentage of alcohol or cocaine consumed or the area under the stress curve.

same amount of alcohol was consumed under both conditions. Thus, rats seem to titrate their alcohol intake quite accurately and specifically up to a certain level regardless of the quantity or concentration of alcohol offered. After a rest period of water only the animals were then reexposed to alcohol. A high correlation was found between the drinking habit at first exposure and that of a subsequent exposure of the same concentration of ethanol. Thus, alcohol consumption is a relatively stable characteristic of a given animal. This conclusion is supported by genetic studies which show that rats can be bred selectively and successfully for alcohol preference or avoidance (6,8).

It has been claimed that alcohol consumption by rats is increased during or after stress, although not all studies agree with this conclusion (3,9, 11–14). In our rats, plasma catecholamine stress responses of a previous stress experience correlate well with voluntary alcohol intake under resting conditions both in terms of group comparisons and correlation analyses. High responders consume more alcohol than do low responders. However, these observations were made under resting conditions. We did not test alcohol consumption under these previously established stressful conditions. However, it can still be concluded that rats with a high stress potential do consume more alcohol voluntarily than do rats with a low stress potential. This is in agreement with data showing that rats selectively bred for high emotionality drink more alcohol than their low emotional counterparts (2) and that alcohol-preferring rats are more anxious and emotionally more reactive as compared with alcohol-nonpreferring rats (15). Thus, a definitive correlation among stress, anxiety and alcohol consumption seems to indeed exist.

It has previously been found that alcohol in moderate doses (1–2 g/kg) has no marked effect on plasma catecholamine levels in resting rats. However, these same doses can significantly antagonize or reduce the stress-induced increases in these biochemicals (1,17). This reduction of the plasma catecholamine stress response has been found to be linked to the stress response without alcohol; the higher a stress response the more of a reduction can be anticipated by alcohol (7). Although we did not test and compare the extent of the alcohol-induced reduction with voluntary alcohol consumption in the same rats, the previous studies on alcohol reduction (1, 7, 17) and this study on voluntary alcohol consumption link both of the variables together in a positive fashion. The higher the stress response the higher the stress reduction and the higher the voluntary alcohol consumption. This suggests that stress reduction might also be linked to voluntary alcohol consumption and will be the subject of a subsequent study.

A comparison between the sexes indicates that female rats show significantly higher stress plasma catecholamine values and consume significantly more fluid than do males. As a matter of fact, their water intake is almost double that of the males. Voluntary alcohol consumption is statistically not different between male and female rats but female rats most often consume more alcohol than do their respective male counterparts.

Our animals also consumed voluntarily a cocaine solution with wide variations in preference and avoidance among individual rats. This is in agreement with a previous study which showed rats to exhibit a selective preference or avoidance of a cocaine solution (4). In our case, cocaine consumption was quite characteristic for a given animal. Again, the high stress responding rats preferred cocaine over the low stress responding counterparts. However, no correlation was observed between preference for or avoidance of alcohol and cocaine; high alcohol consumers were not necessarily high cocaine consumers. Although statistically not significant, female rats drank consistently more cocaine than did the male rats. This is in agreement with human data where heavy alcohol use was found to be sometimes but not always linked to heavy cocaine use (10). At present, it is not clear whether these animals consume cocaine for its gustatory, oral or central effects; however, we could show that the oral consumption of cocaine leads rapidly to appreciable brain levels (unpublished observation).

In summary, preference or avoidance of ethanol is a stable characteristic of a given rat. Independent of the concentrations of ethanol offered, animals titrate their intake to an individually regulated amount. Animals with high plasma catecholamine stress potentials consume more ethanol under resting conditions than do their counter parts with low stress potentials. Under all circumstances, plasma catecholamine stress responses correlate positively with alcohol intake. Our animals also showed large but stable differences for cocaine preference; high stress responders consumed more drug than did low stress responders but no correlations between alcohol and cocaine preference or avoidance were seen in our animals. Sex differences were slight in that female rats consumed more alcohol and cocaine but were statistically not significant.

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