Chronic Ethanol Consumption, Stress, and Hypertension

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SUMMARY **A reliable method of producing physical stress in the rat was developed using heat irradiation, and the possible interaction between chronic ethanol consumption and stress was investigated in a rat model of alcoholism. Chronic heat stress and chronic ethanol consumption each produced mild hypertension in rats. When combined, the two treatments resulted in hypertension more severe than that produced by either stress or ethanol consumption alone. The group of animals receiving both treatments also exhibited high mortality. Investigations into the mechanisms responsible for the apparent additive effects of the two treatments revealed that the animals in this group had the highest circulating norepinephrine levels. The plasma volumes, however, were not different between the stressed groups and their unstressed counterparts. As the plasma norepinephrine level usually reflects overall sympathetic tone of an animal, our results suggest that the additional hypertensive effect of chronic stress on the ethanol-treated animals is associated with increased sympathetic nervous activity and is not a result of expanded plasma volume. These findings may have clinical implications for human alcoholics and in the analysis of cardiovascular risk factors in hypertensive patients. (Hypertension 7: 519–524, 1985)**

KEY WORDS • **ethanol** • **heat stress** • **rodent model** • **blood pressure** • **mortality** • **sympathetic activity** • **catecholamines**

PHYSICIANS have long suspected that chronic alcoholism is associated with hypertension.[1,2] We have developed a rodent model of alcoholism and have shown previously that chronic ethanol consumption is associated with mild hypertension in rats.[3] Parallel observations also have been reported in human alcoholics,[4,5] but variables such as smoking habits, stress, oral contraceptive use, nutritional status, and genetic predisposition make human data difficult to interpret and thus often lead to equivocal conclusions. Animal models allow these variables to be controlled or monitored. Because stress is the most commonly implicated confounding factor in the cardiovascular pathological conditions seen in chronic alcoholism,[6] we have sought an effective method of producing stress in the rat to examine the possible interaction between chronic alcoholism and stress.

In this study we found that chronic heat irradiation most reproducibly elicited an avoidance reaction and a transient hypertensive response in the rat. This method was used to chronically stress the animals in both control and ethanol-treated groups. The resulting blood pressure responses in different groups of rats and the possible mechanisms involved in the hypertension development were analyzed.

Methods

Preparation of Animals

Male Wistar rats (250–300 g) purchased from Charles River Breeding Laboratories (St. Constant, Quebec, Canada) were used in all the experiments reported in this study. To examine the effects of the various methods of stress on the blood pressure of animals in the conscious state without the influence of anesthesia and surgery, with the animal under ether anesthesia, a polyethylene catheter (PE-50) was inserted into the tail artery and exteriorized at the neck by passing through a subcutaneous channel along the back of the animal. The catheter was filled with heparinized saline (20 IU/ml) and kept patent by flushing from time to time. At 24 to 48 hours after operation, the catheter was linked to a pressure transducer outside the cage

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and a continuous blood pressure tracing recorded on a Grass polygraph (Grass Instruments Co., Quincy, MA, USA). The animals were subjected to various forms of stress including repeated exposures to involuntary restraint, swimming, forced hot air, loud noises, and heat lamp irradiation. The treatment that most reproducibly resulted in avoidance reactions as well as transient and inappropriate elevation in blood pressure was picked as the method of stress to be used in the second part of the study.

The details of the ethanol feeding protocol and monitoring procedures used here are published elsewhere.[3,7] Briefly, rats were divided randomly into treatment and control groups after 2 weeks of continuous observation. The animals in the treatment group received ethanol in their drinking water in increasing amounts (from 5 to 20%) for 3 weeks. The control group received tap water during this period, and both groups were given Purina rat chow (Ralston Purina Co., St. Louis, MO, USA) ad libitum. At Week 4 of the ethanol treatment, the rats were randomly subdivided into four groups: control unstressed, control stressed, alcoholic unstressed, and alcoholic stressed. The designated stressed groups (both ethanol treated and control) then received heat irradiation (5 minutes of heat alternating with 5 minutes of rest) for 1 hour each day throughout the remainder of the experimental period. Blood pressure, heart rate, and body weight of each animal were measured weekly. At Week 12, the femoral arterial pressure of each animal was taken while the rat was under light ether anesthesia and one animal from each group (weight matched) was killed for physiological and biochemical studies.

Plasma Volume Measurements

Plasma volumes were measured in animals lightly anesthetized with ether by the standard technique of indicator dilution. A cannula was introduced into the jugular vein of the rat through a small superficial incision on the right ventral cervical region. Radioactively labeled gamma globulins (molecular weight 150,000; methyl-^{14}C, New England Nuclear, Boston, MA, USA) was injected as a bolus (0.1 μCi total) through the jugular cannula, and blood samples (0.5 ml) were taken from the femoral arterial cannula 2, 5, and 10 minutes after the injection. Samples were centrifuged at 2000 g for 5 minutes, 0.2 ml of plasma was withdrawn and mixed with 15.0 ml of Biofluor (New England Nuclear), and the radioactivity was measured. Values were normalized to the body weight of the animal in question and expressed as milliliters per kilogram.

Plasma Catecholamine Measurements

Blood samples obtained from the femoral arterial cannula were centrifuged, and the plasma was quick frozen and stored at −80°C. Analyses were performed by combining 1.0 ml of plasma and 25 μL of 100 ng/ml of dihydroxybenzylamine (DHBA, in 0.1 M $HClO_4$) in a screw-cap conical glass centrifuge tube containing 100 μL of 10% EDTA. To each tube, 50 mg of acid-washed alumina (AAO, Bioanalytical Systems Inc.) was added. Then 1.0 ml of 1.5 M Tris buffer (pH 8.7) was added to each mixture, and the tubes were capped, vortexed, and agitated on a reciprocal shaker for 5 minutes. After the alumina settled, the supernatant was removed and the alumina was washed twice with double distilled water. Catecholamines were then eluted from the alumina with 200 μL of 0.1 M $HClO_4$. This mixture was transferred to a centrifugal filter (MF-1, Bioanalytical Systems Inc.) and centrifuged for 5 minutes at 15,000 g, the clear acid filtrate was used in the following high-performance liquid chromatography (HPLC) procedure.

The HPLC system consisted of a Beckman model 110A pump (Beckman Instruments, Inc., Fullerton, CA, USA), a 50-μL or 100-μL fixed loop injector, and a 4.6- × 125-mm reverse-phase column packed with ODS Hypersil (Shandon Scientific, Runcorn, United Kingdom). An isocratic solvent system containing 0.05 M potassium formate (pH adjusted to 4.3 with formic acid), 200 mg/L of sodium octane sulfonate (Aldrich Chemical Co., Milwaukee, WI, USA), and 60 mg/L of EDTA (Fisher Scientific Co., Pittsburgh, PA, USA) was used in the separation. The flow rate was maintained at 1.0 ml/minute. Amperometric detection was effected with a carbon paste detector electrode (TL-3, Bioanalytical Systems Inc., West Lafayette, IN, USA) packed with a graphite and nujol paste (CP-O, Bioanalytical Systems Inc.). The electrode potential was maintained at +0.60 V against an Ag/AgCl reference electrode (Bioanalytical Systems Inc.) with an LC-4A amperometric detector (Bioanalytical Systems Inc.). Chromatograms were recorded on a strip chart recorder.[8] Typical recovery was in the range of 75%. The following equation was used to calculate the plasma levels from peak height ratios:

$$\text{Concentration of catecholamine} = \frac{(\text{Catecholamine/DHBA})_{\text{sample}}}{(\text{Catecholamine/DHBA})_{\text{standard}}} \times (\text{catecholamine})_{\text{standard}}$$

Values are reported as nanograms of catecholamine per milliliter of plasma.

Results

Method of Stress

Among the many methods of stress tested in this study, we found that short-term irradiation with a 150-watt heat lamp most reproducibly elicited an avoidance reaction and a transient rise in the caudal arterial pressure that did not diminish with time (i.e., no adaptation to the stress was apparent). We assumed that transient and inappropriate blood pressure elevations denote physical stress in the animals. Figure 1 illustrates a typical blood pressure tracing from a rat with a long-term cannula implanted in the tail artery in a preliminary experiment. The blood pressure rose sharply whenever the animal was subjected to the heat stress and returned to normal when the lamp was turned off. The mean core temperature (measured with a rectal

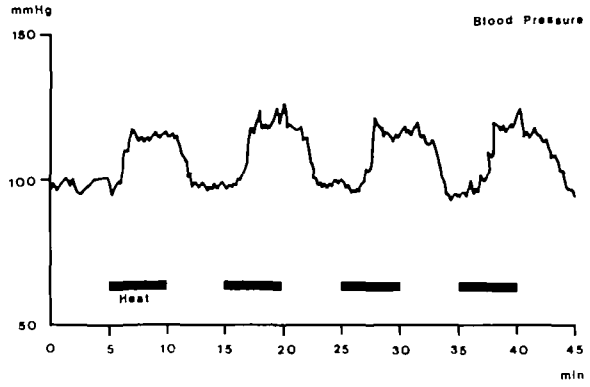

FIGURE 1. *A continuous blood pressure (mean) tracing from a male Wistar rat with a long-term tail artery cannula implant. The thick bars denote heat irradiation with a 150-watt heat lamp and are of 5 minutes' duration. The rat was visibly agitated by the heat, and the square wavelike responses of the blood pressure to the irradiation persisted beyond 180 minutes without signs of adaptation.*

probe; Yellow Springs Instrument Co., Yellow Springs, OH, USA) of the animals was 39.8 ± 0.9°C, and the peak skin temperature was 40.5 ± 0.7°C (n = 4) after 1 hour of intermittent heat irradiation. The surface temperature is below that which is generally thought to be painful.

High Mortality in Alcoholic Stressed Rats

All measurements made before Week 4 indicated that the animals conformed to previously reported results regarding body weight, fluid and food intake, blood ethanol content, plasma and urine ion analysis, and blood pressure values.[3, 7] By Week 12, only 50% of the animals in the alcoholic stressed group were alive. The cause of death appeared to be cardiac failure, as evidenced by pulmonary effusion and gross venous congestion observed at autopsy. The surviving animals in this group were generally very irritable, and many showed seizurelike motor activity. All control stressed rats survived the protocol with no obvious problems. Table 1 lists some of the weights and measurements from the four groups of animals taken at Week 12. The two ethanol-treated groups had slightly reduced total daily caloric intake compared with the two control groups. The alcoholic stressed, alcoholic unstressed, and control stressed animals were all lighter than the control unstressed rats at Week 12, and ethanol consumption was similar between the unstressed and the stressed alcoholic animals (see Table 1).

Blood Pressures

The values of the weekly mean systolic blood pressure of the animals throughout the experiment are illustrated in Figure 2. At Week 4, a difference in the blood pressure was already apparent between the controls and the ethanol-treated animals. By Week 12, the mean blood pressures of the four groups were different, with the alcoholic stressed group having the highest blood pressure values (see Table 1). The mean heart rates of the stressed control group were higher, while those of the unstressed alcoholic groups were lower, than those of the unstressed controls. The stressed alcoholic and unstressed control rats had similar heart rates.

Plasma Volumes of the Different Groups

Plasma volumes were measured in animals lightly anesthetized with ether with the standard technique of indicator dilution. ^{14}C-methyl-gamma globulins were injected as an i.v. bolus, and the radioactivity in the peripheral blood was measured. The advantage of gamma globulins over the more conventional markers such as Evans Blue or ^{125}I-labeled albumin is that the globulins are not appreciably cleared from the circulation within the study period, which makes extrapolation to zero time unnecessary and thereby eliminates the associated errors. Plasma samples taken 2, 5, and 10 minutes after the i.v. bolus contained identical amounts of radioactivity. Because the gamma globulins, unlike albumin, do not enter extravascular spaces, overestimation of the plasma volume is not a problem.[9] Table 2 lists the mean values of the plasma volumes of the four groups of animals. The alcoholic stressed and alcoholic unstressed groups both showed increased plasma volume compared with that of two control groups. There was, however, no difference

TABLE 1. *Measurements Taken at Week 12 on Ethanol-Treated and Control Rats*

	Control		Alcoholic	
Measurement	Unstressed	Stressed	Unstressed	Stressed
Body weight (g)	459 ± 18	442 ± 15	440 ± 10*	428 ± 12*
Ethanol consumption (g/day)	0	0	5.4 ± 1.0	5.9 ± 1.3
Estimated caloric intake (kcal/day)†	138	130	112	108
Mean blood pressure (mm Hg)	98 ± 6	110 ± 8*	122 ± 11*	134 ± 10*
Heart rate (beats/min)	382 ± 14	424 ± 20*	340 ± 22*	386 ± 24

Values are means ± SD, n = 12, except for alcoholic stressed animals, where n = 7.
*Significantly different from unstressed control values, $p < 0.05$.
†As the values are only estimates and not measurements, no standard deviations are given and no statistical analyses were performed

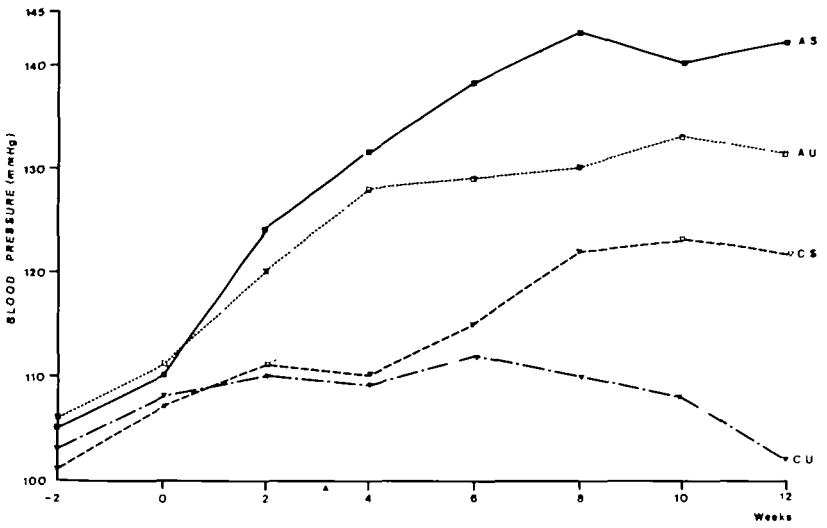

FIGURE 2. *Systolic blood pressure development of four groups of rats during the 12-week protocol. Systolic blood pressures of the animals were monitored weekly with the tail cuff method, and a final intra-arterial pressure was taken at Week 12 while the rats were under light ether anesthesia. Heat stress was implemented during the fourth week and continued until the end of the study. Values are the mean of 12 animals except for the alcoholic stressed (AS) group, in which at Week 12 only 7 animals were left. The standard deviations for the blood pressure values are ±6 mm Hg for the two control groups, control unstressed (CU) and control stressed (CS) and ±11 mm Hg for the two ethanol-treated groups, AS and alcoholic unstressed (AU). The blood pressure values are significantly different ($p < 0.05$) between the four groups of animals from Week 4 onward.*

between the two alcoholic groups themselves, which suggests that the further elevation of blood pressure in the alcoholic stressed group is not the result of plasma volume expansion.

Circulating Catecholamine Levels

The mean plasma catecholamine levels of each group of rats are shown in Table 3. The norepinephrine levels were significantly higher ($p < 0.05$) in the plasma of both groups of alcohol-treated animals when compared with those of unstressed control rats (see Table 3), and a marginal elevation also was noted in the control stressed animals. The stressed alcoholic rats had the highest level of norepinephrine among the four groups of animals, and the mean value was also significantly higher than that of the alcoholic unstressed animals. Plasma epinephrine levels did not differ among the four groups, and plasma dopamine levels were lowest in stressed alcoholic animals.

TABLE 2. *Plasma Volume Estimation*

Group	Mean ± SD (ml/kg)
Control unstressed (n = 8)	49.8 ± 2.2
Control stressed (n = 8)	50.2 ± 1.9
Alcoholic unstressed (n = 8)	59.7 ± 2.1*
Alcoholic stressed (n = 5)	59.9 ± 2.0*

*Significantly different from unstressed control values, $p < 0.05$.

Discussion

In this and previous studies[3, 7] in which rats received ethanol, Wistar rats from Charles River Canada were used. These rats readily ingested 20% ethanol and showed rises in blood pressure. Subsequently, Charles River Canada, Inc., has replaced the old colony of Wistar rats with the new colony of Wistar rats that are relatively pathogen free. We have found that the new colony responds to alcohol quite differently from the old (Chan TCK, Pang CCY, and Sutter MC, unpublished observations, 1983), which suggests that sources and strains of rats are important when the effects of alcohol are studied.

Physical and emotional stress have long been thought to be causally related to hypertension, and experiments in animals and humans provide some supporting evidence.[10-13] Stress also has been regarded as one of the confounding factors in the etiology of the cardiovascular pathological processes found in chronic alcoholics.[4-6, 14, 15] Our results indicate that long-term heat stress elevates the blood pressure of both the con-

TABLE 3. *Plasma Catecholamine Levels*

Group	Norepinephrine (ng/ml)	Epinephrine (ng/ml)	Dopamine (ng/ml)
Control unstressed (n = 6)	0.49 ± 0.04	0.18 ± 0.03	0.11 ± 0.03
Control stressed (n = 6)	0.53 ± 0.05	0.17 ± 0.03	0.08 ± 0.01
Alcoholic unstressed (n = 6)	0.59 ± 0.06*	0.15 ± 0.04	0.10 ± 0.02
Alcoholic stressed (n = 4)	0.68 ± 0.07*	0.16 ± 0.02	0.05 ± 0.02*

Values are means ± SEM.
*Significantly different from unstressed control values, $p < 0.05$.

trol and ethanol-treated rats at a similar rate and to a similar extent above that of the unstressed groups. The finding that the alcoholic stressed animals had higher systolic pressures than the alcoholic unstressed animals suggested an additive effect of stress and chronic ethanol consumption in elevating the blood pressures of these animals. As the blood volumes of the stressed animals were similar to their unstressed counterparts, our results imply that the additional elevation in blood pressure seen in both stressed groups of animals is not the result of plasma volume expansion. Plasma norepinephrine levels of the four groups showed the same stratified pattern as their blood pressure values, which suggests that the additional hypertensive effects of heat stress on the ethanol-treated and control animals is associated with elevated plasma norepinephrine levels. If one assumes that plasma levels of norepinephrine reflect the overall sympathetic nervous activity, then long-term heat stress may increase the sympathetic tone of the animals and therefore contribute to the elevated blood pressure observed in the stressed animals. Our findings are consistent with previous clinical studies in which increased urinary excretion of catecholamines was detected during short-term[16, 17] and long-term[18] ethanol consumption in patients with stressful routines. We do not know whether heat irradiation of our animals altered adrenal steroid secretion, which is sometimes used as a measure of stress.

The reason for the reduced dopamine level in the plasma of the alcoholic stressed rats is not immediately apparent but one can speculate on the basis of previous reports. Darden and Hunt,[19] as well as Major et al.,[20] reported depressed dopaminergic activity in the brains of rodents[19] and humans[20] during withdrawal from chronic ethanol ingestion. Metabolism of other neurotransmitters also has been found to be altered after short-term and long-term ethanol treatments.[21] Unfortunately, we did not measure brain dopamine levels in these rats, but it is possible that stress interacts with chronic ethanol consumption in the alcoholic stressed animals to produce withdrawallike effects. The observations that the alcoholic stressed animals generally were more irritable and had a higher incidence of seizures and higher mortality seem to support this hypothesis.

Earlier results from our laboratory indicated that compared with controls the ethanol-treated rats had cardiac function abnormalities.[7] We found that the mean resting heart rate of the alcohol-treated rats was significantly lower than that of the control animals. In the present study, heat stress elevated the heart rate of the control animals by 11% (see Table 1, unstressed versus stressed controls). The heart rate of the ethanol-stressed animals was 14% greater than that of the alcoholic unstressed animals, but the heart rate of alcoholic unstressed animals was significantly less than that of control unstressed rats. These results suggest that chronic ethanol treatment and heat stress affects the heart rate in an opposing manner. The stressed animals had elevated heart rates, probably as a result of increased sympathetic nervous activity, while long-term ethanol treatment lowered the heart rate because of the ensuing conduction and other cardiac abnormalities.[7] This is a viable explanation for the finding that the heart rate of the alcoholic stressed animals was not different from that of control unstressed rats. It is also possible that the conduction and other cardiac abnormalities in the stressed alcoholic animals may have rendered the hearts of the rats less resistant to the detrimental effects of increased sympathetic drive, which resulted in the high mortality observed.

The findings reported here may have important clinical implications regarding both the mechanisms of hypertension and the analysis of risk factors in cardiovascular and alcohol-related morbidity and mortality.

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References

1 Joske R, Turner CN Studies on chronic alcoholism: 1 The clinical findings in 78 cases of chronic alcoholism. Med J Aust 1952;22.729–734
2 Clark VA, Chapman JM, Coulson AH. Effects of various factors on systolic and diastolic blood pressure in the Los Angeles heart study. J Chronic Dis 1967;20·571–581
3 Chan TCK, Sutter MC. Chronic ethanol consumption and blood pressure. Life Sci 1983;33.1965–1973
4 Barboriak JJ, Rimm AA, Anderson AJ, Schmidhoffer M, Tristani FE. Coronary artery occlusion and alcohol intake. Br Heart J 1977;39 289–293
5 Celentano DD, Martinez RM, McQueen DV. The association of alcohol consumption and hypertension Prev Med 1981; 10:590–602
6. Wallace RB, Lynch CF, Pomrehn PR, Criqui MH, Heiss G. Alcohol and hypertension: epidemiologic and experimental considerations· the lipid research clinic program Circulation 1981,64(suppl III):41–47
7. Chan TCK, Sutter MC. The effects of chronic ethanol consumption on cardiac function in rats. Can J Physiol Pharmacol 1982,60.777–782
8. Davies GC, Kissinger PT. Strategies for determination of serum or plasma norepinephrine by reverse phase liquid chromatography. Anal Chem 1981;53:156–159
9. Woodbury DM. Physiology of body fluids. In: Ruch TC, Patton HD, eds Physiology and biophysics; vol 2. Philadelphia: Saunders, 1973:450–479
10 Forsyth RP, Harris RE. Circulatory change during stressful stimuli in rhesus monkeys. Circ Res 1970:26,27(suppl 1): 13–20
11 Herd JA, Morse WH, Kelleher RT, Jones LG. Arterial hypertension in the squirrel monkey during behavioral experiments. Am J Physiol 1969;27:24–29
12. Brod J Neural factors in essential hypertension. In: Zanchette A, ed. Neural and psychological mechanisms in cardiovascular disease. Milan: Il Ponte, 1972:93–108
13. Editorial. Stress, hypertension and the heart: the adrenaline trilogy. Lancet 1982,2(8313).1440–1441
14. Klatsky AL, Friedman GD, Siegelaub AB. Alcohol use, myocardial infarction, sudden cardiac death, and hypertension. Alcoholism: Clin Exp Res 1979;3:33–39
15. Klatsky AL, Friedman GD, Siegelaub AB, Gerard MJ. Alcohol consumption and blood pressure: Kaiser-Permanente Multiphasic Health Examination data. N Engl J Med 1977; 296:1194–1197

16. Perman ES. The effect of ethyl alcohol on the secretion from the adrenal medulla in man. Acta Physiol Scand 1958;44: 241–247
17. Anton AH. Ethanol and urinary catecholamines in man. Clin Pharmacol Ther 1965;6:462–469
18. Ogata M, Mendelson JH, Mello NK, Majchrowicz E. Adrenal function and alcoholism. II. Catecholamines. Psychosom Med 1971;33:159–180
19 Darden JH, Hunt WA. Reduction of striatal dopamine release during an ethanol withdrawal syndrome. J Neurochem 1977; 29:1143–1149
20. Major LF, Ballenger JC, Goodwin FK, Brown GL. Cerebrospinal fluid homovanillic acid in male alcoholics: effects of disulfiram Biol Psychiatry 1977;12:635–642
21. Hunt WA Neurotransmitter function in the basal ganglia after acute and chronic treatment. Fed Proc 1981,40(7):2077–2081