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Original Article

EFFECT OF ETHANOL ON STRESS INDUCED CARDIOVASCULAR ALTERATIONS IN RATS

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Stress is an inevitable part of life of every living organism. Alcohol has been used and abused for the simple belief of its beneficial effects towards stress suppression. A tension reduction hypothesis of ethanol was not conclusively confirmed for its beneficial effects on different organ system. The present study elucidated the beneficial/adverse effects of two doses ethanol on experimental stress induced alterations of cardiovascular parameters in rats. Male Wistar rats were divided into different control and stress groups (n=10 per group). Stress animals were exposed to isolation or immobilization stress for 7, 15, and 30 days. In separate groups, ethanol (1 and 2 g/Kg body weight) were treated daily once to the isolated groups of rats and 30 minutes prior to the 1-hour immobilization stress group. Vehicle control and ethanol alone without stress (unstressed groups) were simultaneously maintained. At the end of scheduled duration, rats were anesthetized and lead II ECG was continuously recorded. After exsanguinated, blood samples were collected for the estimation of serum SGOT and SGPT levels, the heart and adrenal gland were then removed and weighed. All stress groups exhibited a significant increase in heart and adrenal gland weights, heart rate, and serum SGOT and SGPT ($p<0.05$). Ethanol further enhanced these stress-induced effects in a dose-dependent manner and few cases stress adaptation effects were nullified with the increased duration of stress. This study suggests that chronic ethanol consumption exacerbates the cardiovascular response to stress in a dose-dependent manner.

Key words: isolation, immobilization, stress, ethanol

Stress is a nonspecific response of the organism to demanding internal and external conditions. Hans Selye, the father of stress theory, defined it as 'an exaggerated systematic biological response of an organism to any provoking condition' (Selye, 1936). Stress, a nonspecific response of the body to any demand, is either physical or psychological in nature. It not only needs an environmental event but also involves an individual/organism's unique reaction to the putative stressor (Lazarus and De Longis, 1983). It results in the alteration of biology including major changes in the biochemistry, behavior, and physiology (Vogel, 1993). Hypothalamo-pituitary-adrenal axis is the major central nervous system response to stress by releasing corticotrophin releasing hormone and corticosteroids into the circulation (Dunn

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and Berridge, 1990; Irwin et al., 1990). The cardiovascular system is one of the major systems that are adversely affected by stress.

Alcoholic beverages have been used or abused since the dawn of history and the idea that alcohol decreasing severe anxiety dates back to antiquity. Hippo crates prescription was, "Wine drunk with an equal quantity of water puts away anxiety and terrors" (Hippo crates, 1886). Interaction of stress and ethanol was first suggested by Horton (1943) and further, elaborated by Bales (1946). Masserman and Yum (1946) –reported the improvement of stressed cats treated with ethanol. Acute and chronic types of stressors have proved to be disastrous to the cardiovascular system in experimental animals and human beings (Nagaraja et al., 1999; Ramachandruni et al., 2004). Stress experience has been known to induce alcoholism. However, literature survey reveals a contradicting note on the cardiovascular effect of alcohol; i.e., some report its beneficial effect (Levenson et al., 1980) while others suggest its adverse one (Beilin, 1995). To date, studies highlighting the effects of ethanol on stress induced cardiovascular parameters are limited. Thus, the objective of the present study was to elucidate the beneficial/adverse effects of two doses of ethanol on experimental stress induced cardiovascular parameters in rats.

Materials and Methods

In the present study, healthy inbred adult male albino rats of Wistar strain were used. Rats were used as per the Institutional ethical guidelines. They were housed in metallic cages (4-5 rats per cage) and free access to food and water ad libitum. They were separated into several different groups including control, isolation stress, and immobilization stress with or without ethanol treatment (Et). Several subgroups were as followed: isolation stress for 7 days (Iso-7d), 15 days (Iso-15d), and 30 days (Iso-30d); control with ethanol (1 g/kg or 2 g/kg) for 7 days (ET-1 g or 2 g-7d), 15 days (Et-1g or 2g-15d), and 30 days (ET-1g or 2g-30d); 1g/kg body weight ethanol + 7 days Iso, 15 days Iso, and 30 days Iso; 2 g/kg body weight ethanol + 7 days Iso, 15 days Iso, and 30 days; immobilization stress for 7 days (imm-7d), 15 days (imm-15d), and 30 days (imm-30d) and 1 g or 2 g/kg body weight ethanol + 7 days, 15 days, and 30 days immobilization. Two doses of ethanol (1 g and 2 g per kg body weight) were selected based on previous studies (Pohorecky 1981, 1990).

Isolation stress

Rats were individually housed in a special isolation cage that prevents any external contact by covering each cage with black thick paper. Durations of isolation stress were varied as aforementioned protocol. Ethanol (1g or 2 g per kg body weight) was intraperitoneally injected once daily in the morning (at 10.00 AM).

Immobilization stress

Rats were immobilized one hour per day by using a specific immobilization board. In the ethanol treated immobilized groups, ethanol was intraperitoneally injected 30 min prior to the immobilization stress.

At the end of each study, rats were anaesthetized with sodium pentobarbitone (40mg/kg, Sigma) and their ECG was recorded using lead II. Blood was collected after exsanguinations and the heart and adrenal glands were carefully removed and weighed. Serum samples were used for the estimation of serum glutamate oxaloacetate transaminases (SGOT or AST) and serum glutamate pyruvate transaminases (SGPT) levels by standard methods. ECG was analyzed for heart rate and P-R interval calculation.

Statistical analyses

Data were expressed as mean ± SEM. Comparison of data was done statistically by using one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. $P<0.05$ indicates a significant difference.

Results

There was a significant increase in heart weights following 7 days isolation stress ($P<0.05$); but following 15 and 30 days stress exposure; they were not significantly different from the control group (Table 1). The cardiac hypertrophic effect of isolation stress was significantly amplified by any chronic ethanol treatment. However, ethanol alone without any stress also significantly increased in the heart weight ($P<0.05$, 0.01, Table 1). Weights of the adrenal gland significantly increased following either isolation stress alone or stress with ethanol consumption ($P<0.05$, 0.01, Table 1). Heart rate significantly increased following isolation with ethanol. In few cases, ethanol treatment resulted in nullification of stress-induced adaptation over time (30 days isolation). There was no change in P-R interval following isolation with ethanol treatment. Generalized tissue damage was confirmed by marker enzyme levels, SGOT and SGPT, which their serum levels significantly elevated following either isolation stress with ethanol or ethanol treatment alone ($P<0.01$, Table 1).

Table 1. Isolation stress and ethanol

Treatment (n =10 each)	Heart weight (g/100g)	Adrenal gland weight (g/100g)	Heart rate (bpm)	P-R interval (sec)	SGOT (IU/L)	SGPT (IU/L)
Control	0.319±0.003	0.005±0.001	363±8	0.047±0.003	0.648±0.02	0.58±0.03
Control ET-1g-7d	0.342±0.009*	0.009±0.001**	394±10**	0.048±0.003	6.80±0.46**	2.91±0.2**
Control ET-2g-7d	0.374±0.01**	0.011±0.001**	376±9**	0.044±0.003	4.9±0.13**	4.95±0.3**
Iso-7d	0.343±0.012*	0.011±0.001**	398±18**	0.044±0.003	9.59±0.94**	4.34±0.45**
1gET+Iso7d	0.324±0.016	0.01±0.001**	343±13	0.042±0.002	4.22±0.2**	4.92±0.25**
2gET+Iso7d	0.366±0.012**	0.013±0.001**	460±11**	0.047±0.003	11.13±0.94**	4.29±0.49**
Control ET-1g –15d	0.357±0.011*	0.012±0.002**	394±9**	0.044±0.003	4.7±0.21**	2.9±0.34**
Control ET-2g-15d	0.346±0.011*	0.013±0.001**	430±7**	0.044±0.003	10.24±0.62**	11.63±0.52**
Iso-15d	0.300±0.01	0.009±0.0003**	355±18	0.042±0.002	9.68±0.54**	7.68±0.68**
1gET+Iso15d	0.377±0.014**	0.009±0.001**	353±17	0.04±0.002	11.82±057**	7.004±0.24**
2gET+Iso15d	0.323±0.019	0.012±0.002**	385±9**	0.042±0.002	7.4±0.09**	3.44±0.1**
Control ET-1g-30d	0.369±0.008**	0.011±0.002**	379±8**	0.044±0.003	7.1±0.12**	2.73±0.08**
Control ET-2g-30d	0.343±0.01	0.011±0.001**	390±8**	0.044±0.003	11.6±0.34**	8.65±0.16**
Iso-30d	0.325±0.006	0.008±0.001*	345±6	0.05±0.003	3.7±0.14**	10.10±0.40**
1gET+Iso30d	0.337±0.01*	0.01±0.001**	310±22	0.048±0.003	1.60±0.1**	3.12±0.11**
2gET+Iso30d	0.379±0.02**	0.012±0.001**	363±3	0.042±0.002	8.53±0.39**	4.10±0.05**

Values are Mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ when compared to their corresponding controls.

Immobilization stress with or without ethanol for 7, 15, and days significantly increased heart weight, heart rate, and serum SGOT and SGPT ($P<0.05$, 0.01, Table 2). Ethanol treatment alone also increased significantly heart weight, adrenal gland weight, heart rate, and serum enzyme levels ($P<0.05$, 0.01, Table 2).

Table 2. Immobilization stress and ethanol

(N=10 rats, each group)	Weight of heart (g/100g)	Weight of adrenal (g/100g)	Heart rate (Per min)	P-R interval (Sec)	SGOT (IU/L)	SGPT (IU/L)
Control	0.319±0.003	0.005±0.001	363±8	0.047±0.003	0.648±0.02	0.58±0.03
Control ET-1g-7d	0.342±0.009**	0.009±0.001**	394±10**	0.048±0.003	6.80±0.46**	2.91±0.2**
Control ET-2g-7d	0.374±0.01**	0.011±0.001**	376±9**	0.044±0.003	4.9±0.13**	4.95±0.3**
Imm-7d	0.378±0.006**	0.014±0.001**	430±11**	0.042±0.002	2.82±0.14**	3.77±0.48**
1gET+Imm7d	0.404±0.012**	0.013±0.001**	420±14**	0.040±0.001	5.34±0.27**	6.99±0.17**
2gET+Imm7d	0.341±0.008**	0.013±0.001**	405±12**	0.040±0.003	4.27±0.13**	4.34±0.15**
Control ET-1g –15d	0.357±0.011**	0.012±0.002**	394±9**	0.044±0.003	4.7±0.21**	2.9±0.34**
Control ET-2g-15d	0.346±0.011**	0.013±0.001**	430±7**	0.044±0.003	10.24±0.62**	11.63±0.52**
Imm-15d	0.366±0.016**	0.010±0.001**	345±18	0.044±0.003	6.77±0.69**	4.57±0.45**
1gET+Imm15d	0.361±0.008**	0.011±0.001**	440±7	0.04±0.001	4.23±0.22**	3.634±0.21**
2gET+Imm15d	0.367±0.005**	0.013±0.001**	410±12**	0.048±0.003	4.27±0.13**	2.04±0.16**
Control ET-1g-30d	0.369±0.008**	0.011±0.002**	379±8**	0.044±0.003	7.1±0.12**	2.73±0.08**
Control ET-2g-30d	0.343±0.01**	0.011±0.001**	390±8**	0.044±0.003	11.6±0.34**	8.65±0.16**
Imm-30d	0.335±0.01*	0.009±0.001**	393±9**	0.04±0.001	3.52±0.14**	5.31±0.11**
1gET+Imm30d	0.353±0.007**	0.011±0.001**	405±10**	0.04±0.002	7.99±0.25**	4.36±0.2**
2gET+Imm30d	0.376±0.009**	0.013±0.001**	398±1**	0.04±0.001	4.16±0.37**	3.57±0.21**

Values are Mean ± SEM. *$P<0.05$, **$P<0.01$ when compared to their corresponding controls.

Discussion

"Stress" is an inevitable part of daily life that produces numerous biochemical, neuroendocrine, and physiological changes in an organism (Vogel, 1993). Stressors have frequently been cited as factors contributing to the development of alcoholism in humans. Ethanol is postulated to have anxiety-reducing (anxiolytic) properties (Pohorecky, 1981). The notion that ethanol consumption can reduce stress is taken for granted by most people. Tension or stress reduction hypothesis of ethanol postulated by previous researchers has not been conclusively proved or disproved. Despite intensive research, alcoholism remains one of the oldest and most prevalent health afflictions of humanity (Pohorecky, 1981). The present study indicates that ethanol consumption has deleterious effects on the cardiovascular system and exacerbates stress-induced cardiovascular responses in rats. The cardiovascular system is one of the most vulnerable systems to insults of stress and ethanol. In 1884, Bollinger reported cardiac enlargement in autopsies of persons who had habitually consumed excessive quantities of alcohol (Rubin and Thomas, 1992). Cardiac hypertrophy following chronic stress observed in the present study was similar to previous

reports (Nagaraja et al., 1999). The increased heart weight following both isolation and immobilization stress may be a consequence of an excessive stimulation of the hypothalamo-pituitary-adrenal axis (Nagaraja et al., 1999).

An increase in the adrenal gland weight supports this notion (Spencer and McEwen 1990; Berman et al., 1990). Stress-induced heart and adrenal gland hypertrophy was exacerbated by chronic ethanol treatment. Previous reports suggested that ethanol treatment increased sarcoplasmic reticulum, transverse tubules, mitochondrial volume, and tubular structures in myocardium (Jaatinen et al., 1994; Ahmad, 1995).

Stress of any kind has been proved harmful to the cardiovascular system: various psychological and social stress factors in human beings were found to be the prevailing factors for promotion and development of heart disease (Ramachandruni et al., 2004). Emotional stress like immobilization with or without cold exposure was known to affect the cellular integrity in heart tissue (Sanchez et al., 2002). A significant increase in the serum SGOT and SGPT following stress in the present study is an indication of generalized tissue injury and further SGOT is a more specific marker for disruption of cell integrity of the cardiac musculature (Fontes et al., 1999). Thus damage of cardiac cells by stress exposure and leakage of these enzymes into the blood is more likely. Similar observations were done by Sanchez et al. (2002) in mice following emotional stress.

Ethanol treatment with or without stress in the present study mimicked a stressor response. In most of the cases, ethanol nullified the stress adaptation responses. Heart and adrenal gland weights were accentuated by the treatment of ethanol. Heart rate and serum enzyme levels also exhibited a significant increase following alcohol treatment alone. Myopathic state of the heart due to alcoholism, namely alcoholic cardiomyopathy is manifested by cardiac hypertrophy (Aberle and Ren, 2003). Several mechanisms have been postulated for this cardiomyopathy including oxidative damage, trigylceride accumulation, altered fatty acid extraction, and impaired protein synthesis (Aberle et al., 2003). A primary candidate for the specific toxins of ethanol is its major metabolic products (acetaldehyde and fatty acid ethyl esters) that directly impairs cardiac function by promoting lipid peroxidation and resultant oxidative damage (Aberle et al., 2003).

In the present study, both the doses of ethanol mimic or accentuate the stress-induced alteration in cardiovascular parameters. The present data suggest that ethanol is a stress-mimicking and enhancing agent in a dose-dependent manner with reference to the cardiovascular parameters. Simultaneous estimation of stress hormones and catecholamine levels may add further information to substantiate our findings.

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