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Effect of Ethanol and Thermal Stresses on the Social Behaviour of Male Mice

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ABSTRACT

The effect of ethanol and thermal stresses given simultaneously to male albino mice was studied using social behaviour as the modified assessment parameter in a standard opponent test. The mice were exposed in groups to 0, 10, 20, 30 and 40±2°C for 24 h before subjecting them to behavioural test. The animals were injected with 99% ethyl alcohol at the doses of 1 and 3 g kg^{-1} body weight (i.p.), 24 h after thermal stress. The behavioural activities recorded at 20±2°C were considered as the control activity at normal temperature. All animals were subjected to a modified standard opponent test and their social and non-social behaviour activities were observed and recorded in a thermostatically controlled chamber at all specified temperatures and ethanol exposures. Social activities included durations of threat, attack, fights, defense, naso-nasal and naso-genital contacts whereas the nonsocial behaviour included the durations of elements like wall rears, rears and displacement. These tests showed a significant decrease in all elements of behaviour at low temperatures whereas at high temperatures the effect appeared to be of biphasic nature with an increase followed by a decrease in activities as compared with the controls. Ethanol intoxication inflicted an inhibitory effect on all acts and postures in a dose-dependent manner at all thermal stress. These observations are discussed in the light of the thermal regulatory mechanism in the body and the possibility of studying these effects in perspective of the effects of thermal and alcohol stresses causing injuries in humans.

Key words: Thermal stress, ethanol stress, social behaviour, standard opponent test, male mice, thermoregulation

INTRODUCTION

The normal homeostasis inside a mammalian body is maintained through its normal behavioural and physiological activities. However, stresses of different kinds like physical (environmental) and chemical stresses are important factors and may influence the behaviour and physiology of the mammalian body (Sutoo and Akiyama, 2002). For non-mammalian species also, temperature is one of the most encountered stressful factors in the environment and it affects the biological consequences due to thermoregulatory response and adaptation (Ahmed, 2006). The relationship between various types of stress and catecholamines in the Central Nervous System (CNS) has been extensively investigated in mice and rats (Hata *et al.*, 1991; Hernandez *et al.*, 1986; Sutoo *et al.*, 1991; Sutoo and Akiyama, 2002).

Today, the injuries and adverse effects of heat stress is not only a sports (Bailes *et al*., 2002) and military (Bricknell, 1996) medical problem but, as exemplified by the recent high death toll in France (Dorozynski, 2003) is also a public health issue that may escalate with global warming (Tong *et al*., 2008). Animal models that permit identification of the mechanisms that contribute to heat injury or support heat stress recovery are needed the most to address this potential threat (Leon *et al*., 2005).

Furthermore, ample evidence exist on the effect of various chemical agents like amphetamine, sodium pentobarbital, ethyl alcohol, noradrenaline etc. on the behavioural thermoregulatory activities of rodents by affecting the set point for the control of body temperature through behavioural adjustments (Strek *et al*., 1986; Gordon and Stead, 1988; O'Connor *et al*., 1989; Klein *et al*., 1992; Moshkin *et al*., 1993; Leon *et al*., 1998). Acute administration of ethanol (EtOH) induces hypothermia in rodents maintained in a room-temperature environment (Brownman *et al*., 2000). Ethanol is known to reduce the ability to maintain normal body temperature under cold conditions (Huttunen *et al*., 1998) and it is possible that ethanol-induced hypothermia may involve decreased heat production in addition to increased heat loss (Huttunen *et al*., 1998). Normothermia is maintained by an animal through the thermoregulatory set point in the body (Klein *et al*., 1992), located possibly in the basal forebrain area i.e., the medial preoptic area of the hypothalamus of the rats (Kent *et al*., 1991). Thus, animal studies may provide useful biological insights for a better understanding of human alcohol effects (De Witte *et al*., 2003).

Our main objective in the present study was to determine the social behavioural responses to a range of thermal and ethanol stresses elicited in conscious, unrestrained, freely moving mice that were able to use their behavioral and autonomic thermo effectors to survive and respond to exposure to a variable range of hot and cold environment under controlled doses of ethanol intoxication. Although the present study may be unique to mice or animals of small body mass, elucidation of its mechanisms may reveal aspects of strategy that can be exploited for the prevention and treatment of human thermal and ethanol stress injuries (Leon *et al*., 2005).

MATERIALS AND METHODS

Experimental animals: Male Swiss-Webster strain albino mice weighing 25-30 g and aged 9-10 weeks, were obtained from the animal facility of College of Pharmacy. The mouse were housed in opaque plastic cages (four males per cage) measuring 30×12×11 cm, in the animal facility of the Zoology Department, King Saud University, Riyadh, Saudi Arabia. Animals were kept under reversed lighting conditions with white lights on from 22:30 to 10:30 h local time. The ambient temperature was regulated at 21±1°C. Food (Pilsbury's Diet) and water were available *ad libitum*, except during the experimental sessions when the animals were under visual observations in the thermostatically controlled chambers (Ajarem and Ahmad, 2003). All handlings of the experimental animals and procedures regarding animal care were carried out in compliance with the regulations dictated by the Experimental Animal Care and Use Committee of the Facilities for Experimental Animals of King Saud University. This study was conducted in the Department of Zoology, King Saud University, Riyadh, from September 2006 to September 2007.

Standard opponent test: Eight male subjects were individually housed in fresh cages for 14 days. After this isolation period, these male offspring (test animals) from each experimental category were subjected to standard opponent tests under dim red lighting (ca. 8 lux) as described elsewhere (Ajarem and Brain, 1993) but in a modified manner. The docile and age-matched male

standard opponents were rendered anosmic by applying 25 μL of 4% zinc sulphate solution to the nasal tract under ether anaesthesia for three days prior to encounters (Brain *et al.*, 1981). The anosmic standard opponent intruders were introduced in the home cages of the test animals and the standard opponent test of each test animal was observed visually for 500 sec for only the duration for which the test animal expressed for attack, threat, defense, rear, wall rear etc. Basically the standard opponent test measures aggressive behavior of the test animals in the form of number of attacks, number of threats, number of fights etc. but in the present study all elements of behavior were observed for the duration for which the test animal displayed the observed elements of behavior. This modification has been attempted to simplify the results for presentation and for statistical analyses of all elements of behavior categorized under Social and Nonsocial behavior. The opponents were used only once and the selected elements of behaviour were studied as described elsewhere (Brain *et al.*, 1987; Ajarem and Ahmed, 1991).

Thermal stress protocol: The test animals were exposed to various experimental thermal stress in a large (room sized), thermostatically, light, humidity and fresh air regulated automatically controlled incubator chamber. The temperature of this large chamber was capable of being regulated between a range of 0-60°C. All test animals were transferred in their original cages from the animal facility (20±2°C) to the large incubator chamber which was always initially maintained at 20±2°C. Food and water were available *ad libitum* in the chamber also except during the behavioural observations. Twenty four hours before the commencement of the experimental recording at a specified thermal stress, the temperature of the chamber was set to 0, 10, 20, 30 or 40±2°C. It took approximately 30 min to stabilize the chamber at a required temperature. Thus, the animals were actually exposed to various stabilized temperature for approximately 24 h. The standard opponent animals and the person responsible for conducting the behavioural observations entered the chamber 30 min before the beginning of the recording session at a particular stabilized temperature point. Optimum personal care and protection was taken by the observer from the thermal stress inside the chamber such that the observer remained normal and uninfluenced throughout the observation period.

Ethanol stress protocol: All test animals acclimatized for 24 h at different thermal stress protocol, were exposed to ethanol stress by injecting (i.p.) 99% ethyl alcohol, at the doses of 1 and 3 g kg^{-1} body weight in an injectable volume of 0.5 mL, 30 min before the commencement of the behavioural observations. The control animals were injected with equal volume of distilled water whereas the intruders standard opponent animals were not injected with anything.

Statistical analysis: The data of Standard opponent tests were statistically analyzed using Mann-Whitney U test (Sokal and Rohlfe, 1981) and compared within the experimental groups by Analysis of Variance (ANOVA) using Graph Pad Instat computer programme.

RESULTS

The results of the present study clearly illustrate that all behavioural elements observed at normal control temperature (20±2°C) are drastically and significantly altered at all thermal stress both below as well as at above the normal (control) temperature. Intoxication of animals with ethanol stress, before subjecting to thermal stress, had an inhibitory effect on all behavioural elements in a dose-dependent manner even in the control group (20±2°C). Furthermore, ethanol stress exacerbated the thermal effects in a dose-dependent manner and the pattern of effects at all temperature stress remained almost similar.

Table 1: Effect of thermal and ethanol stresses in male albino mice on their nonsocial behaviour in a modified Standard Opponent test

Ethanol[+] doses (g kg^{-1} b.w.)	Median duration (sec) with ranges of Nonsocial activities[xx] at different thermal stress±2°C				
	0	10	20[#]	30	40
0	27.2**	208.7 *	251.6	357**	196.2*
(Control)	(12-423.9)	(20-508)	(87- 502)	(121-423)	(191-207)
1	59.2[a]	78[a]	170[a]	235.1[a]	127[a]
	(8-120)	(32-213)	(56-339)	(116-487)	(48-270)
3	9.7[b]	33.1[b]	77.2[b]	130[b]	62[b]
	(0-52.6)	(14-197)	(41-212)	(23-331)	(18-259)

[xx] Nonsocial activities included behaviours like wall rears, rears and displacement. [+] Ethanol was injected i.p. 30 min before the behavioural readings. # Normal temperature with control readings. Animals were acclimatized for 24 h at each thermal point before observing the behavioural response. * and ** illustrates statistically significant at $p<0.05$ and $p<0.005$ as compared to the control by Mann-Whitney U-test. [a] and [b] illustrates statistically significant at $p<0.05$ and $p<0.005$ as compared to the control by ANOVA between the groups in a dose-dependent manner

Table 2: Effect of thermal and ethanol stresses in male albino mice on their social behaviour in a modified Standard Opponent test

Ethanol[+] doses (g kg^{-1} b.w.)	Median duration (sec) with ranges of Social activities[xx] at different thermal stress ±2°C				
	0	10	20#	30	40
0	44.3**	108.9 *	134	257**	88.6*
(Control)	(0-351.5)	(48-315)	(73- 414)	(121-423)	(82.3-294)
1	11.4[a]	51[a]	96.8 [a]	129.1[a]	69[a]
	(0-600)	(27-408)	(36-221)	(64-387)	(28-189.5)
3	6.8[b]	43.6[b]	25.9[b]	73[b]	15.6[b]
	(0-421.5)	(19-243)	(14-142)	(11-210)	(4-59.1)

[xx] Social activities included behaviours like threat, attacks, fights, defense, naso-nasal and naso-genital contacts. [+]Ethanol was injected i.p. 30 min before the behavioural readings. # Normal temperature with control readings. Animals were acclimatized for 24 h at each thermal point before observing the behavioural response. *and** shows statistically significant at $p<0.05$ and $p<0.005$ as compared to the control by Mann-Whitney U-test. [a] and [b] shows statistically significant at $p<0.05$ and $p<0.005$ as compared to the control by ANOVA between the groups in a dose-dependent manner

The duration of nonsocial activities expressed by the mice at temperatures lower than the control level, decreased significantly and this decrease was proportional to the temperature, i.e., $p<0.05$ and $p<0.005$ at 10 and 0±2°C, respectively. However, at temperatures higher than the control level, the nonsocial activities expressed by the animals were somewhat biphasic. Initially, the activities increased significantly ($p<0.5$) at 30±22°C but thereafter it decreased significantly ($p<0.05$) at 40±2°C as illustrated in Table 1. The nonsocial activities expressed in ethanol intoxicated mice at different temperatures followed a similar pattern of activity but at a lower inhibited level and the inhibition was dose-dependently significant (Table 1).

The results of the social behaviour also followed a similar pattern as for the nonsocial activities in a dose-dependent manner (Table 2). The duration of social activities expressed by the mice at temperatures lower than the control level, decreased significantly and this decrease was proportional to the temperature, i.e., $p<0.05$ and $p<0.005$ at 10 and 0±2°C, respectively. However, at temperatures higher than the control level, the social activities expressed by the animals were also somewhat biphasic where initially, the activities increased significantly ($p<0.5$) at 30±2°C but thereafter it decreased significantly ($p<0.05$) at 40±2°C as illustrated in Table 1. Further more addition of ethanol stress further aggravated the effects significantly in a dose-dependent manner (Table 2).

DISCUSSION

The present results overall demonstrate clearly that mice do not exhibit a normal social and nonsocial behaviour when exposed to various thermal and ethanol stresses. Considering all social and nonsocial activities at 20±2°C, as the normal or control level and naming it as normothermia, it is observed that hypothermal stresses with ethanol intoxication inflicted a decreased social and nonsocial behaviour in a dose-dependent manner and the mice preferred to remain inactive by showing immobility for longer duration of time. On the other hand, hyperthermal stresses with ethanol intoxication, inflicted somewhat a biphasic effect in a dose-dependent manner. At 30±2°C, the animals illustrated increased activities and thereafter at 40±2°C, the responses dipped to low levels, where the animals become hypoactive by showing a decrease in various social and nonsocial activities and remaining stationery, possibly conserve the body energy due to adverse thermal and chemical (ethanol) stresses. Such biphasic thermoregulatory response due to thermal stress has been reported in mice (Leon *et al*., 2005) and support the present findings. It is well known that a thermoregulatory set point is present in the rodent body and it acts around the normothermia (Klein *et al*., 1992). Any change in the external environment (hypothermia or hyperthermia) might be resulting in the altered thermoregulatory set point, which ultimately compels the animals to regulate its normothermia by changing its behavioural thermoregulatory and/or autonomic thermoregulatory activities (Gordon and Stead, 1986, 1988). These evidences clearly support the present findings. Data of previous studies have illustrated that behavioural thermoregulation is more efficient in minimizing a thermal load than the autonomic thermoregulation (Ajarem and Ahmad, 2003). It is possible that increased social and nonsocial behavioural activities at 30°C cause increased blood circulation and the peripheral vasodilatation causes increased body heat loss as suggested by Kent *et al*. (1991). However, further increase in thermal stress to 40°C incapacitates the animals and compels them render lower expressions of social and nonsocial behaviour response in presence of intruder, so as to conserve their energy for adapting themselves to the adverse stresses. The lower thermal stresses, on the other hand, decrease the social and nonsocial behavioural responsive activities possibly to conserve body heat by remaining inactive for most of the time. In either case, it appears that animals prefer to maintain their normothermia under all challenging thermal stress conditions through behavioural performances and presence of an intruder (opponent) makes differences in only a narrow range of thermal stress.

The present results also illustrate that ethanol stress inflicts a depressive effect on behavioural acts and postures at all thermal stress point. It is known that γ-aminobutyric acid (GABA) receptor A has profound effect on receptor pharmacology (Ebert *et al*., 1997) suggesting the possibility that behavioural sensitivity to ethanol may depend on these receptors in specific brain circuits (Boehm *et al*., 2004). Administration of ethanol (EtOH) induces hypothermia in rodents maintained in room-temperature environment (Brownman *et al*., 2000) it is possible that ethanol-induced hypothermia may involve decreased heat production in addition to increased heat loss (Huttunen *et al*., 1998). However, it should be realized that ethanol is not a molecule with a single clear effect on a particular neurotransmitter system but it may affect multiple stages of the neurotransmission cascade of the large majority of neurotransmitters (De Witte *et al*., 2003). The relationship between various types of stress and catecholamines in the Central Nervous System (CNS) has been extensively investigated (Sutoo and Akiyama, 2002). It has been further elucidated that stress-dependent changes enhances serum and brain calcium levels which in turn enhances dopamine (DA) synthesis in the brain, inducing physiological as well as behavioural changes (Sutoo and Akiyama, 2002). The discussed literature directly and indirectly supports the present

findings, however, further studies on the present lines including neurotransmitters and calcium levels in brain tissue may reveal aspects of strategies that could be exploited for the prevention and treatment of human stress injuries.

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