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HYPOTHALAMIC CATECHOLAMINES AND TOLERANCE FOR SEVERE COLD IN ETHANOL-TREATED GUINEA-PIGS

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1 The effect of ethanol (2 g/kg) on hypothalamic catecholamines in guinea-pigs kept at room temperature (20°C) and in severe cold (−20°C) for 1.5 h was determined. Serum glucose, triacylglycerols and free fatty acids (FFAs), glycogen in liver and skeletal muscle and total lipid and triacylglycerols in the interscapular adipose tissue were also determined.

2 Ethanol increased the noradrenaline and adrenaline content of the hypothalamus at 20°C and reduced the rectal temperature by about 2°C. The hypothalamic noradrenaline content of the ethanol-treated guinea-pigs exposed to cold, in which the fall in rectal temperature was about 8°C, was higher than in the controls, whose rectal temperature decreased only by about 2°C.

3 Cold exposure increased FFA concentration in serum and reduced skeletal muscle glycogen and serum glucose concentrations in both groups, but no significant differences were found in the carbohydrate and lipid values between the groups at −20°C.

4 It is possible that the diminished cold tolerance in the ethanol-treated guinea-pigs might be due, at least in part, to the effect of ethanol on the catecholamines in the hypothalamus.

Introduction

It is commonly stated that ethanol reduces the ability to maintain the normal body temperature under cold conditions, an effect which is dependent on the alcohol dose and the severity of the cold exposure. Small and moderate doses have not been found to have any harmful effects in man under conditions of mild cold exposure, whereas in more severe cold (15°C) a moderate intake of alcohol has resulted in insufficient metabolic compensation and a drop in rectal temperature (Andersen, Hellstrøm & Lorentzen, 1963). Our earlier experiments have shown that ethanol also has a dose-dependent deleterious effect on thermoregulation in severe cold (−20°C) (Huttunen & Hirvonen 1977), and a large dose of ethanol has been found to abolish the improved ability of cold-acclimatized guinea-pigs to endure severe cold (Huttunen, Penttinen & Hirvonen, 1980).

The exact effect which ethanol has on thermoregulatory reactions is nevertheless still unclear, the site of its action being thought to be peripheral and/or central. Since there are several studies suggesting that biogenic amines are involved in the central control of body temperature, the possibility exists that the hypothermic effect of ethanol may be relayed via these amines. Normal temperature is maintained by a delicate balance between the release of adrenaline, noradrenaline and 5-hydroxytryptamine (5-HT) in the hypothalamus (Feldberg & Myers, 1963) and changes in hypothalamic catecholamines have been shown to affect the threshold temperatures at which the responses to cold begin (Zeisberger & Brück, 1971; 1976).

The results presented here show that ethanol affects catecholamine concentrations in the hypothalamus and reduces tolerance to cold in guinea-pigs.

Methods

Adult female guinea-pigs (weighing 600–900 g) were used in the experiments. The animals were housed in groups at a room temperature of 20°C with a 12 h illumination period per day, and were fed on vegetables and pellets (Hankkija Ltd, Helsinki) with water *ad libitum*. After a fast of about 18 h, they were divided into two groups, group 1 (15 animals) being treated with 20% (w/w) ethanol (2 g/kg, i.p.) and group 2 (16 animals) with 0.9% w/v NaCl solution (controls). After 15 min seven animals from group 1 and eight from group 2 were exposed to severe cold (−20°C) and the others kept at room temperature. The fall in rectal temperature in the ethanol-treated guinea-pigs exposed to severe cold was followed to about 30°C, at which point they were killed by a blow on the neck. The ethanol-treated guinea-pigs kept at room temperature and the controls were also killed at the same point in time.

The hypothalamus was removed, immediately

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weighed and homogenized in ice-cold 0.4 M $HC10_4$ (100 mg/0.5 ml) containing 20 µl/ml glutathione (60 mg/ml). The samples were stored frozen at −40°C. No significant differences were found in hypothalamus wet weights between ethanol-treated groups and controls. The catecholamines noradrenaline, adrenaline and dopamine were determined from the diluted homogenate (1:9) using a catecholamine radioenzymatic assay kit (^{3}H) (CAT-A-KIT, Upjohn).

The ethanol concentration in the blood was determined by gas chromatography using propanol as the internal standard (Porapak Q, 120–150 mesh, column temperature 170°C).

Serum glucose and serum triacylglycerols were measured using enzymatic test kits (Roche) and serum free fatty acids (FFAs) by the colorimetric method of Laurell & Tibbling (1967). Glycogen was determined in 100–200 mg samples of skeletal muscle and liver according to the phenol-sulphuric acid colorimetric method of Lo, Russel & Taylor (1970).

Total lipid content was determined according to Folch, Lees & Stanley (1957) and triacylglycerols by the method of Marzo, Chirardi, Sardini & Meroni (1971) in the interscapular adipose tissue, once the lipid classes had been separated by thinlayer chromatography.

Results

After treatment with an alcohol dose of 2 g/kg guinea-pigs showed some drowsiness. The fall in rectal temperature to about 30°C in the ethanol-treated guinea-pigs, in severe cold (−20°C) took about 100 min, during which time the mean rectal temperature of the controls had fallen by about 2°C ($P<0.001$), as also had that of the ethanol-treated guinea-pigs at room temperature (Figure 1 and Table 1).

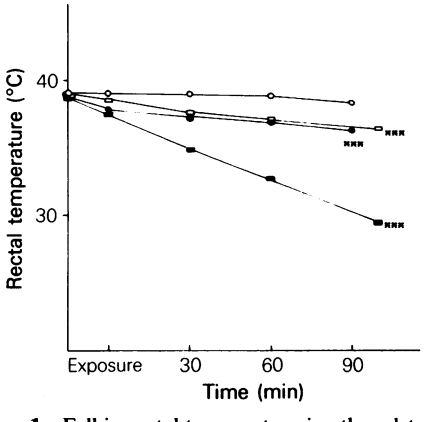

Figure 1 Fall in rectal temperature in ethanol-treated (2 g/kg) guinea-pigs and saline-injected guinea-pigs (controls) in severe cold (−20°C) and at room temperature (20°C). Ethanol-treated guinea-pigs at room temperature (●) and in severe cold (■); Controls at room temperature (○) and in severe cold (□). ***$P<0.001$ compared to initial temperature.

The blood alcohol concentration at death was 1.8 g/l in the animals exposed to cold and 1.4 g/l in those at room temperature. Thus cold affected the elimination of ethanol significantly ($P<0.05$) (Table 1).

Ethanol promoted an increase in the concentrations of noradrenaline ($P<0.05$) and adrenaline ($P<0.01$) in the hypothalamus at room temperature compared with those in the controls, the increase in noradrenaline being about twofold and that in adrenaline about threefold. Cold exposure did not significantly alter the hypothalamic catecholamine concentrations in the controls, but it did reduce the adrenaline content in the ethanol-treated guinea-pigs ($P<0.05$). The noradrenaline content after cold exposure was significantly higher in the ethanol-treated guinea-pigs than in the controls ($P<0.05$) (Table 1).

Ethanol reduced the muscle glycogen content at room temperature ($P<0.01$), but did not affect the serum glucose concentration. Cold exposure reduced the skeletal muscle glycogen content and serum glucose concentration in both groups (Table 2). Severe cold increased the FFA content in the serum both in the ethanol-treated guinea-pigs ($P<0.05$) and in the controls ($P<0.001$), but neither cold nor ethanol affected the serum triacylglycerols or the total lipid and triacylglycerol contents in the interscapular adipose tissue. No significant differences were found in the lipid and carbohydrate values between the groups at −20°C. (Table 2.)

Discussion

The results on the effects of acute intake of alcohol on brain catecholamine contents are controversial. Alcohol has been found to increase brain noradrenaline and dopamine levels (Yamanaka & Kono, 1974) or to decrease noradrenaline concentration in brain (Gursey & Olson, 1960), or to have no effect on these (Häggendal & Lindqvist, 1961; Efron & Gessa, 1963; Corrodi, Fuxe & Hökfelt, 1966; Pohorecky, 1974). These controversial results may arise from the methods used to administer the alcohol, the dose used, the duration of exposure to alcohol and the animal species. Even though no significant changes have been found in endogenous levels of catecholamines, their turnover and metabolites have been found to be affected by acute exposure to alcohol (Corrodi *et al.*, 1966; Pohorecky, 1974; Thadani, Kulig, Brown & Beard, 1975; Thadani &

Table 1 Noradrenaline, adrenaline and dopamine concentration in the hypothalamus of ethanol-treated guinea-pigs exposed to severe cold (−20°C) or room temperature (20°C) for about 100 min (NaCl-injected guinea-pigs were used as the controls)

Guinea-pigs	*Rectal temperature* (°C) *and BAC* (g/l)	*Catecholamines in hypothalamus* (ng/100 mg w.w.) *Noradrenaline*	*Adrenaline*	*Dopamine*
Ethanol-treated (2 g/kg) at 20°C $n = 6$	36.2 ± 0.5 1.4 ± 0.3	38.75 ± 11.76	8.68 ± 3.29	22.54 ± 14.14
NaCl-injected at 20°C $n = 6$	38.3 ± 0.4	18.95 ± 4.76	2.95 ± 1.21	9.23 ± 6.78
Ethanol-treated (2 g/kg) at −20°C $n = 7$	29.4 ± 1.7 1.8 ± 0.2	31.49 ± 12.35	4.19 ± 2.43	17.19 ± 6.95
NaCl-injected at −20°C $n = 6$	36.4 ± 0.9	18.46 ± 4.29	5.50 ± 3.57	18.78 ± 9.92

BAC= blood alcohol concentration.
Values are mean ± s.d.
$^{*}P < 0.05$; $^{**}P < 0.01$. (t test, separate).

Truitt, 1977). More detailed regional studies in rats show that an acute dose of alcohol also affects catecholamine metabolites and turnover in the hypothalamus (Pohorecky, 1974; Bacopoulus, Bhatnagar, & Van Orden, 1978) and an alcohol dose of 4 g/kg has been found to produce a small decrease in the endogenous level of hypothalamic noradrenaline (Pohorecky 1974). In the present study, an alcohol dose of 2 g/kg increased the hypothalamic catecholamine content in adult guinea-pigs at an ambient temperature of 20°C and −20°C compared to controls and reduced tolerance to cold. It could be supposed that the diminished cold tolerance in the ethanol-treated guinea-pigs is due, at least in part, to the increased catecholamine content in the hypothalamus, which has been accepted as the main centre for thermoregulation in mammals.

Behavioural thermoregulatory studies in rats also indicate that the fall in body temperature after ethanol is partly due to a reduction in the thermoregulatory set point (Lomax, Bajorek, Chesarek & Chaffee, 1980).

Table 2 Effect of ethanol (2 g/kg) in severe cold (−20°C) and at room temperature (20°C) on serum free fatty acid (FFA), triacylglycerol and glucose concentrations, liver and skeletal muscle glycogen content, total lipids and triacylglycerols in the interscapular adipose tissue (IAT) in female guinea-pigs (the controls were NaCl-injected guinea-pigs)

Parameters	*Ethanol-treated guinea-pigs* 20°C ($n = 8$)	−20°C ($n = 7$)	*Control guinea-pigs* 20°C ($n = 8$)	−20°C ($n = 8$)	
FFA (mmol/l)	0.52 ± 0.22 — * —	0.87 ± 0.31 — * —	0.62 ± 0.18 — *** —	1.06 ± 0.23	*** vs EtOH + 20°C
Triacylglycerols in serum (mmol/1)	1.01 ± 0.50	1.16 ± 0.30	0.99 ± 0.30	1.28 ± 0.37	
Glucose (mmol/l)	6.33 ± 0.96 — * —	4.60 ± 1.87 — ** —	6.69 ± 0.72 — *** —	4.04 ± 0.79	*** vs EtOH + 20°C
Glycogen in liver (μg/mg)	1.14 ± 0.73	0.80 ± 0.27 — * —	2.07 ± 1.46	0.99 ± 0.47	
Glycogen in muscle (μg/mg)	1.63 ± 0.79 — * — vs. controls **	0.91 ± 0.34 — *** —	2.92 ± 0.81 — *** —	0.77 ± 0.34	* vs EtOH + 20°C
Total lipids in the IAT (%)	74.9 ± 6.9	74.6 ± 3.9	75.1 ± 3.0	77.8 ± 2.5	
Triacylglycerols in the IAT (mg/g)	781.8 ± 128.4	761.3 ± 118.1	730.0 ± 92.0	779.1 ± 94.8	

Values are mean ± s.d.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (Student's t test).

The effect of an intrahypothalamic injection of catecholamines on thermoregulation has been found to depend on the species, on ambient temperature and the dose of drugs used. Most species react with a fall in body temperature after an injection of noradrenaline into the hypothalamic area at an ambient temperature of about 20°C (Bruinvels 1979), this response being due to the suppression of shivering and to peripheral vasodilatation, indicating that both the heat production mechanism and the heat loss mechanism are involved (Feldberg & Myers, 1963). Alcohol is also believed to increase heat loss by means of cutaneous vasodilatation, probably due to a centrally controlled nervous mechanism, and to reduce heat production by reducing metabolism.

The most important sources of heat production in acute cold are shivering and muscle tone. Inputs from cold sensors are integrated in the hypothalamus and transmitted to effector organs such as the muscles. Shivering involves an increased utilization of both carbohydrate and lipid substrates (Himms-Hagen, 1972). The changes noted in muscle glycogen content and blood glucose and FFA concentrations show that severe cold increases the use of fuels in both groups, but the lack of any significant differences in lipid or carbohydrate values between the controls and the ethanol-treated guinea-pigs suggests that the rapid drop in the rectal temperature of the ethanol-treated guinea-pigs was possibly due to the excess of heat loss over heat production, which may have been suppressed by the alcohol, and it is possible that the effects of alcohol on these thermoregulatory responses are relayed via the changes in biogenic amines in the hypothalamus.

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