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The Effect of Ethanol and Cold-Adaptation on the Survival of Guinea Pigs in Severe Cold*

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Summary. An acute ethanol dose of 2 g/kg shortened the survival time at −20° C of guinea pigs both adapted to the cold and those reared in the warm, but no significant difference was observed between the adapted and non-adapted groups. Long-term ethanol treatment of 2 g/kg/day for 4 weeks, lengthened the survival time of the guinea pigs reared at room temperature, but did not affect the survival of the cold-adapted animals. The acute dose of 2 g/kg shortened the survival time of the guinea pigs which had received the 4-week ethanol treatment and had been reared in the warm, but did not have the same effect on the cold-adapted animals. The improved survival rate at severe exposure acquired by adaptation to the cold was abolished by chronic alcohol administration.

Key words: Ethanol effect, in severe cold – Severe cold, effect of ethanol, survival time

Zusammenfassung. Eine akute Äthanoldosis von 2 g/kg verkürzte die Lebenszeit von Meerschweinchen, die entweder akklimatisiert oder nicht-akklimatisiert waren. Von den Tieren, die Äthanol 2 g/kg täglich vier Wochen lang erhalten hatten, lebten die nicht-akklimatisierten Tiere länger als die akklimatisierten. Von den Tieren, die 4 Wochen Äthanol erhalten hatten und vor der Kälteexposition noch eine akute Dosis erhielten, starben die nicht-akklimatisierten schneller, aber bei den akklimatisierten blieb die Dosis ohne Wirkung.

Schlüsselwörter: Alkoholwirkungen, bei Kälte – Unterkühlung, Alkoholwirkungen

Our earlier experiments have shown that ethanol doses which raised the blood alcohol level above 0.12% had a deleterious effect on thermoregulation, and that guinea pigs which had been reared in a warm colony (22–24° C) died at −20° C

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earlier than did guinea pigs which had lived at 17–18° C (Huttunen and Hirvonen 1977). On the other hand, ethanol seemed to have a protective effect on cardiac function, as the heart stopped at 2–3° C lower body temperatures in the animals receiving the larger doses, an observation which has also been made in dogs and rats (White and Nowell 1965; MacGregor et al. 1966).

The idea of a possible detrimental effect of a chronic intake of alcohol on thermoregulation has been raised in connection with hypothermia deaths in skid row alcoholics. The fact that these men are well acclimated to cold is another factor which will determine the end result. The present experiments were thus designed to investigate the effect of cold-adaptation on survival under conditions of severe cold when a large single dose of ethanol is given, and to test the effect of chronic daily ingestion of ethanol on these survival rates.

Material and Methods

Four groups of adult white guinea pigs weighing 660–760 g were used. Group I (14 animals) and group II (18 animals) were kept at a room temperature of 20–21° C and group III (13 animals) and group IV (12 animals) were reared at +4° C for 4 weeks. The animals received pelleted food (Hankkija, Ltd.), vegetables, and water ad libitum. Groups II and IV received ethanol in the stomach (2 g/kg in a 20% solution via a catheter) once daily. After 4 weeks one half of the animals in each group were given 2 g/kg ethanol i.p. and the other half 0.9% NaCl solution, and all the animals were then exposed to severe cold (−20° C) until death.

The body temperature of the animals under severe cold conditions was monitored with an electrical thermometer and the terminal temperature at which the heart stopped was recorded. This was regarded as the moment of death for calculation of the *survival time.*

The ethanol concentration in the blood and brain at death was measured. Red cells and proteins were precipitated with BaOH and $ZnSO_4$ solution and the ethanol in the supernatant measured by gas chromatography (Porapak Q, 120–150 mesh, column temp. 170° C). A similar procedure was applied to the brain tissue after homogenization with BaOH and $ZnSO_4$ solution.

Glucose in the serum was measured using the colorimetric method of Hyvärinen and Nikkilä (1972), in which glucose forms a blue-green color with o-toluidine. The color was measured at 630 nm.

Free fatty acids (FFA) in the serum were determined according to the method of Laurell and Tibbling (1967) in which FFAs form soaps with Cu++ and these are measured colorimetrically at 550 nm.

Catecholamines, adrenaline, and noradrenaline in the urine were measured by the method of Pekkarinen (1969). Catecholamines were absorbed into Al_2O_3 at pH 8.5 and eluted with HCl and NaH_2PO_4 solution. The amines were oxidized to adrenochrome and noradrenochrome and then reduced to adrenolutine and noradrenolutine. The intensity of the fluorescence of these compounds was assayed using an Aminco-Bowman spectrophotofluorometer.

Results

The guinea pigs reared at 20° C died after about 4 h in severe cold (−20° C) when an acute ethanol dose of 2 g/kg was given before the exposure. A 4-week intake of ethanol improved the survival by an average of about 2 h (Table 1).

In the groups reared at +4° C, the acute ethanol dose shortened the average survival time ($P < 0.05$) only in those animals which had received water during adaptation. The guinea pigs which had received ethanol for 4 weeks at +4° C did

Table 1. Effect of an acute ethanol dose (2 g/kg) on the survival time and rectal temperature at death of guinea pigs exposed to −20° C. Groups I and II were reared at +20° C, and groups III and IV at +4° C for 4 weeks. Groups II and IV were given ethanol (2 g/kg) daily

Experimental groups		Given at −20° C	*N*	Survival time, min (mean ± SD)	Rectal temperature at death, °C (mean ± SD)
I	reared at +20° C	Ethanol	8	251.3 ± 87.4 [a]	16.1 ± 2.4
	no ethanol	NaCl	6	336.7 ± 53.8 [a]	17.8 ± 0.6 [b]
II	reared at +20° C	Ethanol	10	356.5 ± 70.9 [a]	15.6 ± 2.2
	with ethanol	NaCl	8	463.8 ± 90.4 [a] [b]	16.9 ± 2.0
III	reared at +4° C	Ethanol	7	403.6 ± 268.0 [a]	16.3 ± 1.4 [b]
	no ethanol	NaCl	6	672.0 ± 129.4 [c]	15.8 ± 1.1
IV	reared at +4° C	Ethanol	6	541.7 ± 454.4	15.5 ± 1.8
	with ethanol	NaCl	6	860.0 ± 611.7	15.5 ± 1.4

[a] $P < 0.05$;
[b] $P < 0.01$;
[c] $P < 0.001$ (Student's *t*-test)

Table 2. Ethanol concentration in the blood and brain of the guinea pigs at death. Groups II and IV were given ethanol (2 g/kg) daily for 4 weeks. The acute ethanol dose in severe cold was 2 g/kg

Experimental groups		Given at −20° C	*N*	Ethanol concentration (‰)	
				Blood	Brain
I	reared at +20° C	Ethanol	8	1.27 ± 0.22	1.11 ± 0.49
	no ethanol	NaCl	6	—	—
II	reared at +20° C	Ethanol	10	1.11 ± 0.52	1.05 ± 0.88
	with ethanol	NaCl	8	—	—
III	reared at +4° C	Ethanol	7	0.93 ± 0.61	0.68 ± 0.51
	no ethanol	NaCl	6	—	—
IV	reared at +4° C	Ethanol	6	1.04 ± 0.69	1.07 ± 0.99
	with ethanol	NaCl	6	—	—

not survive significantly longer in the severe cold than those which had received water (Table 1).

Neither long-term ethanol treatment nor the acute alcohol dose had any effect on the rectal temperature at death (Table 1), and no significant differences were noted in the alcohol concentration in the blood and brain at death between the groups exposed to severe cold (Table 2).

Table 3. Concentrations of glucose and free fatty acids in the serum of the guinea pigs at death. Groups II and IV were given ethanol (2 g/kg) daily for 4 weeks. Ethanol was also given to a half of the animals before cold exposure

Experimental groups	Given at −20° C	*N*	Glucose mmol/l (mean ± SD)	FFA mmol/l (mean ± SD)
I reared at +20° C	Ethanol	8	1.02 ± 0.86	0.92 ± 0.19
no ethanol	NaCl	6	0.88 ± 0.68	0.84 ± 0.36
II reared at +20° C	Ethanol	10	1.55 ± 1.08	1.03 ± 0.30
with ethanol	NaCl	8	1.09 ± 0.49	1.03 ± 0.19
III reared at +4° C	Ethanol	7	1.39 ± 1.00	0.71 ± 0.30
no ethanol	NaCl	6	1.04 ± 0.87	0.92 ± 0.16
IV reared at +4° C	Ethanol	6	4.13 ± 3.99	0.86 ± 0.16
with ethanol	NaCl	6	1.99 ± 0.99	0.68 ± 0.21 [a]

[a] $P < 0.05$ (Student's *t*-test)

Table 4. Urinary excretion of adrenaline (A) and noradrenaline (NA) by the guinea pigs at −20° C expressed as concentration and amount excreted per hour and kg. Groups II and IV had received ethanol (2 g/kg) daily for 4 weeks and a half of the animals in each group had been given ethanol before exposure

Experimental groups	Given at −20° C	*N*	A (μg/ml)	A (μg/h/kg)	NA (μg/ml)	NA (μg/h/kg)
I reared at +20° C	Ethanol	6	0.15 ± 0.12[a]	0.47 ± 0.37[a]	0.01 ± 0.02	0.07 ± 0.10
no ethanol	NaCl	5	0.27 ± 0.25[a]	0.81 ± 0.58[a]	0.03 ± 0.04	0.06 ± 0.09
II reared at +20° C	Ethanol	9	0.39 ± 0.20[b]	1.15 ± 1.19[a]	0.07 ± 0.17	0.23 ± 0.59
with ethanol	NaCl	8	0.20 ± 0.15	0.69 ± 0.66	0.19 ± 0.22	0.52 ± 0.67
III reared at +4° C	Ethanol	7	0.37 ± 0.55	0.95 ± 1.19	0.05 ± 0.07	0.25 ± 0.34
no ethanol	NaCl	6	0.41 ± 0.52	1.21 ± 1.65	0.22 ± 0.21	0.98 ± 0.95
IV reared at +4° C	Ethanol	6	0.23 ± 0.24	0.69 ± 1.11	0.10 ± 0.09	0.24 ± 0.24
with ethanol	NaCl	6	0.32 ± 0.29	0.41 ± 0.23	0.11 ± 0.12	0.25 ± 0.26

[a] $P < 0.05$;
[b] $P < 0.01$ (Student's *t*-test)
Adrenaline values are compared with those for noradrenaline in the same group

The glucose concentrations in the serum at death did not differ significantly between the groups, although a general average trend was observable in that animals which had received the acute ethanol dose and died more quickly showed a higher glucose concentration than their respective controls (Table 3). Serum FFA concentrations were similar in all cases except for the group which had had a long-term ethanol intake at +4° C and no ethanol before exposure to −20° C, in which the value was lower ($P < 0.05$) than in the corresponding control group.

The guinea pigs reared at room temperature excreted more adrenaline than noradrenaline into their urine during severe cold exposure. No significant differences in adrenaline and noradrenaline excretion were observed in the cold-adapted groups (Table 4).

Comments

The acclimation of animals to cold is accomplished by an increased ability to produce heat and conserve it. The guinea pigs reared at +4° C endured severe cold (−20° C) longer than those kept at +20° C when no ethanol was given. On the other hand, no significant differences were observed in the survival time and the rectal temperature between those reared at +4° C and at room temperature when they had received one dose of ethanol before exposure. In other words, cold adaptation did not improve the ability to withstand severe cold after an ethanol dose, an observation which differs from our earlier results with guinea pigs reared at cool ambient temperature (17–18° C). One explanation for this is perhaps the different degree of acclimation achieved at cool vs. cold ambient temperatures and the different duration of acclimation. Ethanol given before exposure shortened the survival time here in both groups, which is an agreement with previous results (Huttunen and Hirvonen 1977).

Ethanol treatment for 4 weeks increased the survival time of the guinea pigs reared at room temperature, but did not have the same effect in the cold-adapted animals. The mechanism by which long-term ethanol intake improves survival under frost conditions is unclear. Some conjectures may be made, but no experimental data are available in the literature regarding ethanol and cold. Ethanol is perhaps used better as a fuel when the organism is adapted to it, and since it is itself a stress factor, it might simply increase the general ability to withstand stress. The fact that the acute alcohol dose which raised the blood ethanol level to about 1.00‰ at death had a deleterious effect on thermoregulation in the group reared at room temperature, but not in the group reared at +4° C, probably reflects the general increased resistance to cold resulting from adaptation as well as from long-term treatment with ethanol. These findings resemble the observation from forensic practice that a young person who is unaccustomed to alcohol is often a victim of accidental hypothermia, but a chronic alcoholic would seldom be.

The fact that the blood and brain ethanol concentrations at death were not lower in the animals which survived the cold for longer may be due to the decreased rate of ethanol oxidation in guinea pigs in hypothermia, since it has been shown that there is a distinct difference in the course of the blood alcohol curve between rats with normal body temperature and cooled rats (Dybing 1945).

Although the animals which received ethanol before exposure died more quickly their serum glucose and FFA values did not differ significantly from those measured in animals which did not receive the ethanol dose and lived longer. This might be the effect of increased consumption of these substances to compensate for the greater heat loss. Hypoglycemia seems in any case to be an important factor in the mechanism of hypothermal death.

Studies on the effects of ethanol on catecholamine metabolism have indicated an increase in urinary excretion after ethanol intake (Perman 1961). The stimulatory effect of ethanol on excretion did not become visible in the present experiments, perhaps because it was masked by the effect of acute severe cold as a strong stimulant of catecholamine excretion (Leduc 1961).

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