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Skeletal Muscle Activity of Alcohol-treated Rabbitts in Immersion Hypothermia: Adenine Nucleotide Concentrations and Phosphorylation State*,**

P. Huttunen, V. L. Kinnula, and J. Hirvonen

Departments of Forensic Medicine and Physiology, University of Oulu, Kajaanintie 52 D, SF-90220 Oulu 22, Finland

Summary. The effects of alcohol injection (0.5 $g \cdot kg^{-1}$ i.v.) on the core cooling and rewarming rates, concentration of the adenine nucleotides, and the phosphorylation state of the adenylate system (ATP/ADP × P) were studied in the skeletal muscle of anesthetized rabbits immersed in ice-cold water. NaCl-injected rabbits immersed in ice-cold water were used as cold controls, alcohol-treated animals at room temperature (20°C) as alcohol warm controls, and NaCl-injected animals at room temperature as anesthesia controls, respectively.

The fall of core temperature to 32°C in the alcohol-treated rabbits and the cold controls took about 40 min. During this time the temperature of the alcohol warm and anesthesia controls fell by about 1°C. No difference in the rewarming rate was observed between the alcohol-treated and cold control rabbits.

Serum glucose concentration was elevated in the cold controls (from 5.9 to 8.3 mmol/l) but not in the alcohol-treated rabbits.

Cold exposure reduced the phosphorylation state in the skeletal muscle of the alcohol-treated rabbits by 32% ($P < 0.05$), but the decrease (6%) was not significant in the cold controls. After rewarming the phosphorylation state decreased in the above groups by 71% and 15%, respectively, as compared with the initial values. No significant changes in the phosphorylation state were found in the warm control animals.

The redox state of the cytosol in the skeletal muscle or liver did not change, nor was there any change observed in the arterial pO_2 or pCO_2 concentrations.

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** Dedicated to Professor W. Janssen on the occasion of his 60th birthday
Offprint requests to: Jorma Hirvonen, DMS (address see above)

In conclusion, the results suggest an increased skeletal muscle activity during immersion hypothermia in alcohol-treated rabbits, but not in the controls.

Key words: Alcohol and immersion hypothermia – Skeletal muscle activity in alcohol-treated rabbits

Zusammenfassung. Es wurden die Effekte von Alkoholinjektionen (0.5 $g \cdot kg^{-1}$ i.v.) auf die Geschwindigkeit der Unterkühlung und Wiedererwärmung des Körperkerns, die Konzentration der Adeninnukleotide und den Phosphorylierungszustand des Adenylatsystems (ATP/ADP × P) im Skelettmuskel von anästhesierten Kaninchen, die im eiskalten Wasser gehalten wurden, studiert. Kaninchen, die NaCl-Injektionen erhielten und in eiskaltem Wasser gehalten wurden, dienten als Kältekontrollen, alkoholbehandelte Tiere, die bei Raumtemperaturen (20°C) gehalten wurden, dienten als Wärmekontrollen unter Alkohol und Tiere, die NaCl-Injektionen erhielten und bei Raumtemperatur gehalten wurden, dienten als Anästhesiekontrollen.

Der Abfall der Kerntemperatur auf 32°C nahm bei den alkoholbehandelten Kaninchen und den Kältekontrollen ca. 40 min in Anspruch. Während dieser Zeit fiel die Temperatur bei den alkoholwarmen und anästhesierten Kontrollen nur um ungefähr 1°C. Es wurden keine Unterschiede der Wiedererwärmungsgeschwindigkeit zwischen den alkoholbehandelten Kaninchen und den Kältekontrollen beobachtet.

Die Serumglukosekonzentration war bei den kalten Kontrolltieren gestiegen (von 5.9 auf 8.3 mmol/l), jedoch nicht bei den alkoholbehandelten Kaninchen.

Die Kälteexposition reduzierte den Phosphorylierungszustand im Skelettmuskel der alkoholbehandelten Kaninchen um 32% ($p < 0.005$), bei den kalten Kontrolltieren jedoch war die Senkung (6%) nicht signifikant. Nach Wiedererwärmung fiel der Phosphorylierungszustand in den oben genannten Gruppen um 71 bzw. 15% verglichen mit den Ausgangswerten. Bei den Wärmekontrollen wurden nur nicht signifikante Veränderungen des Phosphorylierungszustandes gefunden.

Die Laktat: Pyruvatratio veränderte sich weder im Skelettmuskel noch in der Leber. Es waren auch keine Veränderungen in den pO_2- oder pCO_2-Konzentrationen im arteriellen Blut zu beobachten.

Daraus wird gefolgert, daß bei alkoholbehandelten Kaninchen eine Erhöhung der Skelettmuskelaktivität während der Immersionshypothermie eintritt, jedoch nicht bei den Kontrolltieren.

Schlüsselwörter: Hypothermie, Erhöhung der Skelettmuskelaktivität unter Alkoholeinfluß – Alkoholeffekte, bei Unterkühlung

Many drugs with central nervous effects, such as alcohol, are known to cause hypothermia in mammals when exposed to low ambient temperature. The final hypothermic effect of alcohol depends on the dosis, the ambient temperature

and possibly the animal species. It is generally believed that the deleterious effect of alcohol is due to increased heat loss by means of peripheral vasodilation. This seems to have happened in the victims of accidental hypothermia, which were exposed to dry cold, since their skin is reddish also in the non-hypostatic areas. The blood alcohol level has usually been between 1.5‰ and 2.5‰ in such accidents.

Cutaneous vascular dilatation might be less marked in wet exposure, since the stimulus to the skin is more intense by the cold water, and heat loss is thus decreased. Since earlier experiments have shown that alcohol in moderate amounts does not significantly alter cold-tolerance during immersion in cold water (Martin et al. 1977; Kuehn et al. 1978; Fox et al. 1979), it was decided to study the effect of a moderate dose of alcohol on the activity of the skeletal muscle in anesthetized rabbits immersed in ice-cold water. This was done by monitoring the adenine nucleotides and the phosphorylation state of the adenylate system ($ATP/ADP \times P_i$) after alcohol infusion and cold immersion; the phosphorylation state being an important ratio for the regulation of the respiration rate (Klingenberg 1961; Owen and Wilson 1974). The purpose was to obtain information on the degree of heat production during severe exposure leading to distinct hypothermia.

It appeared that the alcohol dose of about 0.5 $g \cdot kg^{-1}$ i.v. did not change the core cooling rate of the rabbits or the rewarming rate, but the phosphorylation state of the skeletal muscle decreased after cooling, indicating that alcohol stimulated the activity in the skeletal muscle of the rabbits immersed in ice-cold water.

Material and Methods

Animal Preparation

Male rabbits weighing about 3,000 g and housed in separate cages in a room with a window and ambient temperature of 20°C were fed on pelleted food (Hankkija, Finland), cabbage, and water ad libitum. All rabbits, after fasting for 16 h, were anesthesized with Hypnorm (10 mg fluanison and 0.2 mg fentanyl/ml) 0.5 $ml \cdot kg^{-1}$ i.m. The femoral artery and vein were cannulated. Arterial pressure was monitored continuously. One animal, in which the systolic pressure fell below 60 mm Hg, was discarded.

The rabbits were divided into four groups. Those in *Group 1* ($n = 7$) were given 10% (w/w) ethanol (about 0.5 $g \cdot kg^{-1}$ i.v.) over 25 min at room temperature (20°C), after which they were immersed in ice-cold water until their core temperature had fallen to 32°C. Then they were rewarmed to 35°C in a warm box at 45°C. Core temperature was measured at the tympanic and rectal sites and expressed as the mean of the individual values obtained. The animals in *Group 2* (cold control group, $n = 6$) were given 0.9% NaCl the same volume as 10% ethanol solution as above. Those in *Group 3* (ethanol-warm control group, $n = 6$) were given ethanol in the same way as Group 1 but, instead of cooling, the animals were kept for the same period at room temperature, and those in *Group 4* ($n = 3$), referred to in the text as the anesthetic control group, were given 0.9% NaCl and maintained at room temperature.

Blood and medial posterior femoral muscle samples were taken by the freeze-clamp technique according to Wollenberger et al. (1960) immediately after cannulation, at a core temperature of 32°C and after rewarming to 35°C, or at the corresponding time intervals in the warm controls. Blood samples were also taken for alcohol determination immediately after the alcohol injection

Biochemical Analyses

Blood alcohol concentration (BAC) was determined by gas chromatography (Porapak Q, 120–150 mesh, column temperature 170°C) and serum glucose concentration using an enzymatic test kit (Roche).

Metabolites were determined from freeze-clamped muscle samples pulverized under liquid nitrogen. Frozen tissue powder was extracted with ice-cold perchloric acid, and the supernatant was neutralized to pH 6.0 with 3.57 *M* K_2CO_3–0.5 *M* triethanol-amine hydrochloride. Neutralized perchloric acid extracts were used for enzymatic assays of lactate (Gawehn and Bergmeyer 1970), pyruvate (Czok and Lamprecht 1970), adenosine-5-triphosphate (Lamprecht and Trautschold 1970), adenosine-5-disphophate (Jaworek et al. 1970), adenosine-5-monophosphate (Jaworek et al. 1970), and inorganic phosphate (Chen et al. 1956). Lactate (Gawehn and Bergmeyer 1970) and glycerol-3-phosphate (Hohorst 1970) were determined from the freeze-clamped lobe of the liver in the last stage of the experiment. The concentrations of the metabolites were expressed as $\mu mol \cdot g^{-1}$ wet weight of the tissue.

Statistical Treatment

The two-tailed Student's *t*-test for dependent and independent means was used for the statistical analysis.

Results

Cooling and Rewarming Rate

The core temperature of the rabbits dropped on average from 39°C to 37°C during cannulation lasting for about 1.5 h. The core temperature drop to 32°C in the alcohol-treated rabbits in ice-cold water took 42 ± 10 min and that in the cold controls 43 ± 10 min. The cooling rate in both groups was thus about 0.1°C per min. During this time the core temperature of the alcohol warm and anesthetic controls fell by about 1°C at a room temperature of 20°C. The rewarming time of the alcohol-treated animals and cold controls was 94 ± 15 min and 92 ± 55 min, respectively, or 0.04°C per min. During this time the core temperature of the warm alcohol and anesthetic controls in the warm box rose to 38.1 ± 1.3°C and 38.1 ± 1.4°C, respectively. The core temperature changes during cooling and rewarming with and without alcohol are shown in Fig. 1.

Blood Alcohol Concentration and Serum Glucose

The mean blood alcohol concentration was 1.5 ± 0.3 $g \cdot l^{-1}$ immediately after alcohol infusion and 0.8 ± 0.1 $g \cdot l^{-1}$ and 0.4 ± 0.3 $g \cdot l^{-1}$ after cooling and rewarming, respectively. The corresponding values for the ethanol warm controls were 1.0 ± 0.4 $g \cdot l^{-1}$ and 0.7 ± 0.4 $g \cdot l^{-1}$, i.e., there were no significant differences in blood alcohol concentrations between the groups.

The serum glucose concentration after cooling was significantly elevated in the cold controls (from 5.9 ± 1.3 to 8.3 ± 2.1 $mmol \cdot l^{-1}$, $P < 0.01$), but not in the alcohol-treated rabbits (from 6.9 ± 1.6 to 7.4 ± 1.1 $mmol \cdot l^{-1}$). After rewarming, the glucose content was still elevated in the controls (9.3 ± 3.0 $mmol \cdot l^{-1}$, $P < 0.05$) but remained unchanged from the initial values in the alcohol-treated animals (6.3 ± 1.3 $mmol \cdot l^{-1}$). No significant changes in serum glucose

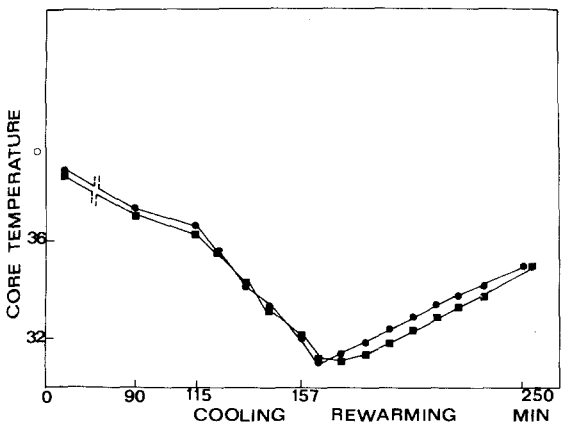

Fig. 1. Cooling and rewarming rates of alcohol-treated and NaCl-injected rabbits. ■ alcohol-treated ($0.5\,g \cdot kg^{-1}$ i.v.), ● NaCl-injected. 0–90 min cannulation, 90–115 min administration of drugs, 115–157 min cooling, 157–250 min rewarming

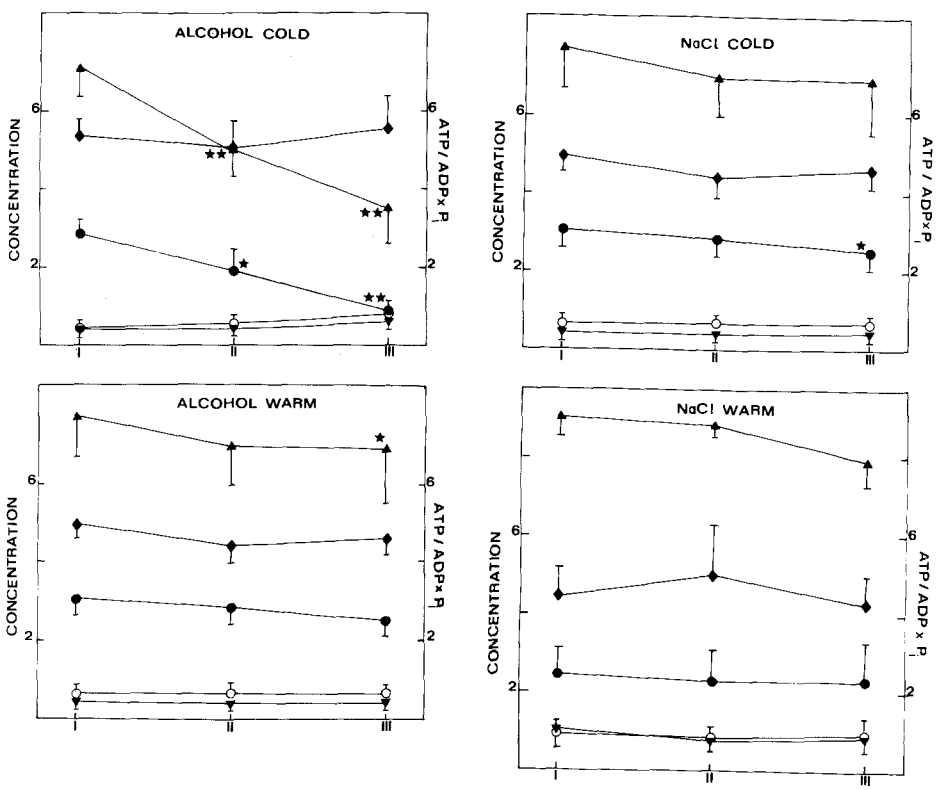

Fig. 2. Adenine nucleotide concentrations and phosphorylation state in the skeletal muscle of rabbits. Values are means ± SE from three to seven separate experiments. Concentrations are expressed in $mmol \cdot g^{-1} \cdot$ wet wt. I, after cannulation; II, after cooling (core temperature 32°C in the rabbits exposed to cold water and 35.5°C in those kept at room temperature); III, after rewarming (core temperature 35°C in the rabbits exposed to cold and 38°C in the warm controls). ▲ ATP, adenosine-5-triphosphate; ◆ Pi, inorganic phosphate; ● ATP/ADP × P_i, phosphorylation state; ○ ADP, adenosine-5-diphosphate; ▼ AMP, adenosine-5-monophosphate; * $P < 0.05$; ** $P < 0.01$

concentrations were observed between the ethanol warm of anesthetic controls at any stage.

Metabolites

The ATP concentration and phosphorylation state (ATP/ADP × P) decreased in the ethanol-treated rabbits after cooling ($P<0.01$ and $P<0.05$), and these decreases were still significant after rewarming as compared with the initial values ($P<0.01$ and $P<0.01$). No significant changes were found in the ADP, AMP, and orthophosphate concentrations (Fig. 2).

In the cold control group adenine nucleotides did not change at any stage, but the phosphorylation state was significantly lower after rewarming than at the beginning of the experiment ($P < 0.05$) (Fig. 2).

No significant changes were found in the phosphorylation state in the ethanol warm control group, but the ATP concentration was lower than the initial value after rewarming ($P<0.05$) (Fig. 2).

Neither adenine nucleotides nor the phosphorylation state changed during the experiment in the anesthetic control group (Fig. 2).

Table 1. Lactate:pyruvate ratio in the skeletal muscle of alcohol-treated and NaCl-injected rabbits exposed to cold water or kept at room temperature

	Alcohol-treated		NaCl-injected	
	Exposed to cold water (3)	Kept at room temperature (6)	Exposed to cold water (6)	Kept at room temperature (3)
I	26.9 ± 11.1	19.6 ± 1.3	29.2 ± 14.8	31.4 ± 10.9
II	25.4 ± 11.7	20.7 ± 5.1	29.9 ± 9.6	30.0 ± 15.8
III	23.9 ± 8.1	23.5 ± 4.6	26.2 ± 3.9	36.9 ± 21.4

I, after cannulation; II, after cooling; III, after rewarming
Values are means ± SD from the number of experiments indicated in parentheses

Table 2. Phosphorylation state, lactate, and sn-glycerol-3-phosphate concentrations in the liver of the rabbits at the end of the experiment

	Alcohol-treated		NaCl-injected	
	Exposed to cold water	Kept at room temperature	Exposed cold water	Kept at room temperature
ATP/ADP × P_i	3.37 ± 2.29 (7)	3.21 ± 1.97 (6)	3.97 ± 2.51 (6)	3.63 ± 3.92 (3)
Lactate $\mu mol \cdot g^{-1}$ wet wt.	2.44 ± 1.00 (6)	2.03 ± 0.98 (6)	2.34 ± 1.44 (6)	1.52 ± 0.73 (3)
Sn-glycerol-3-phosphate $\mu mol \cdot g^{-1}$ wet wt.	0.74 ± 0.09 (4)	1.09 ± 0.43 (3)	0.75 ± 0.46 (6)	0.51 ± 0.12 (3)

t-test for independent means
Values are means ± SD from the number of experiments indicated in parentheses

Arterial pO_2 and pCO_2 values were examined at each stage of the experiment to eliminate anoxia, since no respirator was used. PO_2 values varied between 10.7 and 15.3 kPa and pCO_2 between 2.59 and 4.90 kPa; the values between the different groups did not differ significantly. The ratio of lactate to pyruvate in the skeletal muscle did not change significantly during the experiment in any group (Table 1), nor were there any changes in lactate or pyruvate concentrations observed (data not shown). Alcohol or cold water did not affect the phosphorylation state or concentrations of lactate and glycerol-3-phosphate in the liver (Table 2).

Discussion

Alcohol has been shown to produce a dose-dependent fall in body temperature in mice (Freund 1973), guinea pigs (Huttunen and Hirvonen 1977), and rats (Lomax et al. 1980). Experiments in man show that a moderate dose of alcohol can reduce body temperature in a dry, cold environment (Graham and Dalton 1980; Graham 1981), but not in cold water (Martin et al. 1977; Kuehn et al. 1978; Fox et al. 1979). Fox et al. (1979) have suggested that the absence of an alcohol effect on skin temperature in cold water is due to the very intense stimulation by the water on the receptors, which causes strong vasoconstriction and thereby reduces heat loss due to alcohol, and this is reflected in the unchanged core temperature. On the other hand, a 12%–18% increase in metabolic rate has been seen in alcohol-treated human subjects in cold environments, this probably being an expression of the specific dynamic effect of alcohol (Risbo et al. 1981). In the present work, an alcohol dose of $0.5\,g \cdot kg^{-1}$ i.v., giving a blood alcohol concentration of about $1.5\,g \cdot l^{-1}$, had no influence on the cooling rate of rabbits during immersion in ice-cold water or on the rewarming rate, which is in agreement with the previous results mentioned above.

The importance of the ratio of the concentration of ATP to the production of ADP and the orthophosphate concentration for the regulation of the respiratory rate has been confirmed (Klingenberg 1961; Owen and Wilson 1974), and muscle activity is a mode of thermogenesis in which control operates primarily via changes in the phosphorylation state ratio (Himms-Hagen 1976). Mechanical activity causes a decrease in the concentration of ATP and a large increase in AMP, which will stimulate glycolysis and hence energy production (Beis and Newholme 1975). In exercised animals (carp) ATP concentration in muscle has been found to reduce by about 65% but AMP concentration to remain low and unchanged or the total adenylate pool to decrease during the exercise period (Driedzig and Hochanchka 1976). In cold-acclimated brook trout a decrease in ATP: AMP ratios has also been found, consistent with the activation of glycolysis (Walesby and Johnston 1980). In the present study, the phosphorylation state changed in the alcohol-treated rabbits upon immersion in ice-cold water was consistent with the high muscle activity, which was also observed as strong shivering, but not in the cold controls.

The decrease in the phosphorylation state noted here was due mainly to the reduced ATP concentration, which is in agreement with earlier studies (Beis

and Newsholme 1975), and no significant changes in ADP or AMP concentration were observed. Variations in ADP and AMP content cannot be interpreted with certainty because a substantial fraction of cytosolic ADP is bound to intracellular proteins and because of the rapid decomposition of these nucleotides. The degradation of adenine nucleotides beyond AMP is principally dependent on the high AMP-deaminase activity in rabbit skeletal muscle leading to IMP and inosine (Dixon and Webb 1964; Goodman and Lowenstein 1977) and 5-nucleotidase leading to adenosine.

No significant changes in the orthosphosphate concentrations were observed in this study. This is contradictory because of the orthophosphate release from hydrolysis of ATP. Part of the liberated phosphate could be used to phosphorylate creatine or other metabolites and release of orthophosphate into the bloodstream (Aragón and Lowenstein 1980). A small tendency of the phosphate to increase was, however, observed when the ATP decrease was evident. In the interpretation of the results, the distinction between free and bound adenine nucleotides or between cytoplasmic and mitochondrial concentrations of the metabolites is impossible by a direct technique.

Hypoxia in the present work, in which the anesthetized animals were breathing spontaneously, was excluded by the unchanged arterial pO_2 and pCO_2 values. The well-known effect of ethanol on hepatic metabolites (Bernstein et al. 1973; Savolainen et al. 1977) did not occur most probably because of the short duration of the experiment and low doses of ethanol. A decrease in skeletal muscle ATP-content in the ethanol-cold rabbits indicated an increased muscle metabolism more probably than anoxia of the tissue because of the unchanged lactate:pyruvate ratio. The increased blood glucose concentration in the cold control group is most probably due to the release of catecholamines and cortisol from the adrenal medulla and cortex, stimulated hepatic glycogenolysis and/or the calorigenic effect of insulin in hypothermia (Therminarias et al. 1979).

In conclusion, the activity in the skeletal muscle increased in the alcohol-treated rabbits upon immersion in ice-cold water, which could, at least in part, compensate for the possible heat loss caused by ethanol and explain the similarity in the cooling rate of the groups.

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