Pharmacology Biochemistry & Behavior, Vol. 16, pp. 207–210, 1982. Printed in the U.S.A.

The Interaction of Ethanol and Swimming Upon Cardiac Mass and Mitochondrial Function

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Received 7 May 1981

FARRAR, R. P., C. M. ARDIES, R. L. SHOREY AND C. K. ERICKSON. *The interaction of ethanol and swimming upon cardiac mass and mitochondrial function.* PHARMAC. BIOCHEM. BEHAV. 16(2) 207–210, 1982.—Four groups of female Sprague-Dawley rats received a nutritionally adequate liquid diet formulated for rats. Two groups, one ethanol diet and one control diet swam 6 days/wk for 6 weeks and were designated swim ethanol (SWM-E) and swim control (SWM-C) respectively. Their swimming time increased from 15 min/day on the first day to 2 hrs/day during the final week. One sedentary group received an ethanol diet (SED-E) while another sedentary group received a control diet (SED-C). In the ethanol diet 35% of the calories as ethanol isoenergetically replaced dextrin. The group mean body weights were not different at the end of 6 weeks. The left ventricles of both swimming groups showed similar gains in weight, 13% for the ethanol and 15% for the control. Mitochondrial respiration in the ethanol groups showed a significant depression across substrates and across both populations of mitochondria (subsarcolemmal and intermyofibrillar). The swimming-ethanol interaction in the SWM-E group caused an atrophy of the gastrocnemius-plantaris muscle as evidenced by the 13% loss in weight of the muscle. We conclude that chronic ingestion of ethanol will suppress mitochondrial respiration in sedentary and swimming exercised rats, but will not suppress cardiac hypertrophy in the swimming exercised rats. Muscles that are not chronically overloaded by swimming, such as the gastrocnemius-plantaris muscles will undergo atrophy during the swimming protocol of 6 weeks.

Ethanol Swimming Cardiac mass Mitochondrial function

CHRONIC ethanol consumption has been shown to affect the heart [20, 24, 25, 26] as well as the liver [2,3]. In 8 weeks, muscles from rats consuming 25 percent of calories as ethanol demonstrated a reduction in maximal rate of isometric tension, maximal developed tension, velocity of shortening at zero load and mitochondrial respiration. In addition, acetaldehyde, a by-product of ethanol oxidation, inhibited protein synthesis in the heart [18, 19, 22].

In contrast, swimming for 8 to 10 weeks enhanced contractile performance in the left ventricle of the rat [21] and resulted in increased peak systolic pressure and maximal developed tension. Mitochondrial respiration has not increased in response to swimming regimes [14]. The large aerobic capacity in untrained rats is apparently sufficient to meet the metabolic demands imposed by a swimming program. How much this aerobic capacity must be reduced for a swimming regime to become an overload on the heart is not known.

Recently Palmer *et al.* [17] devised a method for fractioning the cellular mitochondrial populations of the heart into subsarcolemmal and intermyofibrillar regions. Electronmicrographs have shown that the mechanical homogenization released the subsarcolemmal mitochondria, while the intermyofibrillar mitochondria are released only after treating the myofibrillar pellet with a protease, nagarse [16]. The purity of the pellets have been assessed through electronmicroscopic inspection [17], activity of NADH cytochrome c reductase [16], lipolitic enzyme activity [16], Na^+/K^+ ATPase activity (personal communication), as well as by phospholipid content of the membranes contained in the pellets [15]. This technique permits a better quantification of altered aerobic capacity of the heart. The subsarcolemmal mitochondria appear to provide energy for protein synthesis and glucose phosphorylation, while the intermyofibrillar mitochondria provide energy for contraction.

In the present study, we asked several questions: (1) Does chronic ethanol consumption reduce mitochondrial respiration in both populations of cardiac mitochondria? (2) Does swimming enhance mitochondrial function within regions of cardiac cells that may have been masked by considering the mitochondria as one population? (3) Is there an interaction between swimming and ethanol consumption that might offset the ethanol-induced reduction in respiration of the mitochondria? (4) Does the reduced respiration produced by ethanol prevent rats from completing a swimming training

program? (5) Is part of the damage observed in cardiac mitochondria of ethanol treated animals [24] due to dietary insufficiency?

METHOD

Animals

Female Sprague-Dawley rats with an initial mean weight of 225±8 g were divided into four groups; sedentary control (SED-C), N=8; sedentary-ethanol (SED-E), N=8; swim control (SWM-C), N=12; and swim-ethanol (SWM-E), N=12. They were individually housed in stainless steel wire mesh bottom cages in an animal facility maintained at 22°C with alternating 12 hours of dark/light cycle. The animals were weighed and food consumption was measured daily.

Swimming

The rats swam in plastic barrels filled to a depth of approximately 36 inches. The water temperature was 35°C and the rats swam in groups of 6. The animals swam at approximately 8 a.m., 6 days/week. The swimming time increased from 15 min/day on the first day to 2 hrs/day during the 6th week.

Diet

Diets were formulated that met American Institute of Nutrition recommendations [1]. The liquid diets contained 35 percent of energy as ethanol; control diets contained isoenergetic amounts of dextrin. Diets were made fresh daily by the procedure of Miller *et al.* [13] and offered ad lib to all groups. Ethanol tolerance and withdrawal have been demonstrated with this formulation [13].

Tissue Preparation

The rats were anesthetized with an intraperitoneal injection of 50 mg/kg of sodium pentobarbital, the chest cavity was opened and the heart was removed. The heart was placed into a beaker containing ice cold solution B (220 mM mannitol, 70 mM sucrose, 10 mM MOPS (3-[N-Morpholino]propanesulfonic acid), pH 7.4, 1 mM EGTA (ethyleneglycolbis (B-amino-ethyl ether) N, N^1-tetracetic acid) and 0.2% BSA (Bovine serum albumin, fraction V). All subsequent procedures were carried out in a Sherer environmental chamber maintained at 2–4°C. The atria, aorta, and right ventricle were removed from the left ventricle. The left ventricle was cut in half vertically, blotted dry, and weighed. The ventricle was placed into 10 v/w of solution B and the tissue finely minced with scissors. The ventricles were then homogenized for 15 seconds with a Brinkman polytron ST-20 at 5500 rpm and the two populations of mitochondria were isolated according to the procedure of Palmer *et al.* [16]. The mitochondria were washed twice in solution D (100 mM KCl, 50 mM MOPS pH 7.4, 0.2% BSA and 1 mM EGTA) and resuspended in 0.5 ml of solution D. the gastrocnemius-plantaris muscles were excised from the animals, gleaned of connective tissue and fat, blotted dry and weighed

Mitochondrial Respiration

The oxygen consumption was measured polargraphically by a YSI oxygen electrode in a water-jacketed reaction vessel maintained at 30°C by a Haake water bath. The oxygen electrode solution consisted of 250 mM sucrose, 10 mM Tris-HCl, pH 7.4, and 6 mM K_2HOP_4. The substrates were in a final concentration of 16.7 mM pyruvate and 1.67 mM malate, 16.7 mM glutamate, or 16.7 mM succinate. State 3 respiration was initiated with 300 μmoles of ADP.

TABLE 1

BODY, HEART, AND GASTROCNEMIUS-PLANTARIS WEIGHTS AT END OF THE STUDY

	Body (g)*	Heart (mg)	Gastrocnemius-Plantaris (g)
SED-C (N=8)	276±15.5	540.0±27.8	3.31±0.27
SED-E (N=6)	289.3± 8.4	555.0±42.8	3.45±0.24
SWM-C (N=11)	278.4±14.6	620.9±63.5*	3.41±0.38
SWM-E (N=9)	281.3±10.8	627.8±40.6*	3.03±0.23†

*Beginning body weights were 225±8 g. Values are expressed as the group means±the standard deviation.
*Significantly different from sedentary groups at ($p<0.001$) level.
†Significantly different from all other groups at ($p<0.01$) level.

Protein Determination

Protein concentration was determined by the biuret method of Gornall *et al.* [6].

Blood Ethanol

Blood ethanol concentrations on the last week of the experiment were determined on samples taken from the SWM-E group 3, 6, 12, and 22 hours following swimming and at the same time of day for the SED-E group during the last week of the experiment. Blood was drawn into 60 uL heparinized tubes and placed in stoppered vials, and kept in a freezer until they were measured. The concentration of ethanol was measured on a Perkin Elmer F40 gas chromotograph by the method of Erickson *et al.* [4].

Statisitcs

The data were analyzed using 2 way analysis of variance.

RESULTS

Body Weight

As shown in Table 1, final body weights of all animals were greater than initial weights. Differences between groups were not significant.

Heart Weight

The heart weights (Table 1) of both SWM-E and SWM-C groups showed significant hypertrophy ($p<0.0002$).

Gastrocnemius-Plantaris Weight

The combined gastrocnemius-plantaris muscle weights were significantly lower ($p<0.01$) in the SWM-E group than in the other 3 groups. These 3 groups were nonsignificantly different from one another.

Mitochondrial Respiration

The respiration of both populations of mitochondria

TABLE 2

OXYGEN CONSUMPTION OF TWO POPULATIONS OF CARDIAC MITOCHONDRIA WITH DIFFERENT SUBSTRATES

	Pyruvate-Malate	Succinate	Glutamate
SED-C (N=8)			
subsarcolemmal	180.1±55.4*	246.1±95.9*	132.6±50.3*
intermyofibrillar	204.3±29.4*	297.1±36.4*	164.2±43.4*
SED-E (N=6)			
subsarcolemmal	90.9±12.0	122.4±19.5	60.9±12.5
intermyofibrillar	95.4±34.3	163.4±46.1	88.6±30.9
SWM-C (N=11)			
subsarcolemmal	145.6±40.5*	204.8±69.4*	121.8±39.9*
intermyofibrillar	166.5±43.2*	236.7±27.8	151.5±54.4*
SWM-E (N=9)			
subsarcolemmal	106.6±24.8	131.5±41.2	63.2±13.5
intermyofibrillar	119.5±32.0	175.3±56.6	102.8±41.9

Values are for state 3 respiration expressed in ng atoms · mg^{-1} mitochondrial protein · min^{-1}.

*Significantly different from the ethanol groups at the ($p<0.001$) level.

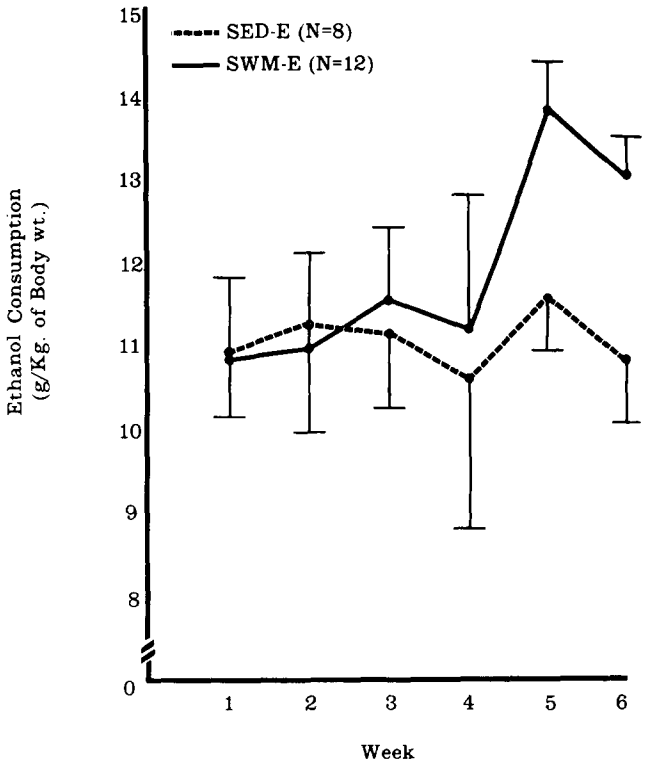

FIG. 1. Ethanol consumption The mean values are expressed as g · of ethanol/kg · of body weight±S.D.

across substrates was significantly ($p<0.0001$) depressed in the ethanol treated animals (Table 2). The intermyofibrillar mitochondria had a significantly ($p<0.001$) higher state 3 respiration across groups when compared to the subsarcolemmal mitochondria in the same group. This is consistent with the original findings of Palmer *et al.* [17]. Swimming did not enhance respiration in either mitochondrial population in the SWM-C group. Swimming did not significantly protect either mitochondrial population from the depressing effects of ethanol consumption upon respiration with all substrates.

Dietary Intake

As can be seen from Fig. 1 the ethanol consumption increased for the trained animals from week one to week five and then fell slightly. The sedentary animals showed a fairly consistent intake over the six week period.

Ethanol Consumption and Blood Ethanol Levels

The ethanol consumption and blood ethanol levels were determined on the SWM-E animals at three, six, twelve, and twenty-two hours after swimming during the last week of the experiment. The time of sampling was noon, 3 p.m., 9 p.m., and 7 a.m.. The consumption pattern revealed that the rats ate 5 g of ethanol/kg body weight at night from 9 p.m. to 7 a.m. and 4 g of ethanol/kg between 3 p.m. and 9 p.m. During these periods of greatest ethanol consumption and blood levels were only 75 mg/dl and 100 mg/dl. Following the 2 hours of swimming, the rats consumed 2.5 g of ethanol/kg and the blood ethanol levels were the highest of any time during the day, 162 mg/dl.

DISCUSSION

The depressing effect that ethanol has upon mitochondrial respiration is consistent with previous literature dealing with cardiac mitochondria [24,25]. In the present study the respiratory control ratios and P:O ratios were not significantly different in the ethanol treated groups, thus mitochondria appear to be tightly coupled, but unable to carry out oxidative phosphorylation as rapidly. Recent changes in diet have been shown to effect the heart mitochondrial membrane

structure [8] and mitochondrial ATPase activity [7]. Ethanol, in addition to altering mitochondrial membrane composition in liver [23] effected the rat of oxidative phosphorylation for all substrates measured. Whether chronic ethanol consumption causes alterations in cardiac mitochondrial membranes that would make them more susceptible to damage during isolation is not known. That rats on ethanol were able to swim for up to 2 hous per day utilizing only 50 percent of the respiratory capacity of the heart, as determined from isolated mitochondrial respiration, is surprising. It was thought that swimming would attenuate the decline in respiratory capacity per mg of mitochondrial protein or induce the synthesis of more mitochondrial protein.

The cardiac hypertrophy that resulted in the SWM-E group is also significant, in light of the depressed respiratory capacity of the heart coupled with the inhibition of protein synthesis by ethanol and its byproducts. An altered ethanol catabolism in the SWM-E animals, as seen from lower blood ethanol levels with higher ethanol consumption may allow for a greater rate of synthesis. While ethanol is primarily metabolized in the liver, an elevated catabolism of ethanol would reduce the deleterious effects throughout the body. The volume overload on the heart produced by swimming, however, may be sufficient to induce this hypertrophy whether ethanol and its by-products are present or not, as was the case with aortic stenosis [26].

The diet used in this study was offered ad libitum to all groups and all groups had normal weight gain. This is in contrast to many diets and means of administering ethanol that have resulted in weight loss in sedentary animals [17, 20, 26]. Even the energy and nutritional needs of the rats swimming up to 2 hours on the ethanol diet appear to be met, although the gastrocnemius-plantaris muscles atrophied. This loss in muscle mass during the 6 weeks of swimming may reflect elevated corticosterone levels due to chronic ethanol consumption [11] coupled with protein catabolism during exercise [5].

The findings of this study suggest that while swimming may alter the catabolism of ethanol in the diet and induce cardiac hypertrophy it is unable to offset the depression in respiration measured in cardiac mitochondria. Whether this depression in respiration measured in vitro reflects the in vivo condition is yet to be determined.

ACKNOWLEDGEMENT

This work was supported in parts with funds from the University of Texas.

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