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Hormonal and metabolic response to physical exercise, fasting and cold exposure in the rat

Effects on ketogenesis in isolated hepatocytes

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Summary. Four groups of rats were subjected to the following conditions: (1) 48 h fasting, (2) 48 h of 4° C cold exposure, (3) 5 h treadmill running, (4) 48 h fasting with 4° C cold exposure. The groups were compared to fed control rats in order to study hormonal and metabolic responses in blood and tissue samples. Isolated hepatocytes were used to evaluate the rate of ketogenesis. Decreases in liver glycogen and increases in blood free fatty acids (FFA) confirmed that glycogenolysis and lipolysis occur in these situations of metabolic stress. Increases in the glucagon/insulin plasma ratio were also noted. Plasma catecholamine levels were only enhanced after running and after cold exposure. Production of blood ketone bodies was stimulated more by running and by fasting than by cold exposure. The low ketone body production observed after cold exposure seems to be linked to increases liver glycogen levels and decreased FFA availability. Liver cells isolated after cold exposure exhibited higher ketogenesis than these isolated after running. This difference in ketogenic capacity could result both from the longer hormonal stimulation by high glucagon/insulin plasma ratios and from the metabolic state of the liver.

Key words: Prolonged physical exercise — Fasting — Cold exposure — Ketogenesis — Pancreatic hormones

Introduction

Ketogenesis is controlled by several hormonal and metabolic changes. The principal hormonal trigger is an increase in the plasma glucagon/insulin ratio, associated with depletion of liver glycogen and increased availability of plasma free fatty acids (FFA). The relative contributions of the various factors have been reviewed by Mac Garry and Foster (1980).

These conditions, which enhance production of ketone bodies, are observed after fasting, physical exercise and cold exposure (Hagenfeldt and Wahren 1968; Mac Garry and Foster 1981; Minaire et al. 1973). However, comparative studies of fasting and physical exercise have revealed differences in the kinetics of the hormonal and metabolic responses. Treadmill running induces change more rapidly than fasting in rats, so that three hours of muscular exercise induces the same decrease in plasma insulin and liver glycogen as 24 h of fasting (Guezennec et al. 1982).

Energy expenditure also varies under different conditions: running and cold exposure enhance metabolism whereas it is depressed by fasting (Drenick and Dennin 1973).

In order to confirm the effects of these time-dependent phenomena and energy expenditure differences on pancreatic hormone secretion and ketogenesis, we carried out a comparative study of physical exercise, fasting and cold exposure in rats.

Materials and methods

Male Wistar rats of mean weight 230±30 g were used in the study. Water and food (UAR 103 carbohydrates: 76%; lipids: 4%; proteins: 20%) were given to the rats until the start of each experimental protocol. The rats were divided into five groups of 15 for the study of the following physiological conditions: 48 h fasting, 48 h cold exposure (4° C), 48 h cold exposure (4° C) with fasting, 5 h treadmill running, and fed unexercised animals as controls. Animals used for physical exercise had

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been previously trained for three weeks, one hour of exercise per day. Each experiment was initiated at 9 a.m. For prolonged physical exercise tests, trained rats were placed on the treadmill and ran for 5 h at a speed of 20 $m \cdot min^{-1}$.

At the end of each test two experimental procedures were carried out. Ten animals from each group were used for the study of hormonal and metabolic responses and five animals were used to obtain isolated hepatocytes.

Experiment 1. Blood and tissue sampling. For one week prio to an experiment, all animals were handled daily to minimize stress responses to handling and to the injections carried out according to the procedure of Avakian and Horvath (1981).

Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (70 $mg \cdot kg^{-1}$ body weight). In running animals, samples were taken 3 min after cessation of running. Blood was sampled by aortic puncture with a heparinized needle. A blood aliquot was immediately deproteinized in $HClO_4$ (6% w/v) for metabolite determination, and the remaining blood was centrifuged at 4° C. Plasma was then frozen at −80° C for hormone assays. In order to determine glycogen concentrations, a small piece of liver was excised and immediately frozen in liquid nitrogen.

Experiment 2. Study of isolated liver cells. In the preparation of rat liver hepatocytes, each rat was anaesthetized according to the procedure described above. The abdomen was opened, the portal vein cannulated and the liver cells isolated and purified according to the protocol described by Berry and Friend (1969). The liver was perfused for 18 min with three successive solutions prepared from Hepes buffer pH 7.4 (Seglen 1973): the first contained EGTA, the second was prepared without EGTA, and the third contained 0.24 mg/ml collagenase (Boehringer, FRG). The solutions were not recirculated or oxygenated. After enzyme perfusion the liver was removed, finely minced, and the cell suspension was filtered through several layers of gauze. Cells were washed with Krebs's solution and were centrifuged twice at 50 g for 2 min. Isolated hepatocytes were suspended in Krebs's medium supplemented with bovine serum albumin (1%) in a 25 ml Erlenmeyer flask under an atmosphere of 5% CO_2 and 95% O_2. Cell counts were performed with a malassez cell, and cell suspensions were diluted to give a protein concentration of approximately 12 $mg \cdot ml^{-1}$.

Incubation procedure. Isolated hepatocytes were incubated at 37° C in a shaking water bath for 60 min in 25 ml Erlenmeyer flasks containing Krebs bicarbonate buffer (pH 7.4), in a final volume of 4 ml. Incubations were performed without addition of substrates in order to study oleate endogenous production, or in the presence of 1 mM oleate. Incubations were stopped by the addition of 0.5 ml of 40% (v/v) $HClO_4$. After centrifugation, ketone bodies were determined in the neutralized $HClO_4$ extracts according to the enzymatic methods of Williamson et al. (1962).

Hormone and metabolite determinations. Plasma insulin was measured by double antibody radioimmunoassay according to the method of Hales and Randle (1963) with a commercial kit (CEA SB INSI 5) containing standard insulin referenced to WHO 66/304. Plasma glucagon was determined according to the method of Faloona and Unger (1974). Plasma catecholamines were assayed according to the method of Brown and Jenner (1981).

Blood glucose, acetoacetate and D-3-hydroxybutyrate were determined in the neutralized $HClO_4$ extracts using enzymatic methods (Bergmeyer 1974; Williamson et al. 1962) and free fatty acids were determined according to the method of Ho (1970). Tissue glycogen was determined according to the method of Roehrig and Allred (1974).

Results were processed by monofactorial analysis of variance and values of $p < 0.05$ or less were considered to be significant.

Results

Blood metabolites

Physical exercise, fasting, cold exposure, and fasting during cold exposure induced several significant changes in blood metabolites (Table 1).

Minor variations in blood metabolites were noted in rats exposed to cold: slight but significant increases ($p < 0.05$) were noted in plasma FFA and blood acetoacetate.

Fasting and running induced similar changes combining decreases in blood glucose ($p < 0.02$), two-fold increases in βOH butyrate and three-fold increases in plasma FFA ($p < 0.01$).

Fasting with cold exposure seemed the most stressful situation: minimal blood glucose combined with maximal total ketone body levels (βOH butyrate + acetoacetate) and plasma FFA.

Table 1. Blood metabolic changes. The effects of 48 h fasting, 48 h fasting with 4° C cold exposure, 48 h cold exposure and 5 h of treadmill running compared to fed control rats. Results are expressed as mean values ± SEM

	Glucose $mM \cdot l^{-1}$	Acetoacetate $mM \cdot l^{-1}$	βOH butyrate $mM \cdot l^{-1}$	Free fatty acid $mM \cdot l^{-1}$
Fasting	6.19 ± 0.68*	0.183 ± 0.03*	1.70 ± 0.15*	1.28 ± 0.09*
Fasting with cold exposure	4.34 ± 0.35*	0.344 ± 0.10*	2.26 ± 0.46*	1.60 ± 0.11*
Cold exposure	7.85 ± 0.32*	0.086 ± 0.01*	0.82 ± 0.06*	0.09 ± 0.08*
Running	5.8 ± 0.67*	0.062 ± 0.01*	1.80 ± 0.18*	1.65 ± 0.07*
Control	8.45 ± 0.37	0.026 ± 0.004	0.71 ± 0.02	0.43 ± 0.11

* Result differs from control value, $p < 0.05$

Table 2. Liver glycogen. The effect of 48 h fasting, 48 h fasting with 4° C cold exposure, 48 h cold exposure and 5 h of treadmill running compared to fed control rats. Results are expressed as mean values ±SEM

	Fasting	Fasting with cold exposure	Cold exposure	Running	Control
Liver glucogen mmol glucose × kg wet wieght^{-1}	9.7±2.6*	1±0.2*	120±22*	2.5±3.5*	213±33

* $p<0.02$

Liver glycogen

Liver glycogen stores were completely depleted after fasting and after fasting with cold exposure and running ($p<0.01$), but were only reduced by half in cold exposed rats ($p<0.01$) (Table 2).

Hormonal response

Cold exposure and running increased both epinephrine and norepinephrine plasma levels compared to control values ($p<0.02$). The greatest increase in norepinephrine was observed after physical exercise, whereas cold exposure induced the largest rise in epinephrine (Table 3).

Fasting did not modify plasma catecholamine levels compared to control rats. This unchanged catecholamine level was also observed after fasting with cold exposure.

All these experimental conditions of metabolic stress induced increases in plasma glucagon and decreases in plasma insulin. The same increases in glucagon levels were noted in all cases, whereas significantly low levels of plasma insulin were observed after the two fasting experiments, as compared to running and to cold exposure ($p<0.05$).

Isolated hepatocytes

The effects of different experimental conditions on ketogenesis in isolated hepatocytes were assessed in two ways (Fig. 1):

(1) without precursor, to evaluate endogenous ketogenesis and (2) with the addition of oleate as substrate to study the production of ketone bodies.

Endogenous ketone body production in liver cells did not differ significantly in fasting, running and cold exposed rats, but was four to five fold greater than in control rats.

The addition of oleate significantly increased ketogenesis by varying amounts in all physiological states ($p<0.02$). Fasting with cold exposure enhanced oleate-induced ketogenesis in isolated hepatocytes as compared to values noted in liver cells from control rats ($p<0.05$). Fasting with cold exposure induced the highest level ($p<0.05$) of ketone body production in isolated hepatocytes (11.7 ± 10.7 μmol $h^{-1}\cdot g^{-1}$ wet material). Cold exposure and fasting enhance ketogenic capacity almost to the same extent. The values observed after incubation with oleate were respectively 80 ± 7 μmol $h^{-1}\cdot g^{-1}$ wet material after fasting and 72 ± 7 μmol $h^{-1}\cdot g^{-1}$ wet material after cold exposure. Ketone body production under these

Table 3. Hormonal levels after 48 h fasting, 48 h fasting with 4° C cold exposure, 48 h 4° C cold exposure and 5 h of treadmill running compared to fed control rats

	Epinephrine ng · ml^{-1}	Norepinephrine ng · ml^{-1}	Insulin μUi · ml^{-1}	Glucagon pg · ml^{-1}
Fasting	0.07 ±0.01	0.42±0.04	32±11.6*	672± 50*
Fasting with cold exposure	0.06 ±0.01	0.63±0.05	24± 3.6*	772±112*
Cold exposure	5.12 ±1.02*	2.11±0.99*	47± 5.3*	705± 51*
Running	4.79 ±1.06*	4.08±1.1*	55± 6.2*	657± 22*
Control	0.100±0.01	0.45±0.04	116± 7.6	258± 29

Results are expressed as mean values ±SEM
* Result differs from control value ($p<0.02$)

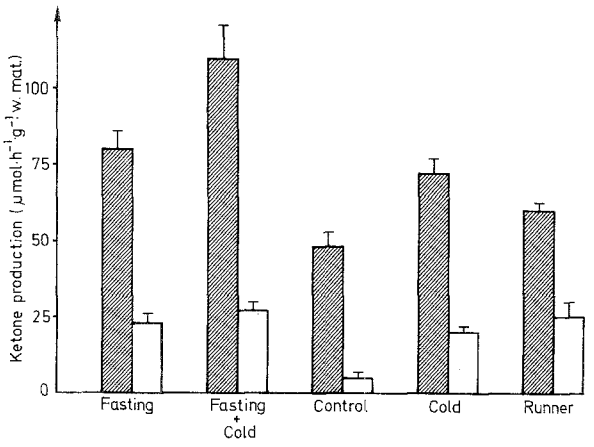

Fig. 1. Total ketone body production (βOH butyrate + acetoacetate) in isolated hepatocytes incubated with ▨ and without □ oleate. Liver cells were isolated from rats after 48 h fasting (F), 48 h fasting with 4° C cold exposure, 48 h cold exposure, 5 h of treadmill running (R) and from fed control group C

conditions was greater than in running rats ($p < 0.05$), (59 ± 4.8 µmol $h^{-1} \cdot g^{-1}$ wet material).

Discussion

The metabolic results of this study are in agreement with previous data showing that fasting, physical exercise and cold exposure enhance the breakdown of tissue glycogen and stimulate the utilization of lipid stores to maintain energy expenditure (Minaire et al. 1973; Guezennec et al. 1982; Guezennec et al. 1984).

Comparison between the four experimental conditions of metabolic stress underlined differences in depletion of liver glycogen, reduction in blood glucose levels and plasma FFA, and increases in ketone body production. The greatest changes were observed after cold exposure when fasting, and confirm the phenomenon previously described as accelerated starvation (Vallerand et al. 1983). In contrast, endogenous glycogen and lipid stores were least used following cold exposure.

Changes in pancreatic hormonal levels followed the same pattern, associating increases in plasma glucagon with decreases in plasma insulin. This observation raises two questions, one concerning the regulation of the secretion of these hormones and the other regarding the metabolic consequences of hormonal changes, particularly for ketogenesis.

According to numerous previous studies, running and cold exposure enhance plasma catecholamine responses (Galbo 1979; Jansky et al. 1976; Galbo et al. 1976).

On the other hand, as observed previously, fasting decreases sympathetic nervous system activity and suppresses catecholamine response to cold exposure in rats (Avakian and Horvath 1981). This suggest that glucopenia played the principal role in the control of pancreatic hormone secretion during the two fasting experiments.

Conversely, maintenance of blood glucose after cold exposure underlines the effect of increases in plasma catecholamines and, indirectly, in autonomic nervous system activity in suppressing insulin secretion and increasing glucagon secretion.

During running, both glucopenia and enhanced catecholamines act synergistically in pancreatic cells (Galbo et al. 1979). These simultaneous modifications explain the rapid changes in plasma pancreatic hormone levels observed following physical exercise.

Although running, fasting and cold exposure act differently on mechanisms of pancreatic hormone secretion, the plasma glucagon insulin ratio increased in all three cases. There is much evidence to show that this increase is the primary mediator in short-term control of ketogenesis (Mac Garry and Foster 1980). However, our results indicate a large difference in blood levels of ketone bodies.

Fasting with cold exposure produced an almost three-fold increase in blood ketone bodies above the levels in cold-exposed rats. Plasma glucagon levels did not differ significantly in these two cases. Two metabolic events can be evoked to explain low-level ketogenesis in cold-exposed animals: the persistence of liver glycogen stores and insufficient FFA availability. A hormonal factor could be involved, since plasma insulin levels were higher after cold exposure than after fasting, with or without cold exposure. However, the role of insulin in the acute regulation of ketogenesis is still controversial (Witters and Trasko 1979; Beynen et al. 1980).

Data from the study of isolated liver cells provide information on the role of FFA availability and the duration of exposure to high plasma glucagon/insulin ratios.

The incubation of hepatocytes with oleate provided an index of maximal liver cell ketogenesis. Under these conditions, the results obtained in isolated liver cells did not exactly reflect the differences observed in blood sampling. As in vivo, the two fasting experiments induced higher keto-

genesis in liver cells, but in contrast to the in vivo results, the isolated cells from cold-exposed rats produced more ketone bodies than those from running rats. This difference suggest that the ketogenic pathway in liver cells is more stimulated by cold exposure than by running.

The experimental protocol, which compared 5 h of running to 48 h of cold exposure before isolation of liver cells, involved differences in the time of exposure to hormonal stimulation.

Increases in plasma glucagon/insulin ratios have been observed after 24 h cold exposure in rats (Smith 1983) whereas one hour of running was sufficient to produce the same change (Galbo et al. 1979); Guezennec et al. 1982). The liver is therefore subject to ketogenic hormonal stimulation for at least 20 h before incubation of isolated liver cells. One could hypothesize that this time-related difference could involve the activity of a key enzyme such as acyl carnitine transferase. The persistence of liver glycogen stores and low levels of FFA in the blood suggests that the metabolic state of the liver after 48 h cold exposure is intermediate between rats that are fed and running and those that are starved. In these circumstances, it is likely that higher levels of malonyl CoA will be found in the livers of cold-exposed rats.

Ketone body production in isolated hepatocytes incubated without substrate was similar under the four conditions of metabolic stress. This indicates comparable ketogenesis from endogenous triglycerides in the liver cells (Debeer et al. 1979), and confirms that the ketogenic differences in oleate medium noted between running and cold-exposed rats in linked to the availability of exogenous lipids. The enhanced catecholamine responses noted after running and cold exposure and the low catecholamine levels resulting from fasting confirm that there is no marked effect of catecholamine on rat ketogenesis either in vivo or in vitro (Bahnsen et al. 1984).

The present comparative study shows that physical exercise, cold exposure and faster induce different metabolic and hormonal responses, which in turn influence ketogenesis.

The cold exposure results show that although the hormonal stimulations noted are the same as those seen after fasting and running, the blood level of ketone bodies is correlated with glycogen store levels and FFA availability. Data from the isolated liver cell study suggest that maximal ketogenesis is due both to the time of exposure to hormonal stimulation and to the metabolic state of the liver. Nevertheless, further study is necessary in order to verify the role of liver cell (fatty acyl CoA) : (malonyl CoA) ratios on acyl carnitine transferase activity after exposure to these different conditions of metabolic stress.

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