Investigations of the mechanisms of glycolytic control during hibernation

KENNETH B. STOREY
Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, Ont., Canada K1S 5B6
Received January 19, 1987

STOREY, K. B. 1987. Investigations of the mechanisms of glycolytic control during hibernation. Can. J. Zool. **65**: 3079–3083.

Molecular mechanisms of glycolytic rate control during hibernation were investigated in the meadow jumping mouse, *Zapus hudsonius*. The content of fructose-2,6-bisphosphate, a potent activator of phosphofructokinase, decreased significantly in brain, heart, and fat pad after 5–8 days of hibernation, rose in kidney, and was unchanged in skeletal muscle. Apparent covalent modification of regulatory enzymes of glycolysis during hibernation was examined in brain, heart, kidney, and skeletal muscle but occurred only in selected instances. Hibernation led to a significant reduction in the percentage of glycogen phosphorylase in the phosphorylated *a* form in brain and produced kinetic changes (altered K_a AMP, I_{50} citrate) in phosphofructokinase from heart indicative of enzyme covalent modification. No evidence for covalent modification of pyruvate kinase during hibernation was found in any tissue. Covalent modification of enzymes and alterations in fructose-2,6-bisphosphate content offer organ-specific control over glycolytic rate during hibernation in response to both the general metabolic rate depression of the hibernating state and the individual adjustments in organ fuel use.

STOREY, K. B. 1987. Investigations of the mechanisms of glycolytic control during hibernation. Can. J. Zool. **65** : 3079–3083.

Les mécanismes moléculaires du contrôle de la glycolyse ont été étudiés au cours de l'hibernation chez la Souris sauteuse des champs, *Zapus hudsonius*. Après 5–8 jours d'hibernation, le contenu en fructose-2,6-diphosphate, un puissant activateur de la phosphofructokinase, diminue significativement dans le cerveau, le coeur et le coussin adipeux, augmente dans le rein et reste stable dans le muscle squelettique. La modification covalente apparente des enzymes régulateurs de la glyclolyse durant l'hibernation a été examinée dans le cerveau, le cœur, le rein et le muscle squelettique, mais cette modification ne se produit que dans des conditions particulières. L'hibernation aboutit à une réduction significative du pourcentage de la glycogène-phosphorylase présente dans sa forme phosphorylée *a* dans le cerveau et provoque des changements cinétiques (de K_a AMP et de la concentration I_{50} de citrate) dans la phosphofructokinase venant du cœur, ce qui indique une modification covalente de l'enzyme. Il ne semble pas y avoir de modification covalente de la pyruvate kinase au cours de l'hibernation, dans aucun des tissus. La modification covalente des enzymes et les changements dans le contenu de fructose-2,6-diphosphate permettent un contrôle du taux de glycolyse spécifique à chaque organe au cours de l'hibernation, en réaction à la diminution du taux métabolique général due à l'état d'hibernation d'une part, et aux ajustements individuels de l'énergie utilisée dans chaque organe d'autre part.

[Traduit par la revue]

Introduction

Winter survival for many types of animals depends upon the induction of a hypometabolic state to conserve fuel reserves and limit energy expenditures. Profound metabolic rate depression characterizes winter dormancy and diapause in ectotherms and hibernation in endotherms. A similar depression of metabolic rate is the basic strategy for survival under other environmental stresses (e.g., low oxygen, low water) and appears in facultative anaerobiosis, anhydrobiosis, estivation, and torpor. The molecular mechanisms underlying the depression of metabolic rate in cells have only recently begun to be studied (reviewed by Storey 1988; Hochachka and Guppy 1987). Principles are emerging that cut across phylogenetic lines. Molecular controls involved in glycolytic rate depression during hypometabolism include: (*i*) covalent modification (via phosphorylation–dephosphorylation reactions) of regulatory enzymes to produce less active enzyme forms, (*ii*) dissociation of enzymes from complexes bound to subcellular particles, and (*iii*) withdrawal of fructose-2,6-bisphosphate (F-2,6-P_2) activation of phosphofructokinase (PFK) to limit the anabolic uses of carbohydrate in the depressed state (Storey 1985*a*, 1985*b*, 1988; Plaxton and Storey 1984, 1986). Low intracellular pH accompanies metabolic depression in many instances and appears to help trigger and (or) facilitate these responses (Busa and Nuccitelli 1984). These mechanisms are widely applicable to facultative anaerobiosis in both vertebrates and invertebrates (marine molluscs, goldfish, turtles) and also appear to be involved in estivation in snails (Storey 1988, and unpublished data). Do they also participate in metabolic depression in hibernating mammals?

Small mammal hibernators abandon homeothermy and strongly depress their metabolic rate (to levels ranging from 1.9 to 4.6% of euthermic controls) and body temperature to survive the winter with limited fuel reserves (Deavers and Musacchia 1980; Snapp and Heller 1981). The result is energy savings of about 90% compared with the energy that would be required to maintain euthermia throughout the winter (Wang 1978). Both nervous and endocrine triggers appear to be involved in entry into hibernation (Hudson and Wang 1979), and a respiratory acidosis may facilitate the metabolic changes occurring (Snapp and Heller 1981; Malan 1985). A lowering of the hypothalmic set point and peripheral vasodilation promote the drop in body temperature (Snapp and Heller 1981). At the cellular level, rates of metabolic processes must be regulated with respect to two needs. (*i*) Thermogenesis must be sharply reduced: rates of exothermic reactions must be reduced, particularly those whose prime function is metabolic heat generation; and (*ii*) metabolism must be reorganized for the hypometabolic state: rates of ATP-producing, ATP-utilizing, and passive processes must be rebalanced and hibernation-specific metabolic requirements (e.g., reorganization of fuel use) must be instated. Neither of these can be the result of simple Q_{10} effects on rate processes during entry into hibernation because low temperature is the consequence (not the cause) of the depressed metabolic state. Rather, specific biochemical controls must be activated to regulate key processes and produce the coordinated action required to reestablish homeostasis in the hypometabolic, hypothermic state (Willis 1979). Evidence of the specific depression of various metabolic processes in the hibernator is now accumulating (Yacoe 1983; Fedotcheva et al. 1985).

We begin an investigation of the molecular mechanisms

underlying metabolic rate depression in hibernators by analyzing the roles of covalent modification of regulatory enzymes and fructose-2,6-bisphosphate control of PFK in glycolytic control in the meadow jumping mouse, *Zapus hudsonius*. This species spends about 7 months of the year in profound hibernation without feeding during periodic bouts of arousal (Whitaker 1972). An initial study of liver metabolism revealed that glycogen phosphorylase, PFK, and pyruvate kinase (PK) all exhibited altered kinetic properties in hibernating animals, changes consistent with the covalent modification inactivation of these enzymes, and serving metabolic rate depression and (or) a metabolic reorganization to preserve carbohydrate fuel reserves in the hibernating state (Storey 1987). The present study extends this same analysis of glycolytic control during hibernation to other organs.

Materials and methods

Animals

Meadow jumping mice, *Z. hudsonius* Zimmerman, were collected by livetrapping in southern Ontario and were held in a laboratory colony at York University for 2–3 months before use. Details of animal holding are as in Collins and Cameron (1984). Briefly, for long-term holding, environmental conditions were 23°C, "natural" day length (adjusted once per week to reflect outdoor conditions), and *ad libitum* access to water and a diet of sunflower seeds, Purina rabbit chow, and rolled oats. Control mice were sampled from these conditions on the same day that hibernators were sampled. To induce hibernation, mice were moved to a cold room with a daily temperature cycle between 6 and 12°C. Natural day length was maintained, and water and rabbit chow were available *ad libitum*. Most animals entered hibernation within 24 h under these conditions. For enzyme studies, mice were sampled after 5–8 days of continuous hibernation (variation in the length of hibernation reflected the individual behaviour of animals). For F-2,6-P_2 determinations, a group of short-term hibernators were also sampled after less than 24 h of hibernation.

Animals were killed by decapitation. Tissues were immediately excised and frozen in liquid nitrogen. For long-term storage, tissues were transferred to a freezer at −80°C.

Chemicals

Biochemicals and coupling enzymes were from Boehringer Mannheim Corp., Montreal, or Sigma Chemical Co., St. Louis. Potato PP_i-phosphofructokinase was from Sigma.

Measurement of fructose-2,6-bisphosphate

Samples of frozen tissue were ground to a powder under liquid nitrogen. Extraction and assay of F-2,6-P_2 followed the method of van Schaftingen (1984) involving tissue extraction in hot (80°C) 50 mM NaOH followed by determination of the content of bisphosphate based on the ability of extracts to activate the PP_i-linked phosphofructokinase from potato tubers. That enzyme activation was due to F-2,6-P_2 alone was confirmed when activation was lost after acid treatment of samples.

Enzyme assay

Small portions of tissue (100–200 mg) were ground to a powder under liquid nitrogen, rapidly weighed, and then immediately homogenized (1:5 w/v) in ice-cold 50 mM imidazole–HCl buffer, pH 7.0, containing 15 mM 2-mercaptoethanol, 100 mM NaF, 5 mM EDTA, 5 mM EGTA, and 0.1 mM phenylmethylsulphonyl fluoride (PMSF), using a Polytron PT10 homogenizer. A 50-μL aliquot of the homogenate was withdrawn for measurement of glycogen phosphorylase activity. Remaining homogenate was centrifuged at 27 000 × *g* for 20 min at 4°C. Supernatant was removed and dialyzed for 2 h at 4°C against homogenizing buffer minus PMSF and was then used for studies of PFK and PK.

All assays were performed on a Pye Unicam SP8-100 recording spectrophotometer at 23°C. An assay temperature intermediate between euthermic and hibernating body temperature was used but this did not affect the detection of covalent modification because phosphorylation and dephosphorylation are stable enzyme modifications.

TABLE 1. Effect of hibernation on fructose-2,6-bisphosphate concentrations in tissues of the meadow jumping mouse, *Zapus hudsonius*

	Control	Hibernating	
		Short term	Long term
Brain	6.20±0.53	5.93±0.26	4.45±0.54**
Kidney	1.58±0.37	1.76±0.36	5.48±1.08**
Heart	0.39±0.07	0.23±0.08	0.10±0.03*
Skeletal muscle	1.62±0.17	1.37±0.21	1.04±0.16
Fat pad	0.58±0.17	—	0.19±0.03**

NOTE: Two lengths of hibernation were tested: short term was less than 24 h of hibernation; long term was 5 to 8 days of hibernation (tissues from long-term animals were studied for enzyme properties (Tables 1, 2, and 4)). Values are means ± SEM (in nmol/g wet weight), $n = 5$ for control and short-term hibernation, $n = 8$ for long-term hibernation.
*Significantly different from control, by Student's *t*-test, $P < 0.01$.
**Significantly different from control by Student's *t*-test, $P < 0.05$.

Glycogen phosphorylase

Phosphorylase *a* activity was determined in 50 mM potassium phosphate buffer, pH 7.0, 2 mg/mL glycogen (previously dialyzed), 0.4 mM NADP, 0.25 mM EDTA, 4 μM glucose-1,6-bisphosphate, 15 mM $MgCl_2$, and excess dialyzed phosphoglucomutase and glucose-6-phosphate dehydrogenase. For measurement of total phosphorylase, 1.6 mM AMP was added. Enzyme activity was measured in the original homogenate after allowing large particles to settle.

Phosphofructokinase

Conditions for maximal activity were 50 mM imidazole–HCl buffer, pH 7.0, 10 mM fructose-6-phosphate (F-6-P), 0.5 mM ATP, 0.2 mM NADH, 5 mM $MgCl_2$, 50 mM KCl, and excess dialyzed aldolase, triosephosphate isomerase, and glycerol-3-phosphate dehydrogenase.

Pyruvate kinase

Conditions for maximal activity were 50 mM imidazole–HCl buffer, pH 7.0, 5 mM phosphoenolpyruvate, 5 mM ADP, 0.2 mM NADH, 10 mM $MgCl_2$, 100 mM KCl, and excess dialyzed lactate dehydrogenase.

$S_{0.5}$ and n_H values were determined from Hill plots using experimentally measured V_{max} values. I_{50} values were determined from plots of v versus [inhibitor], and apparent K_a values from plots of v versus [activator] at subsaturating F-6-P or phosphoenolpyruvate.

Results

Fructose-2,6-bisphosphate

Levels of the bisphosphate were measured in tissues of control animals and in animals hibernating for short (less than 1 day) or long (5–8 days) periods (Table 1). During short-term hibernation, F-2,6-P_2 content did not change in any of the tissues tested. However, long-term hibernation led to a significant drop in F-2,6-P_2 content in brain, heart, and fat pad (to levels 72, 26, and 33% of control values, respectively), whereas F-2,6-P_2 content rose 3.5-fold in kidney.

Glycogen phosphorylase

The effect of long-term hibernation (5–8 days) on the activity of glycogen phosphorylase in four tissues of *Z. hudsonius* is shown in Table 2. In brain, the percentage of glycogen phosphorylase in the active, phosphorylated *a* form decreased during hibernation, from 57% in control to 25% in hibernating mice, suggesting reduced glycogenolysis in the brain of the hibernator. Kidney, heart, and skeletal muscle, however, showed no significant changes between euthermic and hibernating animals in either the total activity of phosphorylase expressed or the percentage of enzyme in the *a* form.

Phosphofructokinase

Selected kinetic parameters, known to be altered by covalent modification of PFK in other mammalian systems, were chosen for analysis (Foe and Kemp 1982; Sakakibara and Uyeda

TABLE 2. Effect of hibernation on glycogen phosphorylase in tissues of the meadow jumping mouse, *Zapus hudsonius*

	Control			Hibernating		
	a	*a* + *b*	% *a*	*a*	*a* + *b*	% *a*
Brain	0.73±0.12	1.26±0.13	57.2±6.5	0.33±0.06*	1.13±0.19	25.3±3.2*
Kidney	0.21±0.07	0.32±0.09	63.6±5.0	0.27±0.07	0.58±0.07	46.1±8.4
Heart	1.33±0.15	5.34±0.47	25.3±1.2	1.68±0.61	6.59±1.30	23.7±3.7
Skeletal muscle	1.58±0.35	9.13±0.45	13.3±3.6	1.84±0.42	10.8±1.32	17.1±3.0

NOTES: Activities of phosphorylase *a* and total (*a* + *b*) phosphorylase are given as micromoles glucose-1-phosphate produced per minute per gram wet weight. Values are means ± SEM; $n = 4$ for kidney and muscle, $n = 3$ for heart, and $n = 5$ for control and 7 for hibernating, for brain.

*Value significantly different from control by Student's *t*-test, $P < 0.01$.

TABLE 3. Effect of hibernation on the kinetic properties of phosphofructokinase in tissues of the meadow jumping mouse, *Zapus hudsonius*

	Brain	Kidney	Heart	Skeletal muscle
V_{max}, U/g wet wt.				
Control	6.28±1.40	3.16±0.60	13.4±1.30	21.0±8.5
Hibernating	6.96±0.42	3.22±1.30	12.3±1.20	18.3±4.2
$S_{0.5}$ fructose-6-phosphate, mM				
Control	0.27±0.04	1.55±0.35	0.20±0.04	0.16±0.06
Hibernating	0.34±0.06	0.99±0.19	0.19±0.02	0.12±0.03
n_H				
Control	2.50±0.29	1.51±0.28	1.15±0.20	2.45±0.47
Hibernating	2.40±0.11	1.56±0.21	1.40±0.20	3.90±0.33
K_a AMP, μM				
Control	60±8	33±9	37±3	20±28
Hibernating	54±8	42±2	60±2*	17±2
K_a fructose-2,6-bisphosphate, nM				
Control	115±21	65±16	50±5	19±2
Hibernating	86±12	72±20	45±3	25±14
I_{50} ATP, mM				
Control	1.10±0.09	2.57±0.70	3.34±0.73	1.80±0.30
Hibernating	0.91±0.08	1.94±0.37	2.64±0.45	1.71±0.26
I_{50} citrate, mM				
Control	0.05±0.003	0.62±0.21	0.49±0.10	0.25±0.04
Hibernating	0.06±0.016	1.27±0.47	1.05±0.10**	0.37±0.11

NOTE: Values are given in units of micromoles fructose-6-phosphate utilized per minute. Fructose-6-phosphate concentrations were subsaturating for K_a and I_{50} determinations: 0.1 and 0.2 mM for brain, 0.1 and 0.8 mM for kidney, 0.05 and 0.8 mM for heart, and 0.05 and 0.4 mM for skeletal muscle, respectively. For I_{50} determinations, inhibitors were added, mixed with magnesium in the following ratios: 1:1 for Mg^{2+}: ATP and 2:1 for Mg^{2+}: citrate. Data are means ± SEM; $n = 4$ for heart and skeletal muscle, and $n = 4$ and 7 for brain and 3 and 4 for kidney, for control versus hibernating, respectively.

*Value significantly different from control by Student's *t*-test, $P < 0.01$.

**Value significantly different from control by Student's *t*-test, $P < 0.05$.

1983). Enzyme maximal velocity (V_{max}), affinity for F-6-P ($S_{0.5}$ and n_H), K_a for activators (AMP, F-2,6-P_2), and I_{50} for inhibitors (ATP, citrate) were compared in tissues from control versus hibernating *Z. hudsonius* (Table 3). PFK from heart of hibernators showed significantly different kinetic properties than that of controls, both K_a for AMP and I_{50} for citrate being increased 2-fold. The kinetic properties of PFK in brain, kidney, and skeletal muscle, however, were unaffected by the hibernating state.

Pyruvate kinase

Analysis of selected kinetic properties of PK in organs of control versus hibernating *Z. hudsonius* is shown in Table 4. The PK activity in kidney showed allosteric properties although none of the kinetic parameters measured were affected by the hibernating state. Mammalian kidney typically contains a mixture of three PK isozymes (K, M, and L forms) (Engstrom 1978); covalent modification of only one of these forms (e.g., the L form, as determined for *Z. hudsonius* liver PK (Storey 1987)) might, perhaps, have been undetected in the present study but more likely should have produced some measurable change in the kinetic properties assayed, even in a mixture of isozymes. Heart, skeletal muscle, and brain all showed nonallosteric enzymes (unaffected by F-1,6-P_2 or alanine), the typical M-type found in these tissues in other mammals (Engstrom 1978). Neither V_{max} nor $S_{0.5}$ for phosphoenolpyruvate in these tissues was altered as a result of hibernation.

Discussion

Hibernation requires a variety of metabolic adaptations including those that regulate and coordinate a profound metabolic depression as well as those that reorganize metabolism for

TABLE 4. Effect of hibernation on the kinetic properties of pyruvate kinase in tissues of the meadow jumping mouse, *Zapus hudsonius*

	Brain	Kidney	Heart	Skeletal muscle
V_{max}, U/g wet wt.				
Control	114±23	64±16	150±46	342±89
Hibernating	127±16	76±16	182±43	301±72
$S_{0.5}$ phosphoenolpyruvate, mM				
Control	0.09±0.01	0.61±0.06	0.13±0.05	0.09±0.01
Hibernating	0.01±0.02	0.64±0.04	0.10±0.03	0.08±0.01
K_a fructose-1,6-bisphosphate, μM				
Control	NE	0.16±0.11	NE	NE
Hibernating	NE	0.21±0.14	NE	NE
I_{50} L-alanine, mM				
Control	>50	2.76±0.35	NE	NE
Hibernating	>50	3.00±0.80	NE	NE

NOTE: Values are given in units of micromoles phosphoenolpyrvate utilized per minute. Values for the Hill coefficient, n_H, were not significantly different from 1.0 in any case. K_a and I_{50} values were determined at subsaturating (0.05 mM) concentrations of phosphoenolpyruvate. Data are means ± SEM; n = 3 for kidney and heart, and n = 5 and 7 for brain and 4 and 5 for skeletal muscle, for control and hibernating, respectively. NE, the modifier F-1,6-P_2 or alanine had no effect on pyruvate kinase.

long-term survival at a reduced body temperature. Changes in fuel use are well known: lipid oxidation becomes primary, ketosis occurs, carbohydrate is spared, and gluconeogenesis from amino acids is increased (Hochachka and Somero 1984). Glycolytic rate in most or all tissues should be reduced. Glycolytic rate control in other instances of facultative hypometabolism has been linked (to date) to three regulatory mechanisms: covalent modification of regulatory enzymes, enzyme complex formation, and F-2,6-P_2 control of PFK (Storey 1985*a*, 1988; Plaxton and Storey 1984, 1986) influenced, perhaps, by a low pH environment occurring in the hypometabolic state (Busa and Nuccitelli 1984). Glycolysis in liver of hibernating *Z. hudsonius* is certainly affected by covalent modification of enzymes (Storey 1987). Kinetic changes indicative of enzyme inactivation appeared within 24 h for both glycogen phosphorylase (an 11.4-fold drop in activity of phosphorylase *a*) and PFK (I_{50} values for ATP and citrate dropped 4- and 3.7-fold, respectively), and persisted with long-term hibernation. For PK, similar indications of covalent modification appeared over the long term (K_a F-1,6-P_2 increased 4.4-fold, I_{50} alanine dropped 6.3-fold). These changes in liver may be related both to metabolic depression and to changes in fuel use in the hibernating state. Thus, the effect on liver PK in the hibernator was equivalent to that typically occurring during starvation and probably has the same function: phosphorylation inactivation of PK promotes gluconeogenesis (Engstrom 1978). The responses of glycogen phosphorylase, PFK, and F-2,6-P_2 in liver of the hibernator were not, however, those typical of starvation, and are probably linked, instead, to hibernation-specific functions such as metabolic depression.

The present study extended the analysis of glycolytic control during hibernation to determine whether covalent modification of glycolytic enzymes and F-2,6-P_2 control of PFK were general mechanisms of metabolic regulation in other organs of the hibernator. The data do not indicate a widespread involvement of covalent modification in altering the activity of glycolytic enzymes for the hypometabolic, hibernating state. Rather, specific instances of enzyme covalent modification in individual organs probably underlie organ-specific adjustments in metabolic activities or fuel use during hibernation. Similarly, glycolytic control via hibernation-induced changes in organ F-2,6-P_2 content does not occur in all instances. F-2,6-P_2 content is depressed in three tissues (brain, heart, and fat pad), raised in one (kidney), and unaltered in two others (skeletal muscle and liver) (Table 1) (Storey 1987). Such changes are again probably related to organ-specific metabolic adjustments during hibernation that may include, for three tissues, a role in reducing PFK activity and, with it, glycolytic rate.

A dephosphorylation inactivation of glycogen phosphorylase was apparent in brain. The probable function of this modification is in preserving brain glycogen reserves at a time when exogenous substrate availability is changing and brain oxidative metabolism switches from a total dependence on blood glucose to an increasing use of ketones (Krilowicz 1985).

Probable covalent modification of heart PFK during hibernation was also indicated by a 2-fold rise in both the K_a AMP and I_{50} citrate of the enzyme in hearts of hibernators. Epinephrine-stimulated phosphorylation of rat heart PFK produces a more active enzyme form (opposite to the response of liver PFK to phosphorylation), which is less sensitive to inhibitors (Narabayashi et al. 1985). The kinetic changes in heart PFK from *Z. hudsonius* as a result of hibernation suggest, therefore, a dephosphorylation of the enzyme to produce a less active enzyme form in the hibernating state. However, full determination of the physiological significance of covalent modification of PFK in hearts of hibernators requires complete studies of the purified enzymes (control versus hibernating) to analyze the kinetic properties of both high and low phosphate forms with respect to high and low temperature, pH change, and known changes in the levels of effectors (particularly F-2,6-P_2) during hibernation.

Fructose-2,6-bisphosphate is an extremely important regulator of glycolytic flux. In general, high F-2,6-P_2 signals the abundance of glucose and facilitates the use of carbohydrate for biosynthetic purposes (Hue 1983). Cellular content of the compound is modulated in response to a wide variety of extracellular stimuli that modulate 6-phosphofructo-2-kinase activity via phosphorylation–dephosphorylation reactions (Hue 1983). F-2,6-P_2 content is rapidly reduced under conditions (e.g., starvation, anoxia) in which carbohydrate must be preserved as an energy reserve (Hue 1982; Storey 1985*b*). The present study shows that modulation of F-2,6-P_2 is also an

important factor in organ-specific glycolytic control during hibernation. Although a role in the initiation of metabolic depression is not indicated (short-term hibernation, <24 h, did not alter F-2,6-P_2 in any organ), decreased content of F-2,6-P_2 in brain, heart, and fat pad over the longer term would contribute to a reduction in PFK activity and a depression of glycolytic activity as hibernation became prolonged. This may be related to changes in fuel use (promoting carbohydrate sparing) with long-term hibernation. In addition, reduced F-2,6-P_2 levels can leave PFK susceptible to the negative effects of the lower pH environment (relative acidosis) of the hibernating state. PFK is highly sensitive to pH changes over the physiological range and shows pronounced inactivation below pH 7.5 (Bock and Frieden 1976); F-2,6-P_2 reversal of this low pH sensitivity appears to be the mechanism that allows continued PFK function at reduced pH in working muscle (Dobson et al. 1986). In the hibernator, however, interacting effects of reduced F-2,6-P_2 and low cellular pH, perhaps combined with the tendency of low pH and low temperature to cause dissociation of the active tetramer (Hand and Somero 1983), could sum to reduce PFK activity and glycolytic flux during hibernation.

In general, then, the present study indicates that changes in the phosphorylation state of glycolytic enzymes and changes in the concentration of the PFK activator, F-2,6-P_2, are not broad-based mechanisms of glycolytic control in organs of a hibernating mammal as they are in other instances of facultative hypometabolism. Rather, these controls are applied more selectively, in an organ-specific manner, to individualize the response of glycolysis with respect to both the hypometabolic state and the relative role of carbohydrate catabolism, compared with other fuels, in supporting energy requirements during hibernation. It is interesting to speculate, however, that covalent modification would be an extremely useful mechanism for controlling other hibernation-specific events such as the reversible inactivation of ion pumps and ion channels as a means of reducing thermogenesis.

Acknowledgements

The author is indebted to Dr. D. M. Cameron of York University for providing the animals, protocols, and facilities used for the hibernation experiments. The excellent technical assistance of Mrs. S. A. Walters is greatly appreciated. This study was supported by operating grants from the Natural Sciences and Engineering Research Council of Canada and from Carleton University Faculty of Graduate Studies and Research.

BOCK, P. E., and FRIEDEN, C. 1976. Phosphofructokinase. I. Mechanism of pH-dependent inactivation and reactivation of the rabbit muscle enzyme. J. Biol. Chem. **251**: 5630–5636.

BUSA, W. B., and NUCCITELLI, R. 1984. Metabolic regulation via intracellular pH. Am. J. Physiol. **246**: R409–R438.

COLLINS, V. E., and CAMERON, D. M. 1984. The effects of diet and photoperiod on hibernation in the woodland jumping mouse, *Napaeozapus insignis* (Miller). Can. J. Zool. **62**: 1938–1945.

DEAVERS, D. R., and MUSACCHIA, X. J. 1980. Water metabolism and renal function during hibernation and hypothermia. Fed. Proc. Fed. Am. Soc. Exp. Biol. **39**: 2969–2973.

DOBSON, G. P., YAMAMOTO, E., and HOCHACHKA, P. W. 1986. Phosphofructokinase control in muscle: nature and reversal of pH-dependent ATP inhibition. Am. J. Physiol. **250**: R71–R76.

ENGSTROM, L. 1978. The regulation of liver pyruvate kinase by phosphorylation–dephosphorylation. Curr. Top. Cell. Regul. **13**: 29–51.

FEDOTCHEVA, N. J., SHARYSHEV, A. A., MIRONOVA, G. D., and KONDRASHOVA, M. N. 1985. Inhibition of succinate oxidation and K^+ transport in mitochondria during hibernation. Comp. Biochem. Physiol. B, **82**: 191–195.

FOE, L. G., and KEMP, R. G. 1982. Properties of phospho and dephospho forms of muscle phosphofructokinase. J. Biol. Chem. **257**: 6368–6372.

HAND, S. C., and SOMERO, G. N. 1983. Phosphofructokinase of the hibernator *Citellus beecheyi*: Temperature and pH regulation of activity via influences on the tetramer–dimer equilibrium. Physiol. Zool. **56**: 38–388.

HOCHACHKA, P. W., and GUPPY, M. 1987. Metabolic arrest and the control of biological time. Harvard University Press, Cambridge, MA.

HOCHACHKA, P. W., and SOMERO, G. N. 1984. Biochemical adaptation. Princeton University Press, Princeton, NJ.

HUDSON, J. W., and WANG, L. C. H. 1979. Hibernation: endocrinologic aspects. Annu. Rev. Physiol. **41**: 287–303.

HUE, L. 1982. Role of fructose-2,6-P_2 in the stimulation of glycolysis by anoxia in isolated hepatocytes. Biochem. J. **206**: 359–365.

———— 1983. Role of fructose-2,6-P_2 in the regulation of glycolysis. Biochem. Soc. Trans. **11**: 246–247.

KRILOWICZ, B. L. 1985. Ketone body metabolism in a ground squirrel during hibernation and fasting. Am. J. Physiol. **249**: R462–R470.

MALAN, A. 1985. pH as a control factor in hibernation. *In* Living in the cold. *Edited by* H. C. Heller, X. J. Musacchia, and L. C. H. Wang. Elsevier Science Publishing Co., New York. pp. 61–70.

NARABAYASHI, H., RANDOLPH-LAWSON, J. W., and UYEDA, K. 1985. Regulation of phosphofructokinase in perfused rat heart. Requirement for fructose-2,6-bisphosphate and a covalent modification. J. Biol. Chem. **260**: 9750–9758.

PLAXTON, W. C., and STOREY, K. B. 1984. Purification and properties of aerobic and anoxic forms of pyruvate kinase from red muscle tissue of the channeled whelk, *Busycotypus canaliculatum*. Eur. J. Biochem. **143**: 257–265.

———— 1986. Glycolytic enzyme binding and metabolic control in anaerobiosis. J. Comp. Physiol. B, **156**: 635–640.

SAKAKIBARA, R., and UYEDA, K. 1983. Differences in the allosteric properties of pure low and high phosphate forms of phosphofructokinase from rat liver. J. Biol. Chem. **258**: 8656–8662.

SCHAFTINGEN, E. VAN. 1984. D-Fructose 2,6-bisphosphate. *In* Methods of enzymatic analysis. Vol. 6. *Edited by* H. U. Bergmeyer. Verlag Chemie, Weinheim. pp. 335–341.

SNAPP, B. D., and HELLER, H. C. 1981. Suppression of metabolism during hibernation in ground squirrels (*Citellus lateralis*). Physiol. Zool. **54**: 297–307.

STOREY, K. B. 1985*a*. A re-evaluation of the Pasteur effect: New mechanisms in anaerobic metabolism. Mol. Physiol. **8**: 439–461.

———— 1985*b*. Fructose-2,6-bisphosphate and anaerobic metabolism in marine molluscs. FEBS Lett. **181**: 245–248.

———— 1987. Regulation of liver metabolism by enzyme phosphorylation during mammalian hibernation. J. Biol. Chem. **262**: 1670–1673.

———— 1988. Suspended animation: the molecular basis of metabolic depression. Can. J. Zool. **66**. In press.

WANG, L. C. H. 1978. Energetic and field aspects of mammalian torpor: The Richardson's ground squirrel. *In* Strategies in cold. Natural torpidity and thermogenesis. *Edited by* L. C. H. Wang and J. W. Hudson. Academic Press, New York. pp. 109–145.

WHITAKER, J. O. 1972. *Zapus hudsonius*. Mammal. Species, **11**: 1–7.

WILLIS, J. S. 1979. Hibernation: cellular aspects. Annu. Rev. Physiol. **41**: 275–286.

YACOE, M. E. 1983. Adjustments of metabolic pathways in the pectoralis muscles of the bat, *Eptesicus fuscus*, related to carbohydrate sparing during hibernation. Physiol. Zool. **56**: 648–658.