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Metabolic Support of the Flight Promptness of Birds

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ABSTRACT. Promptness in the flying of birds has a chemical support in the metabolic design of glycolysis. We present here results that demonstrate a good concordance between metabolic response time (transition and relaxation times) of breast muscle glycolysis and the kind of flight behaviour in four species of birds: rock dove and turtle dove as long-flight birds and hen and red-legged partridge as short-flight sprinter birds. Glycolysis in long-flight birds has a very high basal activity, but its activation (by means of increasing hexokinase activity) is low and very slow. On the contrary, glycolysis of the short-flight sprinter birds has a low basal activity, but its activation is large and very quick, showing a high metabolic reprise. These results demonstrate that the development of either aerobic or anaerobic energetic metabolism has been two different evolutionary targets that make different physiological roles possible. COMP BIOCHEM PHYSIOL 113B, 439–443, 1996.

KEY WORDS. Transition time, flight promptness, metabolic response time, metabolic promptness, glycolysis

INTRODUCTION

Response time of muscular mechanical work is one of the most interesting features that differentiates the behaviour of animals and determines a number of different adaptability possibilities. Regarding the role of cellular metabolism, as the chemical machinery behind every biological function, one should expect to find a close relationship between physiological time and metabolic response time of glycolysis, a very important metabolic pathway in providing the energy supply for mechanical work.

It is well known that there are two kinds of animal tissues with respect to their oxygen consumption (aerobic and anaerobic); it is also well known that there is a concordance between such cells and their particular design of glycolysis: aerobic cells are well coupled with the Krebs citric acid cycle and anaerobic cells are coupled with an important flux towards lactate (7). There is also a good concordance with the most abundant lactate dehydrogenase (LDH) isoenzyme in such tissues. In aerobic tissues, such as the brain, heart and lung, LDH-1 (B_4) is the most abundant, whereas LDH-5 (A_4) is the main isoenzyme in anaerobic tissues, such as liver and many skeletal muscles. Wilson *et al.* (20) (see also ref. 10) studied the LDH activity in the breast muscle of several duck species. They classified those birds into two classes regarding their flight behaviour: migrating and non-migrating. The analysis of LDH isoenzymes showed that migrating ducks habituated to long-distance flights and had ≥45% of LDH-1 (aerobic) in their breast muscle, whereas non-migrating ducks, which do not necessitate such a high activity in aerobic metabolism, had <5% of this isoenzyme.

Anaerobic glycolysis requires a big glycogen reserve and cannot be maintained for long periods of time, because glycogen storage is quickly depleted in this way. However, this procedure has an important advantage that is decisive for fitness and is impossible to obtain through other pathways of energetic metabolism: it is a very quick way to get ATP. Meléndez-Hevia *et al.* (14) demonstrated recently that in metabolic processes under the same enzyme kinetic features, the shorter the pathway, the shorter its transition time, allowing a quicker metabolic response. The response time of anaerobic glycolysis (which is not coupled to the Krebs cycle and the respiratory chain) is shorter than any aerobic pathway. It has few steps and few intermediates, and thus it needs less time to reach a new steady state under any activation. Optimization of glycogen structure for a large glucose storage and its quick breakdown has been demonstrated by Meléndez-Hevia *et al.* (13).

Animal movement can be classified into two different groups: distance runners (or flyers) and sprinters. The first group is characterized by a capacity to maintain a large consumption of energy over a prolonged period of time. On the other hand, the main feature of the second group is a short response time and a capacity for rapid acceleration. In contrast to their advantages, the distance runners are slow to accelerate and the sprinters cannot maintain a high velocity for long

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time. It is well known that long-distance running animals have a high aerobic metabolism activity (see ref. 11). Anaerobic glycolysis should be, therefore, an ideal mechanism to serve as the chemical support of a quick macroscopic response of the sprinter animals. In accordance with all of the above reasoning, a relationship should also exist between metabolic response time of the glycolytic pathway and the kind of flight conduct in birds adapted to these two different roles. Metabolic response time of glycolysis should be short in short-flight birds and long in long-flight birds. The aim of the present work was to investigate this concordance by means of a comparative study of the metabolic response time behaviour of the glycolytic pathway among long-flying and short-flying birds.

The theory of the metabolic response time and its control has been well developed in the last years (1,5,8,12,19), but there has been few experimental works presenting an application of it to a particular metabolic system (18) and none deriving any relationship between the microscopic metabolic response time and the macroscopic physiological response behaviour. Here, we use the term "metabolic response time" to express the general time features of a metabolic system that turns to a different state under a given stimulus. This has been described in the literature by means of two variables (8,9,12,16,18,19): transition time t, whose definition, based on mass conservation, is the quotient between the whole pool of intermediates ($\sigma = \Sigma S_j$) over the flux (J) at steady state, that is, $\tau = \sigma/J$, and t_{99} (relaxation time), which is the time necessary for the flux of the end product release to reach the 99% of its steady-state value (Fig. 1). Here, we analyse the value of these variables in glycolysis of breast muscles from different flying and non-flying birds. In this work, we analyse these variables in the response of glycolysis under a high activation in the breast muscle of four species of birds. Our results show a good concordance between the flight promptness of each one of the studied species and their particular metabolic features with regard to the response time of glycolysis: long-flying birds have a high basal glycolytic activity in breast muscle but a low and slow activation capability. On the contrary, sprinter birds have low basal glycolytic activity, but their response to stimulus is much larger and quicker.

MATERIALS AND METHODS

The experimental model for this study was designed to determine the differences between response times in metabolic functions that can point out the specific features of the kinetic structure of glycolysis by modifying one previously described by us (17). This consists of a soluble fraction of breast muscle that converts glucose into triose phosphate through the first steps of glycolysis by means of the enzymes of bird muscle cells.

The functioning of this metabolic system was assayed in a spectrophotometer by a continuous recording of NADH decay in the glycerol phosphate dehydrogenase reaction from reaction start time, thus obtaining values of flux and response time. The system was fed with glucose, and its conversion into triose phosphate was shortened by driving the carbon flux towards glycerol phosphate by means of auxiliary enzymes and NADH. Two different kinds of transients can be considered when a metabolic system evolves towards a given steady state (Fig. 1): when the system starts "empty" of intermediates and when it changes from a given state to another one. The first is a "non-real" transition, usually promoted by the addition of the first substrate, that does not have any real physiological meaning but might tell us some information about the kinetic features of the system (see, e.g., 8,19). The second usually occurs as a consequence of a given change in any enzyme activity that promotes a certain difference in the steady-state flux. This transient between two different steady states has a real physiological meaning. In any case, both of the transition times can give information about different aspects of the kinetic features of the system and its behaviour. The relationship between empty transition times of the two different steady states (τ_A and τ_B) and the transition time between them (τ_{AB}) has been derived by Easterby (8):

$$\tau_{AB} = \tau_B - \tau_A \cdot \frac{J_A}{J_B} \qquad (1)$$

where $\tau_{AB} = \Delta\sigma/J_B = (\sigma_B - \sigma_A)/J_B$; τ_A, τ_B, σ_A and σ_B are the transition times and whole pools of the two states A and B, respectively (Fig. 1). We analyse in this work these three different transients. The stimulus used for increasing the flux

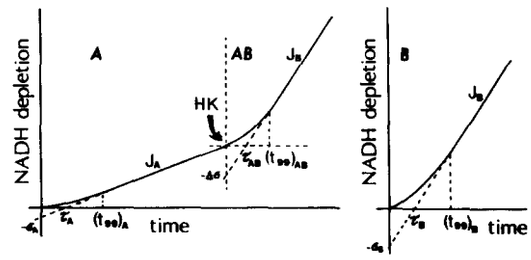

FIG. 1. Graphic recording of a typical experiment showing progress curves in the three transients of the metabolic system studied (obtained by recording NADH decay in the shortened glycolysis system described in the text). (A) Transient from the system "empty" of intermediates until reaching its first (basal) steady state A; this was achieved by triggering the reaction with the addition of the glucose substrate. (AB) Transient from the basal steady state A to the activated steady state B; this was promoted by the addition of hexokinase (five times its basal V_{max}/K_m value in every case) on the system being at A steady state (see the arrow). (B) Transient from the initial situation (empty of intermediates) directly to the B state, triggered by adding glucose to the system that had the hexokinase previously added. This figure shows a typical set of experiments, where all these stages can be seen. Metabolic fluxes at steady state (J) were taken from slopes of the straight lines. Response times (τ and t_{99}) were taken as shown in the figure for each case.

of the pathway was the addition of hexokinase. This step (the supply of phosphorylated glucose) has a critical activation role in this system as it has been previously shown (17).

Four different species of birds were selected for this experiment: the rock dove (*Columba livia*) and turtle dove (*Streptopelia turtur*) as long-flying birds and the hen (*Gallus domesticus*) and red-legged partridge (*Alectoris rufa*) as flight sprinters (and non-long-flying birds). Breast muscle, as the organ mainly responsible for the flight work, was used to assay the glycolytic behaviour. The birds were killed by jugular cut, and 10 g of breast muscle was immediately taken for tissue processing. These muscles were bright red in doves, rose-coloured in partridge and clearly white in hen, in good agreement with their role as aerobic and anaerobic, respectively. The tissues were finely chopped and homogenized in a Potter-Elvehjem with Teflon pestle at 1 g/4 ml in 50 mM HEPES/10 mM sodium phosphate buffer (pH 7.4) containing 100 mM KCl, 10 mM $MgCl_2$ and Trypsin inhibitor (Sigma type III) at 1 mg/3 ml. Homogenates were clarified by centrifugation at 20,000 **g** during 30 min, in a Beckman J2-21 centrifuge with a JA-20 rotor. All these operations were made at 3–5°C. The supernatants were used immediately for kinetic experiments and protein assays.

Kinetic experiments were carried out in 1-ml cuvettes in a Beckman DU-70 spectrophotometer at 37°C, following the reactions by means of a continuous recording of the NADH decay at 340 nm. Each sample contained ~0.3–2 mg of protein of muscle extract (25–100 μl) per ml, according to their activity. The metabolic system *in vitro* was shortened as previously described (17) by including in the mixture triose phosphate isomerase and glycerol phosphate dehydrogenase as auxiliary enzymes and 0.6 mM NADH, which guarantees the immediate conversion of triose phosphate into glycerol phosphate. ATP was buffered with creatine phosphate and creatine kinase. Whole composition of the incubation mixture is shown in Table 1. Reactions were triggered by addition of glucose (6 mM in the final mixture). Incubation mixtures were previously depleted of intermediates by allowing the systems to run for 10–20 min before the glucose addition, when a good basal line was achieved. The behaviour of the metabolic system was assayed under two different conditions: basal, without any other enzyme added besides the auxiliaries mentioned above, and under addition of commercial hexokinase, five times its basal activity (V_{max}/K_m). The addition of hexokinase promotes an important activation of the system, increasing its capacity of transformation, with a consequent increase of flux.

TABLE 1. Composition of the incubation mixture used in kinetic assays

ATP	1.0 mM
Glucose	6.0 mM
Fructose 2,6-bis-phosphate	10.0 μM
NADH	0.6 mM
Creatine phosphate	10.0 mM
Triose-phosphate isomerase	5.0 Units/ml
Glycerol-phosphate dehydrogenase	1.0 Unit/ml
Creatine kinase	5.0 Units/ml

These products were dissolved in the same buffer used for homogenization described in Materials and Methods.

This kind of activation means the availability of phosphorylated glucose from any particular source, with no effect on ATP concentration, because of its phosphocreatine buffering; it has been demonstrated by several groups (3,15,17) that the availability of phosphorylated glucose is the most critical step in controlling the glycolytic flux in muscle. Steady-state fluxes were obtained by recording the progress of the reaction, from starting time (triggering or activating the reaction) until steady state, with a constant slope; these slopes were taken as steady-state fluxes. t_{99} values were taken from the graphs by means a computer program of non-linear regression that gives the slope of the curve (as the derivative) at any point; t_{99} is the abscisa (time) where the derivative is 99% of the slope at steady state. Transition times (τ) were taken from the graphs, as Fig. 1 shows. The three different transients mentioned above (A, B and AB) were studied. Reaction progress plots of a typical set of experiments are shown in Fig. 1. Enzymes, metabolites and other chemicals were obtained from Sigma Chemical Company (St Louis, MO, U.S.A.).

RESULTS

Table 2 shows the results of these experiments. They clearly demonstrate the differences in the glycolysis behaviour of the four species studied, as well as the close relationship of the birds with their flying mode. Three features can be seen. First, the values of the fluxes (both basal and stimulated) are much higher in the two long-flying birds, indicating their greater glycolytic activity (basal flux is ~6–13 times those of the non-long-flying birds). Second, the increase of flux under the hexokinase stimulus is about two times that of the aerobic birds and 7–13 times that of the anaerobic ones. Third, metabolic response times are clearly shorter in the anaerobic birds. It must be noted that these differences have been observed by studying the behaviour of glycolysis common to aerobic and anaerobic pathways, which indeed demonstrate the particular features of each kind of glycolytic design. It is clear from the data that doves, as aerobic birds, have a good chemical machinery for flying, with a very high glycolytic activity, which is a good support of its continuous mechanical work. Hen and partridge are shown to be birds prepared for quick responses to stimuli rather than for a continuous energy supply. In effect, hen and partridge have a very poor basal glycolytic activity, but their response to a stimulus is large for the flux and short for the time. In fact, "metabolic reprise" ρ defined as

$$\rho = (J_B/J_A)/t_{99} \quad (2)$$

is high in the anaerobic birds and very low in the aerobic ones.

TABLE 2. Steady-state fluxes and metabolic response times of the three transients studied

	A-Transient				B-Transient				AB-Transient				
	J_A	τ_A	σ_A	t_{99}	J_B	τ_B	σ_B	t_{99}	J_B/J_A	τ_{AB}	$\Delta\sigma$	t_{99}	ρ
Rock dove	2.23	0.417	55.83	55	5.26	0.350	110.60	70	2.3	0.183	54.77	34	0.07
Turtle dove	2.63	0.133	21.06	27	5.16	0.483	149.83	51	1.9	0.383	128.77	40	0.05
Partridge	0.38	0.008	0.19	1	2.70	0.250	40.50	35	7.0	0.250	40.32	24	0.29
Hen	0.20	0.003	0.04	1	2.65	0.100	15.90	32	13.2	0.220	15.86	15	0.88

Fluxes (J) are given in nmol of NADH depleted per sec, per mg of protein; times [transition times (τ) and relaxation times (t_{99})] are given in seconds (also corresponding to 1 mg of protein). Each value (reported as a mean) was obtained from two different animals, carrying out three experiments with each, giving a coefficient of variation $V = (s/\bar{X}) \cdot 100 = 3.1$. Breast muscles of these animals had approximately the same amount of protein (between 75 and 100 mg of soluble protein per gram of tissue). It can be seen that these results well account for Easterby's (8) formula, which relates the transition times, $\tau_{AB} = \tau_B - \tau_A \cdot (J_A/J_B)$. Time values presented in this table divided into the dilution factor of each tissue from its original situation *in vivo* to this analysis allows us to have an estimate of the actual metabolic response times. Dilution factors per protein mg were rock dove, 80; turtle dove, 73; partridge, 111; hen, 111. This gives the following values of t_{99} for the AB-transient: rock dove, 425 msec; turtle dove, 547 msec; partridge, 216; hen, 135 msec.

Symbols: J, steady-state flux; τ, transition time (total pool of intermediates (σ) over the flux at steady state); t_{99}, relaxation time (time that the system spends to reach 99% of the output flux at steady state; ρ "metabolic reprise," defined as flux increase factor (J_B/J_A) over t_{99} of the AB-transient.

DISCUSSION

Our conclusions on metabolic promptness are based on t_{99} values, because τ_{AB} does not really express the response time that can have a general significance. In effect, as τ is the ratio of two steady-state values (σ/J), it can be known without knowing the transient phase, and as state variable, it cannot have information about how such a steady state has been achieved. τ is the time the system spends to reach a certain fraction of the steady-state flux, but such a fraction is not the same in every system, so it is not very informative as response time, and this feature is shown in our results.

There are many well-known cases of closely related species occupying very different niches. They are clear examples of divergent evolution from a common origin promoted by the different places available to be occupied. This phenomenon was first observed by Darwin (6) studying the famous finches from the Galapagos Islands (see also Ref 4). This is equivalent to Kaplan's ducks mentioned above, where again animals very closely related have been diversified playing different roles. Evolution is opportunistic, because if these niches had not been available, such a diversification would not have taken place. Nevertheless, this would not have been possible if metabolism were not capable of adapting to support all these different roles. This is strongly reinforced by our observations of the concordance between the metabolism and the macroscopic behaviour of these birds.

Alexander (2) pointed out that some organisms with exceptional performance have clear inevitable costs. He also discussed that it is often not easy to know the costs to a great aerobic muscle machine, such as the pronghorn antelope. We believe that the results presented here can contribute to an answer: anaerobic metabolism can account for a quick response, whereas aerobic metabolism has the burden of a very long response time. These results show a good correlation between these features and can contribute to an understanding of how different metabolic designs can make an appropriate chemical support of the different physiological behaviours that make evolutionary diversity possible. These results are a good example of what a specific metabolic design means for different physiological roles, concluding that anaerobic work has also an interesting evolutionary meaning.

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