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Seasonal Changes in the Activities of Glycolytic Enzymes from Liver and Skeletal Muscle of *Rana perezi*

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Glycogen phosphorylase (GP), Hexokinase (HK), Phosphofructokinase (PFK), Pyruvate kinase (PK) and Lactate dehydrogenase (LDH) activities from skeletal muscle and liver were measured in *Rana perezi* for the four seasons of the year. Skeletal muscle showed a decrease in PFK, PK and LDH activity during winter and summer. Liver displayed an increase in GP activity in spring and in PK and LDH in autumn.

Key words: Seasonal changes, *Rana perezi*, Glycolytic enzymes.

Seasonal climatic changes affect amphibian physiology and activity remarkably. These animals try to compensate the ambiental conditions through metabolic and behavioural responses.

Changes in animal metabolism take place during the reproduction and hibernation periods. In autumn, animals accumulate, mainly in liver and fat bodies, reserve substances which are consumed throughout the winter, when reduced photoperiod and cold temperatures induce hibernation or torpor with limited activity and metabolism. In spring, animals cease torpor and apply their physiological processes to breeding, using the reserve substances that remain after hibernation. Amphibians recover their usual activity and assume an active growth period during summer (6, 8, 18, 21, 23, 24, 26).

Several reports have appeared concerning seasonal oscillations of enzyme activities in different tissues from anuran species (2, 4, 15, 19-22). They suggest, in general, a high metabolic activity during summer (9, 20-22). In fact, an enhanced glyconeogenesis in liver can be predicted during this season (2, 19). On the other hand, a high lactate dehydrogenase activity has been measured in muscle, liver and heart from *Discoglossus pictus* during autumn (4) and in liver from *Rana esculenta* during winter (22). Glycogen phosphorylase (GP) have shown its high-

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est values in liver from *Rana temporaria* during spring and summer (21), while there has not been any change in muscle and heart from *R. esculenta* (9). In addition, HK from muscle of this species has its highest values during summer (9).

In this paper, glycogen phosphorylase (GP), hexokinase (HK), glucokinase (GK), phosphofructokinase (PKF), pyruvate kinase (PK) and lactate dehydrogenase (LDH) activities from both skeletal muscle and liver have been measured as an approach to a biochemical basis of above described seasonal oscillations.

Materials and Methods

Rana perezi Seoane (1885) specimens with average weight 22.5 g (15-42 g) were trapped from their natural environment, the edges of the river Segura in Orihuela (Alicante, Spain), in February, April, July and November.

Preparation of extracts. — Animals were anaesthetized with 4% Urethan and gastrocnemic muscle and liver removed. Portions of tissues (200-400 mg) were homogenized in cold in a Polytron PTA 10S at 10,000 rpm for 30 sec with 10 volumes of 2.35 mM maleate-NaOH-NaF buffer pH: 6.5 for GP or 58 mM potassium phosphate pH: 7.5 for PFK, PK and LDH measurements. Homogenates were centrifuged at 12,000 *g* and 4° C for 15 min in a Sorvall RC 5B centrifuge. For HK and GK, particulate plus soluble activities, portions of tissue (200-400 mg) were homogenized in 5 volumes of 85 mM Tris-HCl buffer pH: 7,5, 0.8 mM EDTA, 8 mM $MgCl_2$, 1 mM dithioerythritol (DTE), then centrifuged at 1,700 *g* for 5 min. Afterwards the precipitates were resuspended and centrifuged in the same conditions.

Determination of enzyme activities. — All activities were measured at 25° C using a spectrophotometer Kontron Uvikon 810 with recorder and connected to a thermostatized bath. The assay conditions for the individual enzymes were as follows: Glycogen phosphorylase (GP; E.C. 2.4.1.1) activity was determined according to the method of HEDRICK & FISCHER (11) at final concentrations: 100 mM Maleate-NaOH buffer pH: 6.5, 0.15 mM NaF, 1% glycogen, 5 mM DTE, 1 mM AMP, for total GP activity, 75 mM G-1-P and 0.2 ml of extract in a final volume of 0.4 ml, during 5 min. Form (a) without AMP and blanks without G-1-P were also carried out. The Pi liberated was determined as by LOWRY and LÓPEZ (16).

Hexokinase (HK, E.C. 2.7.1.1) and Glucokinase (GK, E.C. 2.7.1.2.) activities were measured for 3 min coupling their reactions with the reduction of NADP in presence of an excess of G-6P dehydrogenase (G-6PDH). The final concentrations in 1,165 ml of final volume of medium were: 85 mM Tris-HCl buffer pH: 7.5, 0.8 mM EDTA, 8 mM $MgCl_2$, 1 mM DTE, 2.5 mM ATP, 0.4 mM NADP, 0.5 mM glucose for HK activity and 20 mM glucose for glucokinase measurements, 2.1 U G-6-PDH and 0.05 ml of extract of tissue. Blanks without glucose were run and their activity subtracted.

Phosphofructokinase (PFK, E.C. 2.7.1.11) activity was measured coupling fructose-1,6-biphosphate formation with the oxidation of NADH using aldolase, triosephosphate isomerase (TPI) and glycerol-3-phosphate dehydrogenase (G-3PDH). The final concentrations in a total volume of 1.16 ml were: 50 mM Tris-HCl pH: 8, 2 mM $MgCl_2$, 5 mM KCl, 1.5 mM ATP, 0.1 mM AMP, 1 μM F-2, 6-bisP, 0.15 mM NADH, 1 mM NH_4Cl, 0.04 U aldolase, 3.8 U TPI, 0.37 U G-3PDH, 2.4 mM F-6-P and 0.05 ml of extract conveniently diluted to get a linear reaction rate. The change of absorbance was recorded during 5 min to over-

come the initial delay. Controls had no added F-6-P.

Pyruvate kinase (PK, E. C. 2.7.1.40) activity was determined as by ZAMMIT *et al.* (29) in 1.5 ml of medium at final concentrations: 100 mM trietanolamine buffer pH: 7.4, 80 mM KCl, 10 mM $MgCl_2$, 0.2 mM NADH, 4.7 mM ADP, 2 mM PEP, 14.5 U LDH and 0.1 ml of extract conveniently diluted. The absorbance change was recorded during 2 min. Blanks without PEP were run in parallel.

Lactate dehydrogenase (LDH, E.C. 1.1.1.27) activity was measured according to BERGMEYER and BERNT (1) in a cuvette with 3.05 ml of medium at final concentrations: 58 mM potassium phosphate buffer pH: 7.5, 0.33 mM pyruvate, 0.15 mM NADH during 1 min. Blanks without NADH were run in parallel.

Proteins were determined according to LOWRY *et al.* (17) using bovine serum albumine as standard.

All enzymatic activities are expressed as U per gram of wet tissue and U per mg of protein. U refers to one μmole (PFK, PK and LDH) or nmole (HK, GK, GP) of transformed substrate, or formed product, per minute. Statistical analysis for differences consisted of one way analysis of variance ($p < 0.05$).

Results

Table I and figure 1 show the values obtained for enzymatic activities of muscle referred to g of wet weight and mg of protein, respectively. Total and form (a) glycogen phosphorylase activities do not show significant differences with respect to the season. HK and GK activities show their highest values in summer and the lowest in spring. These differences are significant when activities per wet tissue are considered. PFK and PK exhibit their

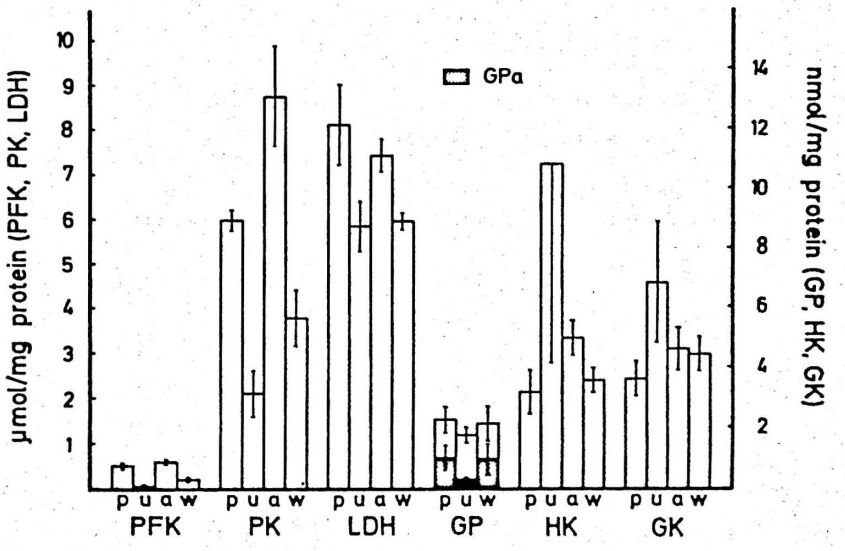

Fig. 1. *Seasonal variations in specific activities of several glycolytic enzymes from* Rana perezi *gastrocnemic muscle.*

Vertical bars are standard error of the mean. p: Spring; u: Summer; a: Autumn; w: Winter. Units: μmol (PFK, PK and LDH) or nmol (GP, HK and GK) of substrate consumed (or produced) × min^{-1} × mg^{-1} protein.

Table I. *Enzymatic activities from gastrocnemic muscle of* Rana perezi *in different seasons of the year.* Values (mean ± s.e.) are expressed per minute and gram of wet weigth.

	SPRING	SUMMER	AUTUMN	WINTER	$p < 0.05$
GPt	143.2 ± 20.6 (7)	92.2 ± 11.8 (8)	—	159.4 ± 31.1 (8)	NS
GPa	53.8 ± 19.4 (7)	13.5 ± 3.9 (8)	—	60.2 ± 20.3 (8)	NS
HK	115.8 ± 27.3 (7)	265.5 ± 58.9 (7)	202.5 ± 26.2 (9)	162.0 ± 18.4 (11)	S
GK	123.9 ± 17.9 (7)	349.7 ± 58.4 (7)	190.4 ± 37.1 (9)	198.6 ± 20.6 (11)	S
PFK	24.4 ± 2.8 (7)	3.6 ± 1.6 (7)	25.3 ± 1.4 (7)	12.5 ± 3.1 (11)	S
PK	288.2 ± 30.4 (7)	125.0 ± 21.6 (7)	376.0 ± 47.5 (7)	231.9 ± 37.2 (11)	S
LDH	380.9 ± 48.6 (7)	428.2 ± 51.5 (7)	325.3 ± 23.0 (7)	406.4 ± 33.8 (10)	NS

Number of animals in brackets. S = significant, NS = no significant differences at $p < 0.05$.

Table II. *Enzymatic activities from liver of* Rana perezi *in different seasons of the year.* Values (mean ± s.e.) are expressed per minute and gram of wet weigth.

	SPRING	SUMMER	AUTUMN	WINTER	$p < 0.05$
GPt	27.3 ± 3.5 (7)	6.1 ± 4.3 (8)	—	15.6 ± 4.2 (8)	S
GPa	14.7 ± 3.9 (7)	3.6 ± 2.6 (8)	—	8.8 ± 2.0 (8)	S
HK	18.5 ± 7.5 (7)	195.3 ± 93.2 (7)	8.9 ± 6.5 (9)	52.5 ± 24.6 (11)	S
GK	0.0 (7)	126.8 ± 67.6 (7)	87.1 ± 42.9 (9)	94.3 ± 39.0 (11)	NS
PFK	0.45 ± 0.14 (7)	0.47 ± 0.14 (6)	0.57 ± 0.09 (7)	0.72 ± 0.18 (12)	NS
PK	21.6 ± 3.1 (7)	13.1 ± 1.7 (6)	28.3 ± 1.6 (7)	24.9 ± 2.3 (12)	S
LDH	40.8 ± 7.4 (7)	70.7 ± 10.1 (8)	53.3 ± 4.1 (7)	55.1 ± 4.5 (10)	S

Number of animals in brackets. S = significant, NS = no significant differences at $p < 0.05$.

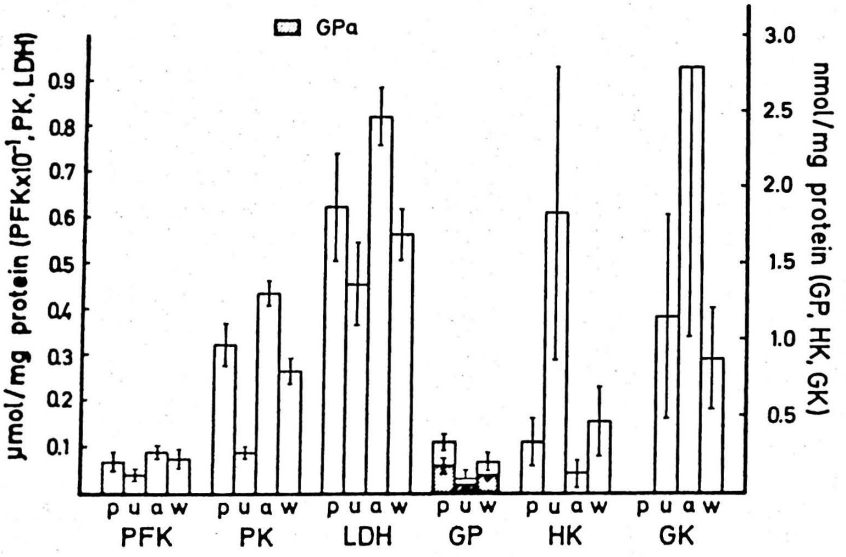

Fig. 2. *Seasonal variations in specific activities of several glycolytic enzymes from* Rana perezi *liver.* Vertical bars are standard error of the mean. p: Spring; u: Summer; a: Autumn; w: Winter. Units as in fig. 1. PFK values are represented as 10 × actual data.

maximum during autumn and their minimum during summer, no matter how their activities are calculated. LDH shows variation in its activity per mg of protein, being low in summer and winter. There is also a tendency for muscle glycolytic activities to rise (PFK, PK) in autumn, while the lowest values are found in summer. In contrast, HK and GK activities are maxima in summer and minima in spring.

Glycogen phosphorylase activities, total and form (a), in liver (table II and fig. 2), can be observed to diminish in summer. Other liver glycolytic enzymes, mainly PK, show a tendency to increase in autumn and, as happens in muscle, diminish in summer. On the other hand, HK and GK activities have their highest values in summer. GK activities could not be detected in spring.

Discussion

Seasonal changes in carbohydrate reserves of anuran amphibians have been reported abundantly (3, 6, 8, 12-14, 18, 21, 26, 27). Liver and muscle glycogen are more abundant in autumn and winter than in spring and summer. Blood glucose levels increase in the breeding season in some species (3, 12, 26, 27), and another hyperglycemic peak could be detected, when glycogen is being stored, in summer or autumn, perhaps depending on the distribution of the species, in the North or South. These peaks of circulating glucose have been related to glycogen store mobilization for the former and gluconeogenesis for the latter (22).

Muscle GP activities did no change seasonally *in vitro*. Nevertheless, it is probable that variations in environmental temperature (21) and in concentrations of regulatory metabolites throughout the year can cause seasonal changes in these activities *in vivo*. On the other hand, liver GP activities showed an increase in spring, mainly in total GP activity. A similar increase was observed in *Rana temporaria* (21). This could provide a big glycogenolytic capacity which would be the origin of the above mentioned hyperglycemia (3, 12, 26) related to high energetic demands of breeding.

HK and GK activities from both muscle and liver showed low values. This observation correlates well with the fact that sugar levels in amphibian blood are lower than in other vertebrates (5, 28). These enzyme activities in muscle had a tendency to increase in summer. This also was observed in *Rana esculenta* (9) and is probably related to high blood glucose in this season (7). In liver, a decrease in HK activity was observed in autumn. This would be not in agreement with the enhancement in glycogen synthesis, as a preparation for winter, expected in this season. However, the maintenance of GK activity could assure the uptake of blood glucose by liver. In addition, it is probable that glycogen accumulation occurs in a later period to fat and protein stores, as reported in other species of *Rana* (21, 23).

Taking into account that enzyme activities rise with temperature, the observed decreases in PK and LDH, in both tissues, and in PFK in muscle, would compensate positively the change produced directly by temperature variation throughout spring, summer and autumn (10). However, these enzymes show inverse compensation from autumn to winter and from winter to spring in muscle, while in liver only show such a compensation from autumn to winter and no compensation between winter and spring. All this can produce a reduced glycolytic capacity for muscle during winter, a period of limited muscle activity, and an enhancement during spring, the breeding season of this species, that induces a high behavioural activity. The results obtained here are in agreement with those reported for *Discoglossus pictus* LDH (4).

Anyway, seasonal variations in glyco-

lytic enzyme activities reported here are not great because *Rana perezi* is a species living in temperate zones and then it suffers lesser fluctuations in energetic reserves throughout the year, as indicated by somatic indices (fat bodies and liver weights, personal observations), than Northern species (21, 23, 24).

Resumen

Se determinan las actividades de las enzimas glucolíticas glucógeno fosforilasa (GP), hexoquinasa (HK), fosfofructoquinasa (PFK), piruvato quinasa (PK) y lactato deshidrogenasa (LDH) en el músculo esquelético y en el hígado de *Rana perezi*, en las cuatro estaciones del año. El músculo esquelético presenta un descenso de actividades PFK, PK y LDH durante el invierno y el verano. El hígado muestra un incremento en la actividad GP en la primavera, y en la PK y en la LDH durante el otoño.

Palabras clave: Variaciones estacionales, *Rana perezi*, Enzimas, glucolíticas.

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