REVISTA ESPAÑOLA DE FISIOLOGIA, 44 (2), 157-162, 1988

The Effect of Temperature on Immunoreactive Glucagon Plasma Level in Carp *Cyprinus carpio*

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(Received on October 30, 1987)

J. BLASCO, J. GUTIERREZ, J. FERNANDEZ and J. PLANAS. *The Effect of Temperature on Immunoreactive Glucagon Plasma Level in Carp* Cyprinus carpio. Rev. esp. Fisiol., 44 (2), 157-162, 1988.

Plasma immunoreactive glucagon levels (IRG), plasma glucose levels and brain and liver glycogen concentrations were analyzed in carp (adapted to 15° C) subjected to short-term temperature changes (1.6 or 11 h, at 5° C or 28° C, respectively) and to long-term temperature changes (21 months at 28° C). The high temperature (28° C) produced significant increases in IRG in both short and long-term experiments. Brain glycogen also decreased in both experiments whereas liver glycogen only changed in the long-term experiment. Low temperatures did not provoke any changes either in IRG or in liver glycogen, whereas brain glycogen decreased in the 1 h exposure. In short, under these conditions in carp, IRG did not respond to low temperature but could play an important role in high temperature acclimation.

Key words: Immunoreactive glucagon (IRG), Temperature, Glucose, Glycogen, *Cyprinus carpio*.

When the thermal environment is altered artificially or by natural conditions, physiological changes are produced in poikilotherms initiating long-term adjustments which enable the animal to operate more efficiently at the new temperature (7).

Initial response of most poikilotherms to thermal variation is mainly a corresponding change in metabolic rate (9) and a variation in oxygen consumption (3). Corticoid stress response in fish is affected by increasing or elevated temperatures (19) in a similar way to catecholamine levels in plasma (12). Several authors have studied the glycogenolytic role of these hormones in fish (15, 22). The glycogenolytic role of glucagon in liver of higher vertebrates is well known (23). In fish, the effects of mammalian glucagon injection were similar to those found in homeotherms: hyperglycemia, gluconeogenesis and glycogenolysis (17, 20, 24). However, there is very little information on glucagon secretion in fish.

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UMMINGER and BAIR (21) observed a degranulation of the alpha cells of the pancreas in *Fundulus heteroclitus* subjected to subzero temperatures. Furthermore, plasma glucagon levels in mammals increase in cold acclimation (10, 11).

In this work, the effect of temperature on plasma immunoreactive glucagon levels (IRG), plasma glucose, and glycogen mobilization in the brain and liver of carp were studied.

Materials and Methods

Two experiments were carried out: *1) Short-term experiment:* Forty five carp *(Cyprinus carpio* L.), with average weights and sizes of 273.1 ± 39.8 g and 23.4 ± 0.5 cm mean ± s.e., were maintained in tanks (250 l) with a closed circuit system, and adapted to a natural photoperiod at 15° C for one month (December). Fish were fed with commercial fish food (Bioter-Biona) once a day, but were fasted the day before the experiment.

The experiment was carried out for five consecutive days. Each day 9 animals were transferred to separate tanks, 3 fish to 28° C, 3 to 5° C and 3 to 15° C. This last group was used as a control (in order to produce the same handling effect as in experimental fish). Thus, the tanks were exactly the same in both shape and conditions except for temperature. At 1 h (9 am) after transfer, one fish from each temperature group (28° C, 5° C and 15° C, controls) was anaesthetized with MS-222 (0.5 g/l) and sampled. This was repeated at 6 and 11 h (2 and 7 pm, respectively) after tank change, and for 5 consecutive days. Blood samples were taken from the caudal vein. The fish were quickly sacrificed and samples of liver and brain were extracted and frozen in liquid nitrogen for glycogen analysis as rapidly as possible. Blood samples were centrifuged at 3,000 rpm for 15 minutes. Plasma aliquots were distributed in separate plastic tubes for the following assays: glucose (glucose oxidase method, Boehringer Mannheim, kit) and immunoreactive glucagon (mammalian glucagon RIA) (6). Trasylol (1,000 IU/ml plasma) was added to the samples for the glucagon assay before freezing. The purification of glycogen was done according to GOOD *et al.* (5) and the glycogen content was assayed with a modified anthrone-reagent method (4). *2) Long-term experiment:* Five carp were maintained under natural conditions at the Aquarama Zoo Barcelona (Spain) with natural fluctuations in temperature and photoperiod. Another five carp were maintained at 28° C for 21 months. Carp were sacrificed in December and the water temperature of the control group was the same as that of the other experiment (15° C). The sampling procedure employed was the same as in the short-term experiment.

All data were expressed as mean ± standard error (s.e.). Differences between groups were tested with Duncan multiple range test (18) in the short-term experiment and with Student's t-test in the long-term ones.

Results

In the 28° C experimental group, plasma IRG levels were significantly higher ($p < 0.01$) than in the control group at 1.6 and 11 h after transfer (fig. 1). These results were confirmed in the long-term study where the IRG levels of carp acclimated to 28° C were also significantly higher ($p < 0.05$) than those in the control group (fig. 2).

However, the IRG levels in the 5° C experimental group were very similar to those in the control group at 1, 6 and 11 h of exposure (fig. 1).

The plasma glucose levels in the 28° C experimental group did not show any significant changes after the short-term

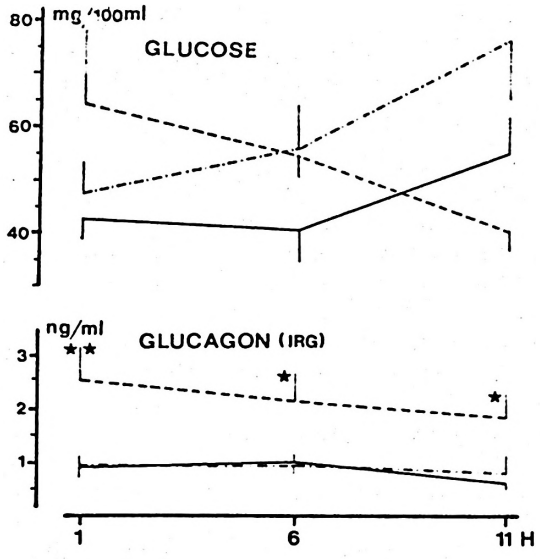

Fig. 1. *Effects of acute temperature changes on plasma glucose and immunoreactive glucagon levels in carp.*
Values given as the mean ± S.E. (———) Control, (- - -) 28° C Exp and (· · ·) 5° C Exp. ★ $p < 0.05$ with respect to control values. ★★ $p < 0.01$ with respect to control values.

or long-term acclimation. However, a marked increase (51 %) in plasma glucose levels at 1 h after transfer to 28° C was observed (fig. 1). In the other experimental group (5° C), the plasma glucose levels at 1, 6 and 11 h were very similar to those in the control group (fig. 1).

The levels of brain glycogen decreased significantly ($p < 0.01$) at 1 h of exposure to 28° C (fig. 3) and also after long-term acclimation ($p < 0.05$) (fig. 2). Hepatic glycogen only decreased significantly ($p < 0.05$) after the long-term acclimation (fig. 2). In fish transferred to 5° C, the brain glycogen was significantly lower ($p < 0.05$) at 1 h than that of the control group, although at 6 h the levels were very similar. Hepatic glycogen did not change (fig. 3).

Discussion

The high levels of plasma IRG in carp at 1, 6 and 11 h of exposure to 28° C (an immediate temperature increase of 13° C) indicate a rapid response to the high tem-

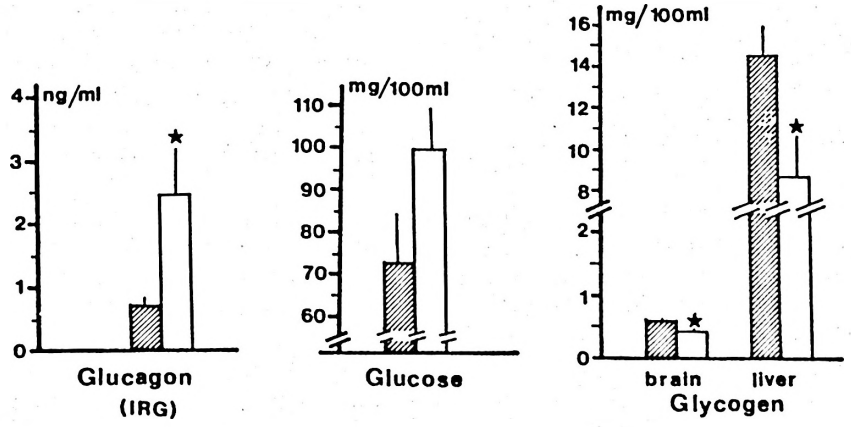

Fig. 2. *Effects of long-term acclimation (28° C) on plasma glucose and immunoreactive glucagon levels, and brain and liver glycogen concentrations in carp.*
Values given as the Mean ± S.E. Glycogen is expressed as mg/100 mg wet weight of tissue. Control (striped bars), experimental group (open bars). ★ $p < 0.05$ vs. control values.

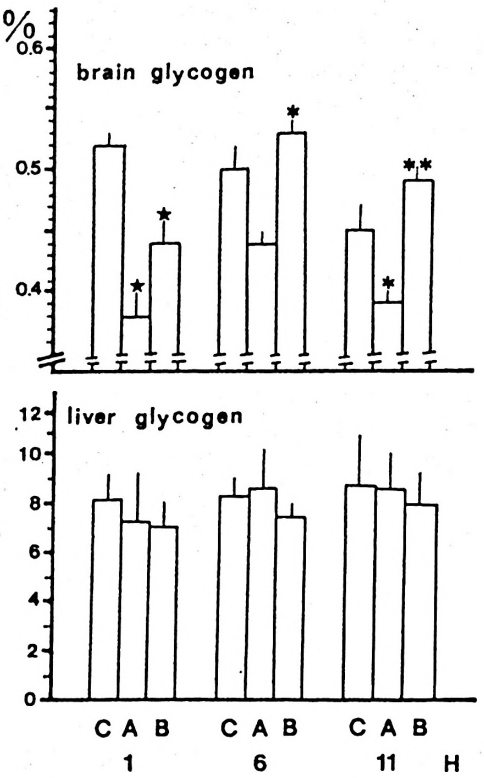

Fig. 3. *Effects of acute temperature changes on brain and liver glycogen in carp.*
Values given as the Mean ± S.E. Glycogen is expressed as mg/100 mg wet weight of tissue. C (Control group); A (28° C Exp.); B (5° C Exp.).
★ $p < 0.05$ vs. control values. ⁎ $p < 0.05$ vs. values from the preceding hour. ⁎⁎ $p < 0.01$ v.s. to values from the preceding hour.

perature change. Moreover, the maintenance of such high levels of this hormone in the long-term experiment could mean that IRG plays a role in the new steady state of carp as a consequence of high temperature.

In fish, there are no studies on this subject; in mammals, however, glucagon has been shown to be involved in the metabolic acclimation to cold (11). Under such conditions, glucagon contributes to the mobilization of reserves which are used for thermogenesis. Exposure to high temperature produces an increase of the metabolic rate in carp (3, 13) and IRG could favour the mobilization of reserves to maintain this increased metabolism. A tendency of the plasma glucose levels to increase not only at the beginning of short-term exposure but also in the long-term experiment was observed. Many authors have reported the hyperglycemic effect of glucagon injection in fish (2, 8, 14).

The significant fall of liver glycogen in the long-term acclimation to high temperature suggests that a higher energetic demand exists under this condition and coincides with the higher IRG levels, whereas short-term exposure to high temperature seems to be too short a period to observe this glycogen mobilization. These results are consistent with what has been reported for other cyprinids (1) where hepatic glycogen did not decrease either after several hours of exposure to high temperatures or after 10 days of acclimation.

In *Carassius auratus*, the brain glycogen concentration decreases after transfer to high temperature, both in short or in long-term acclimation (1). These results coincide with our observations in carp. PLISETSKAYA (16) explained that the higher reserves of brain glycogen in several species of fish, in comparison with mammals, could provide an immediate energy supply to this vital organ in adverse conditions.

In this experiment, no changes in IRG levels in response to cold were found. However, this does not mean that IRG secretion would not be produced under more extreme conditions. UMMINGER and BAIR (21) observed hypertrophy and degranulation of alpha cells in the islets of Langerhans when *Fundulus heteroclitus* was exposed to −1,5° C for periods of 1 and 10 days.

In conclusion, high temperature conditions in carp provoked an earlier brain glycogen mobilization while liver glyco-

gen needed a more prolonged exposure to be mobilized. A short exposure to 5° C did not provoke a response in plasma IRG levels, but an increase of this hormone was observed at 28° C. This hormone probably contributes to maintaining the increase in the metabolism caused by the high temperatures.

Acknowledgements

We would like to acknowledge the assistance of Dr. A. San Miguel (Aquarama, Zoo, Barcelona) in obtaining study specimens. We also wish to express our sincere thanks to Dr. F. Rivera and Dr. R. Casamitjana of the «Hospital Clínico y Provincial de Barcelona», for their invaluable help.

Resumen

Se estudian los niveles plasmáticos de glucagón inmunorreactivo (IRG), glucosa y glucógeno en cerebro e hígado en carpas, adaptadas a 15° C, sometidas a un cambio brusco de temperatura (1, 6 y 11 h a 5° C ó a 28° C). Temperaturas de 28° C producen aumentos significativos de IRG tanto a corto como a largo plazo. El glucógeno cerebral disminuye en ambos experimentos y el del hígado sólo a largo plazo. Las bajas temperaturas no provocan cambios de IRG ni de glucógeno hepático, mientras que el glucógeno cerebral disminuye tras una hora de exposición. En estas condiciones en carpa, el IRG no responde a las bajas temperaturas pero podría tener un papel importante en la aclimatización a elevadas temperaturas.

Palabras clave: Glucagón inmunorreactivo, Temperatura, Glucosa, Glucógeno, *Cyprinus carpio*.

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