REVISTA ESPAÑOLA DE FISIOLOGIA, 50 (4), 219-228, 1994

Direct Transfer of Rainbow Trout to Seawater Induces Several Changes in Kidney Carbohydrate Metabolism

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(Received on May 17, 1994)

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The levels of glycogen and glucose, and the activities of several key enzymes of glycogenolysis, glycolysis, gluconeogenesis and the pentose phosphate shunt were assessed in kidneys of rainbow trout *(Oncorhynchus mykiss)* of two sizes (80 and 140 g) after transfer to seawater (28 p.p.t.) during 7 days. The results indicated changes, mainly size-independent, in kidney carbohydrate metabolism during transfer of rainbow trout to seawater. An enhanced glycogenolysis and a concomitant increase in gluconeogenic enzyme activity were clearly observed in kidneys of both sizes of animals during transfer to seawater. Changes are suggested to be related to the known role of kidney as a glucose producer tissue thus satisfying, at least in part, the high energetic requirements of the osmoregulatory work performed by other tissues using glucose as fuel, such as the gills, during adaptation to seawater.

Key words: Rainbow trout, Seawater adaptation, Kidney, Glycogenolysis, Gluconeogenesis.

In fish an increased energetic cost usually takes place associated with seawater adaptation. This cost probably arises from increases in the rate of active transport of ions at the gill, kidney and/or intestine, in response to increments in passive ion and water fluxes (8, 9, 19). The number of studies performed in analyzing the possible existence of changes during seawater adaptation in energetic substrates and/or in the activity of enzymes involved in their metabolism are scarce (27) and far from conclusive, except for lipid metabolism (37). In such studies decreased liver glycogen levels (39, 47) and increased plasma glucose levels (1, 25) have been usually reported.

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There are several references regarding changes at both osmoregulatory (21, 26) and endocrine (3) levels during seawater adaptation of rainbow trout *(Oncorhynchus mykiss)*, but the metabolic changes associated with seawater adaptation have been scantily studied concerning either metabolic rates (33) or metabolic intermediates and/or enzyme activities (12, 15, 16, 25). The change in salinity during adaptation can be performed in a direct way, thus studying the changes due to the osmotic shock, or in a gradual way, which resembles more the adaptation found in nature by euryhaline species. Previously we studied the effect of direct and gradual adaptation of rainbow trout to seawater on carbohydrate metabolism in liver (44), muscle (40, 43), and gills (41) observing a general gearing up of metabolism to provide energy for osmoregulation.

Many different changes occur in kidney osmoregulatory function during seawater adaptation including changes in renal morphology (5), an increased excretion of divalent ions such as magnesium and sulphate (34), decreased glomerular filtration rate (4), and decrease in urinary production (7, 36). Most of these changes need energy in the form of ATP and, therefore, may be associated with an increased energetic demand that would lead to changes in kidney intermediary metabolism. However, few studies have been performed regarding metabolic changes in kidney where only the activities of some respiratory enzymes (28, 29) and enzymes involved in amino acid catabolism (15, 16) have been reported hitherto. Therefore, it would be of interest to examine the existence of changes in kidney metabolism during direct adaptation of rainbow trout to seawater. Size was included as an additional factor since it could also be expected to influence the parameters assessed in the experiment due to the seemingly size-dependency of seawater adaptation in rainbow trout (13, 14).

Materials and Methods

Animals and experimental design.– Two experiments were performed with domesticated rainbow trout of two different weights, obtained from a hatchery in Soutorredondo (Noia, Galicia, Spain) just as it has been previously detailed (43, 44). The first experiment was performed on 80 g average b. w. trout (denoted as small) during October-November 1991, whereas the second experiment was performed on 140 g average b. w. trout (denoted as large) during November-December 1991. The month's delay between transfer of small and large fish could not be avoided because of tank limitations. In both experiments fish were randomly assigned (10 fish per tank) to two identical but separate systems of 10 fiber glass tanks (150 l) each for acclimatization during 10 days in well aerated, recirculating and dechlorinated tap water. The water was recirculated in both systems being physically filtered and purified. After 10 days of acclimatization, salinity was gradually increased (during 1 h) to 28 p.p.t. with artificial seawater (Instant Ocean®) in one of the systems, whereas the other remained, along with the control animals, as freshwater. The fish were fed once daily throughout the experiment with commercial dry pellets (Sterling Silver Cup, Spain. Proximate analysis: 50 % crude protein, 20 % fat, 21 % carbohydrate, and 9 % ash in the dry matter), with a ration equivalent to 1.5 % of body wt/day, and were fasted 24 hours prior to sacrificing. The handling and care of the fish were maintained unchanged throughout the sampling period, under natural conditions of temperature (from 10 °C to 14 °C), pH (7.6-7.9, once in seawater) and photoperiod. The common water quality criteria (pH, hardness, and the levels of oxygen, carbon dioxide, hydrogen sulphide, nitrite, nitrate, ammonia, calcium, chlorine and

suspended solids) were assessed throughout the experiment with no major changes being observed. The fish were sampled at the same time of day (10 a. m.) to avoid interference by circadian rhythms in the measured parameters.

Three samplings were carried out for each experiment 1, 4 and 7 days after transfer to seawater. On each sampling date, 20 fish (10 controls and 10 fish transferred to seawater) were quickly dip-netted and anesthetized with buffered MS-222 (pH 7.4; 50 mg/l; no effect on the parameters assessed was seen due to the time spent in the tank with anesthetic). The caudal kidney was quickly removed, weighed, frozen on dry ice and stored at –80 °C until further assay. The average time invested in processing the samples from one trout was 3 min.

Analytical procedures.– Kidney glycogen and glucose levels were determined following the method of KEPPLER and DECKER (18). Enzymatic analyses were all carried out at maximun rates with the reaction mixtures set up in preliminary tests to render optimal activities (data not shown). Kidney samples were homogenized using a Potter-Elvejhem teflon-in-glass homogenizer held on ice with 10 vols of ice-cold homogenization buffer (50 mM imidazole-HCl, pH 7.4). The homogenate was centrifuged (2 min, 9,000 g, Kubota microcentrifuge KM 15200) and the supernatant (0.1 ml) was used directly in enzyme assays. The reactions were started by the addition of homogenates, at a preestablished protein concentration, omitting the substrate in control cuvettes.

Glycogen phosphorylase (EC 2.4.1.1; GP) and fructose 1,6-biphosphatase (EC 3.1.3.11; FBP) activities were assayed colorimetrically using the methods of MORATA *et al.* (32) and VILLANUEVA and MARCUS (46), for GP and FBP, respectively, but inorganic phosphate was quantified according to LE BEL *et al.* (22). Glycogen synthetase activity (EC 2.4.1.11; GS) was assayed using the method of SHERIDAN *et al.* (38) with the amount of pyruvate being colorimetrically determined using 2,4-dinitrophenyhydrazine as described by KATSUKI *et al.* (17).

The activities of 6-phosphofructo 1-kinase (EC 2.7.1.11; PFK), hexokinase (EC 2.7.1.1; HK) and glucose 6-phosphate dehydrogenase (EC 1.1.1.49; G6PDH) were assessed after incubation of the supernatant sample in a final volume of 1 ml. The activities were assayed by recording the reduction/oxidation of NADP or NADH at 340 nm in a Uvikon 930 spectrophotometer. The enzymatic methods used were the modified versions (43, 44) of methods previously described (10, 24, 31).

Data analyses.– All the assessed variables were normally distributed (Kolmogorov-Smirnov test) and data are shown as means ± standard error of means. Group variance homogeneity was assessed using the Cochrans' C test. Logarithmic or inverse transformations of the data were performed, where necessary, to fulfil the homocedasticity conditions of the analysis of variance but data are shown in their decimal values in favour of simplicity. Statistical differences were tested using a three-way analysis of variance, with treatment (freshwater vs. seawater), size of fish (small vs large), and time after transfer (1 through 7 days) being the main factors. The differences were considered to be statistically significant at $P < 0.05$. All analyses were performed with the statistical software SPSS/PC+.

Results

Due to technical reasons, completely beyond experimental design, impaired

sampling of small animals occurred with the fish in freshwater on day 7 of experiment. The significant effects obtained from the analysis of variance are shown in table I. No second order interaction, i.e. treatment × size of fish × time after transfer was found in any of the measured parameters and, therefore it was omitted in the ANOVA table.

A decrease was observed in kidney glycogen levels of both sizes of fish after transfer to seawater (fig. 1A), with treatment and size of fish being the factors found to influence kidney glycogen levels (table I). In contrast, kidney glucose levels were not affected by treatment (fig. 1B) with the changes observed being only attributable to the effect of the different size of fish evaluated (table I). When the activity of those enzymes involved in glycogen metabolism such as GP (fig. 2A) and GS (fig. 2B) was assessed their changes were due to the effect of size of fish and time after transfer, but not to the treatment effect (table I). Nevertheless, their trends of activity were in agreement with those observed in glycogen levels.

Besides glycogen levels, two other sources of glucose were evaluated in kidney during transfer of rainbow trout to seawater such as the use of exogenous glucose (evaluating HK activity) and gluconeogenesis (evaluating FBP activity). A clear increase was observed in FBP activity for both sizes of animals transferred to seawater (fig. 3A) that can be exclusively attributed to the effect of treatment (table I). In contrast, changes observed in HK activity (fig. 3B) did not indicate a treatment effect, though a size effect as well as a size-treatment interaction were clearly established (table I).

Finally, two different uses of glucose were evaluated in kidney, i.e. through gly-

Table I. *Significant effects obtained after three-way analysis of variance of parameters assessed in kidney of rainbow trout.*

Treatment (freshwater vs seawater), size of fish (small vs large) and time after transfer (1 through 7 days) are the main factors; treatment × size, treatment × time, and size × time are the first order interactions. df, degrees of freedom.

Parameter	Source of variation	df	Mean Square	F	P
Glycogen	Treatment	1	0.509	4.760	0.032
	Size	1	1.203	11.24	0.001
Glucose	Size	1	0.045	5.612	0.023
Glycogen Phosphorylase	Size	1	40.76	5.821	0.018
	Size x Time	2	32.14	4.596	0.013
Glycogen Synthetase	Size	1	0.066	10.76	0.002
	Time	2	0.002	3.057	0.050
Fructose 1,6-biphosphatase	Treatment	1	1.043	15.09	0.001
Hexokinase	Size	1	0.154	4.660	0.035
	Treatment x Size	2	0.128	3.870	0.026
Phosphofructokinase	Size	1	8.467	107.9	<0.001
Glucose 6-phosphate dehydrogenase	Size	1	61.97	3.967	0.050
	Time	2	67.16	4.299	0.017
	Treatment x Size	1	79.08	5.062	0.027

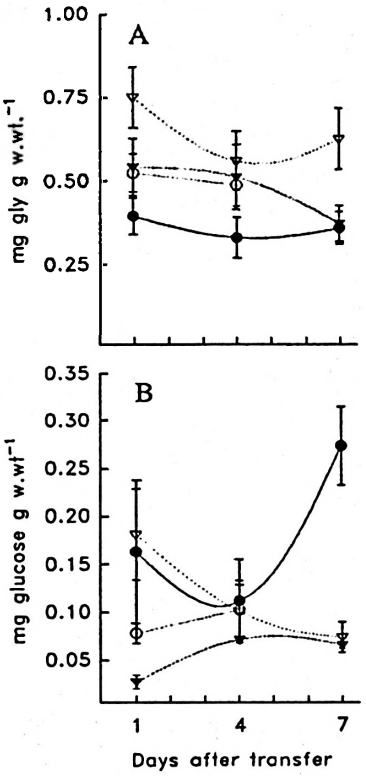

Fig. 1. *Effect of seawater (filled symbols) on kidney levels of glycogen (A) and glucose (B) in rainbow trout of two different sizes, small (circle) and large (triangle) sampled on days 1, 4 and 7 after transfer.* The control animals (open symbols) stayed in freshwater and were sampled on the same days of experiment. The values are the means ± S.E.M. of N = 10.

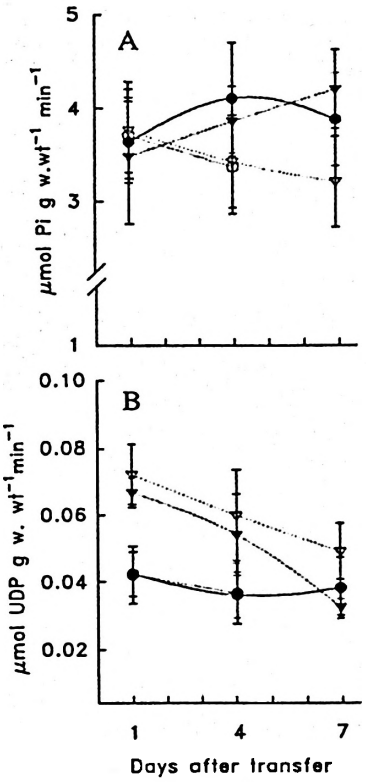

Fig. 2. *Effect of seawater (filled symbols) on kidney glycogen phrosphorylase (A) and glucosgen synthetase (B) activities in rainbow trout of two different sizes, small (circle) and large (triangle) sampled on days 1, 4 and 7 after transfer.* Legend as in fig. 1.

colysis or through the pentose phosphate pathway, with no changes being attributed in any case to the effect of treatment as a main factor (table I). The glycolytic enzyme PFK displayed changes (fig. 4A) that can be strictly associated with the different size of fish assessed (table I), though a lower activity was always detected in animals transferred to seawater. The changes observed in G6PDH activity (fig. 4B) can be due to the effect of size and time as well as to the interaction between treatment and size (table I).

Discussion

Reduced liver glycogen levels, accompanied by elevated blood glucose levels have both been reported during smoltification (27) and adaptation to seawater (1, 12, 25, 44, 47). This mobilization to peripheral tissues must be directed to satisfy the increased energetic demand observed in osmoregulatory organs during adaptation to seawater (8, 19, 21) and can be due to the action of those hormones, such as cortisol and/or GH,

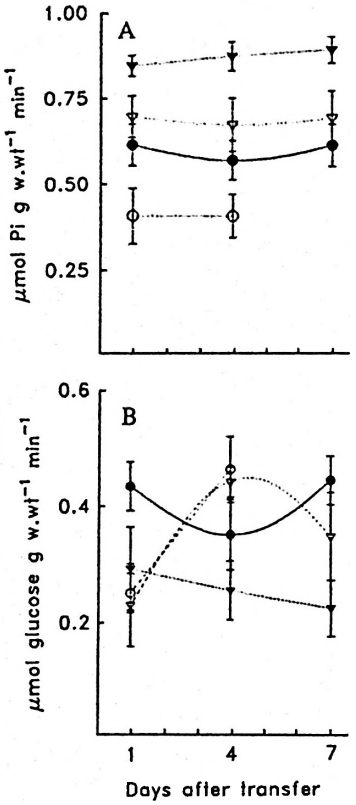

Fig. 3. *Effect of seawater (filled symbols) on kidney fructose, 1,6-biphosphatase (A) and hexokinase (B) activities in rainbow trout of two different sizes, small (circle) and large (triangle) sampled on days 1, 4 and 7 after transfer.*
Legend as in fig. 1.

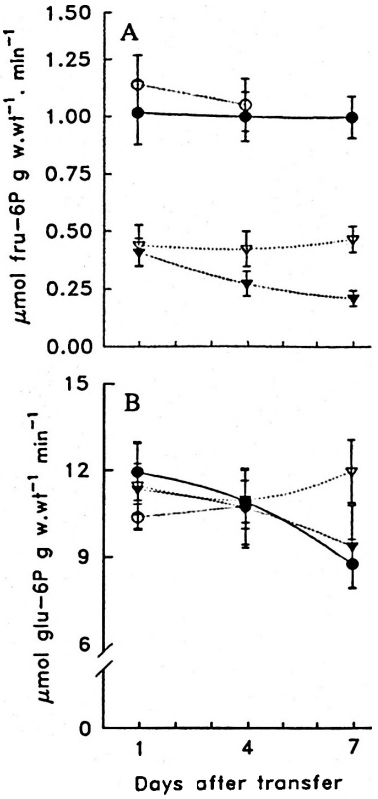

Fig. 4. *Effect of seawater (filled symbols) on kidney 6-phosphofructo 1-kinase (A) and glucose 6-phosphate dehydrogenase (B) activities in rainbow trout of two different sizes, small (circle) and large (triangle) sampled on days 1, 4 and 7 after transfer.*
Legend as in fig. 1.

involved in the control not only of carbohydrate metabolism (2, 23) but also of osmoregulation during adaptation to seawater (3).

Of the osmoregulatory organs, gills, kidney, urinary bladder and intestine, few studies have been made up to now regarding changes in kidney metabolism during adaptation to seawater. Na^+/K^+-ATPase activity is known to increase in gills during seawater adaptation (21) but this is not the case in kidney where no changes have been observed in such enzyme activity (15, 28). Of all the processes activated in kidney during seawater adaptation (4, 7, 34, 36) the major energetic cost arises from producing a concentrated urine (11). These increased energetic requirements during seawater adaptation have been studied in kidney only by the assessment of cytochrome C oxidase and cytrate synthetase respiratory enzymes, that displayed an increased activity in smolts of Atlantic salmon (29).

Kidney could rely upon different fuels such as glucose, lactate or amino acids (30). One or several of those fuels may be used in kidney to perform its osmoregula-

tory work during seawater adaptation. Glucose can be obtained from three different sources: from kidney glycogen stores; from gluconeogenesis, or via the blood stream. The amount of glycogen in the kidney of fish is quite elevated in comparison with other tissues (6, 45). A decrease in kidney glycogen levels during seawater adaptation of rainbow trout indicating for the first time that kidney internal stores may be used during the osmotic adjustments of hypoosmoregulation. The decrease in glycogen levels can be attributed, at least in part, to the changes observed in GP and GS activities though the treatment effect was not significant in both enzymes. Also a sharp increase in kidney FBP activity was observed in rainbow trout transferred to seawater. A similar finding had been previously observed in livers of the same species during adaptation to seawater (43). This parallel increase is not surprising considering that kidney is besides liver the main tissue involved in gluconeogenesis (20, 45), and a similar increase had been already found associated with the seasonal changes of hypoosmoregulatory ability in rainbow trout (42). Another evidence supporting an increased gluconeogenesis in kidney comes from the increased activity of aspartate aminotransferase and especially glutamate dehydrogenase observed in kidneys of rainbow trout transferred to seawater by JÜRSS *et al.* (15, 16). These increased enzyme activities point to an increased amino acid catabolism i.e. more amino acids would be used as gluconeogenic substrates in kidney during adaptation to seawater. Since an increase of blood glucose is a well known feature during adaptation of rainbow trout to seawater (1, 25), also in the stock used in this study (44), increased levels of glucose are available to the tissues, kidney for instance, where HK activity is sufficient to allow the use of exogenous glucose (20). Despite this, no significant increase in kidney HK activity was noted in the present study as the fish were transferred to seawater. Therefore an increased glucose production takes place in kidney of rainbow trout during adaptation to seawater through an increase in both glycogenolysis and gluconeogenesis.

If possible uses of glucose are considered, a slight decrease (though non significant) was seen in the activity of the PFK glycolytic enzyme during transfer to seawater. This is not striking considering the increased FBP activity observed at the same time and the fact that glycolysis does not operate at high rates in kidney compared with other tissues (6, 20). Glucose, besides being used in glycolysis, may be used via the pentose phosphate pathway, which seems to operate at important rates in fish kidney (30), but no major changes were detected in the activity of G6PDH which could be related to the effect of seawater transfer. Finally another possible pathway of glucose utilization, i.e. being exported to other tissues, cannot be discarded to occur in our case since the activity of the enzyme involved in glucose export, G6P, is important in kidney owing to its clearly established gluconeogenic role (6, 45). Therefore we hypothesize, taken into acccount the increased glucose production and the lack of increased glucose use in kidney, that most of the glucose obtained in kidney during seawater adaptation of rainbow trout is destined to be exported to other tissues in which increased demand for fuels such as glucose occur at the same time as seawater adaptation. On the other hand, kidney energetic requirements during seawater adaptation have to be ascertained since they should be supported via fuels other than glucose. In this way, amino acids are also known to be an excellent fuel for kidney (30), and a higher pro-

tein demand is known to occur in rainbow trout during seawater adaptation (15, 16).

To sum up, during transfer of rainbow trout to seawater, several changes were observed in the kidney carbohydrate metabolism. These changes were mainly size-independent in contrast to other studies in which metabolic changes occurring in liver (44) diferred between small and large fish. An enhanced glycogenolysis was observed in both sizes of animals and there was a concomitant increase in gluconeogenic enzyme activity. Both changes are suggested to be related to the known role of kidney as a glucose producer tissue. This production would be related, at least in part, to the high energetic requirements of osmoregulatory work performed by other tissues using glucose as fuel, such as the gills (35, 41), during adaptation to seawater.

Acknowledgements

This work was supported by a research grant from the "Xunta de Galicia" (XUGA 20001B90) to M. Aldegunde and J. L. Soengas and J. Fuentes were recipients of a predoctoral fellowship. We are indebted to Patricia Barciela by her expert technical assistance and useful advices.

J. L. SOENGAS, J. FUENTES, M. D. ANDRÉS y M. ALDEGUNDE. *La transferencia directa de la trucha arco iris al agua de mar induce graves cambios en el metabolismo renal de los hidratos de carbono.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (4), 219-228, 1994.

Se miden en riñón de trucha arco iris *(Oncorhynchus mykiss)* de dos tamaños diferentes (80 y 140 g) los niveles de glucógeno y glucosa, así como las actividades de una serie de enzimas clave de la glucogenolisis, la glucólisis, la gluconeogénesis y la ruta de los fosfatos de pentosa, para detectar la posible existencia de cambios en el metabolismo de carbohidratos asociados a la transferencia al agua de mar (28 por mil) durante 7 días. los resultados muestran la existencia de cambios en el metabolismo de carbohidratos independientes del tamaño del animal. Se observa un marcado incremento en la glucogenolisis renal, con activación de la gluconeogénesis, para ambos tamaños de animales, durante la adaptación al agua de mar. Se sugiere que estos cambios metabólicos estarían relacionados con la función del riñón, como tejido productor de glucosa, para abastecer el aumento de combustible en otros tejidos, como las branquias, durante la adaptación al agua de mar.

Palabras clave: Trucha arco iris, Adaptación al agua de mar, Riñón, Glucogenolisis, Gluconeogénesis.

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