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Seasonal Environmental Changes Modulate the Prolactin Receptor Expression in an Eurythermal Fish

Rody San Martín, Pilar Cáceres, Rodrigo Azócar, Marco Alvarez, Alfredo Molina, María I. Vera, and Manuel Krauskopf*

Millenium Institute for Fundamental and Applied Biology, Universidad Andrés Bello, Santiago, Chile

Abstract Eurythermal fish have evolved compensatory responses to the cyclical seasonal changes of the environment. The complex adaptive mechanisms include the transduction of the physical parameters variations into molecular signals. Studies in carp have indicated that prolactin and growth hormone expression is associated with acclimatization, suggesting that the pituitary gland is a relevant physiological node in the generation of the homeostatic rearrangement that occurs in this adaptive process. Here, we report the cloning and characterization of a full-length carp prolactin receptor cDNA, which codes for the long form of the protein resembling that found in mammalian prolactin receptors. We identified up to three receptor transcript isoforms in different tissues of the teleost and assessed cell- and temporal-specific transcription and protein expression in carp undergoing seasonal acclimatization. The distinctive pattern of expression that carp prolactin receptor (cPRLr) depicts upon seasonal acclimatization supports the hypothesis that prolactin and its receptor are clearly involved in the new homeostatic stage that the eurythermal fish needs to survive during the cyclical changes of its habitat. J. Cell. Biochem. 92: 42–52, 2004.

Key words: prolactin receptor; acclimatization; teleost

The study of the physiological and molecular processes that sustain life demands, as Somero commemorating Peter Hoschachka's key work in elucidating the mechanisms of biochemical adaptation revived, to keep the organism and its environmental relationships squarely in focus [Somero, 2002].

Eurythermal fish, as the carp (*Cyprinus carpio*), have evolved mechanisms to compensate for the naturally occurring changes in environmental temperatures and photoperiod. The consequent seasonal acclimatization processes are genetically programmed and involve cyclical rearrangements of molecular and cellular functions and the modulation of gene expression [Dietz and Somero, 1992; Vera et al., 1993; Kausel et al., 1999; Fletcher et al., 2001; Alvarez et al., 2003]. Likewise, temperature acclimation, even though a distinctive process [Hochachka and Somero, 2002], also imposes gene expression adjustments to provide the homeostatic state that fish needs to survive [Segner and Braunbeck, 1990; Goldspink, 1995; Tiku et al., 1996; Arends et al., 1998; Watabe, 2002].

Acclimatization of the carp starts when the fish senses the gradual physical parameter changes of its habitat and transduces them into molecular signals that may coordinate the adaptive physiological and cellular responses. We have been working with the hypothesis that this process encompasses a neuroendocrine cascade triggered by the slow changes in ambient temperature and entails transcriptional modulation in the hypothalamic-pituitary axis [Figueroa et al., 1997; Arends et al., 1998; Kausel et al., 1999]. In this context we searched for environmentally-induced pituitary gland genes and found a high level of prolactin (PRL) mRNAs in the rostral pars distalis (RPD) of

Dedicated to the memory of Prof. Peter Hochachka (1937–2002).

Abbreviations used: PRL, prolactin; cPRLr, carp prolactin receptor; RPD, rostral pars distalis; PI, pars intermedia.

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*Correspondence to: Manuel Krauskopf, Facultad de Ciencias de la Salud, Universidad Andrés Bello, República 237, Santiago, Chile. E-mail: mkrausk@unab.cl

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summer-acclimatized carp [Figueroa et al., 1994]. Conversely, the winter acclimatized-fish exhibited a negligible content of PRL mRNAs transcripts. Furthermore, we showed that the transcription factor Pit-1 involved in the regulation of PRL expression as well as the transcription of other anterior pituitary hormones is transcribed at significantly higher levels in summer-acclimatized carp with respect to the winter-adapted fish [Kausel et al., 1999]. While growth hormone (GH) seems to be also environmentally induced in the carp [Figueroa et al., 2000], somatolactin, a piscine pituitary hormone belonging to the PRL/GH family appears not to be associated to the seasonal acclimatization of this teleost [Lopez et al., 2001]. Thus, the pituitary gland may be a relevant node in coordinating the seasonal adaptive adjustment in the eurythermal carp and prolactin an important signal molecule in this process.

PRL has over 300 different functions in vertebrates [Freeman et al., 2000]. In euryhaline fish is clearly the fresh water-adapting hormone [Pickford and Phillips, 1959; Manzon, 2002].

Some teleosts exhibit two PRL forms. While in some fish such as salmon, carp, and eel the two forms are highly homologous [Manzon, 2002], in tilapia they differ in structure and biological activity [Yamaguchi et al., 1988; Auperin et al., 1994].

The presence of PRL receptors in various tissues of fish were established since Edery et al. [1984]. Having observed that in the carp pituitary gland a cell- and temporal-transcription of Pit-1 [Kausel et al., 1999] occurs concurrently with the higher summer-acclimatized carp levels of PRL mRNAs, and to understand the possible role of PRL as a temperature-environmental-adapting signal hormone we deemed important to isolate and characterize a carp cDNA PRL receptor (cPRLr) and assess its expression upon acclimatization. Here, we describe and analyze a full-length cDNA encoding for a cPRLr and report about its gene expression, examining transcripts and protein in fish that undergo natural environmental adaptation. We show that the seasonal differences in the amount of PRLr may be playing one of the underlying mechanisms of temperature acclimatization in the carp and supports the working hypothesis that PRL, in addition to be an osmoregulatory hormone in fish, is also an essential molecule for temperature acclimatization.

MATERIALS AND METHODS

Animal and Tissues

Male carp weighing 1,000–1,500 g were caught and maintained in a fixed 3×4 m cage submerged 2 m in an affluent of the same river with temperatures 18–20°C (summer) and 8–10°C (winter). Tissues from summer- and winter-acclimatized fish were dissected and either frozen as described elsewhere [Sarmiento et al., 2000].

Cloning of Carp PRLr cDNA

A cDNA fragment (904 bp) was obtained by RT-PCR using the sense 5′-GAGACATTCACATGCTGGTG-3′ and antisense 5′-AATCTTGATGGCTTTTGGAC-3′ primers derived from known PRLr cDNA sequences including goldfish [Tse et al., 2000]. Reverse transcription (MMLV RT, Gibco-BRL, Carlsbad, CA) was performed using carp kidney RNA (TRIzol) as template and the antisense oligonucleotide. The PCR amplification was carried out at low stringent annealing temperature and the product cloned into the pGEM-T Easy Vector (Promega, Madison, WI) and sequenced. The full-length 5′ region, including the transcription start site, and the 3′ cDNA end was obtained using the First Choice RLM-RACE Kit (Ambion, Austin, TX) and carp kidney RNA. The 5′ RACE reaction utilized the cPRLr3 (5′-AATCTTGATGGCTTTTGGAC-3′) and cPRLr4 (5′-CTATATAGGCCACATCCACC-3′) gene-specific primers for nested PCR. The cPRLr5 (5′-CAATCGAACAGTGGGATGCC-3′) oligonucleotide was used for the 3′ RACE reaction. The PCR products were cloned into the pGEM-T Easy Vector (Promega) and fully sequenced.

Northern Blots

Total RNA (25 μg) from different tissues (brain, gills, intestine, kidney, liver, and pituitary gland) of winter- and summer-acclimatized carp was fractionated on a 1.2% denaturing agarose/formaldehyde gel, transferred to Hybond-XL nylon membrane (Amersham Pharmacia Biotech, Buckinghamshire, England) and hybridized with a ^{32}P labeled cPRLr cDNA probe (derived from the extracellular domain coding region). Prehybridization and hybridization was carried out at 42°C and the membranes were washed up to 55°C for 30 min in 0.1× SSC, 0.1% SDS.

Competitive RT-PCR

To obtain a competitor RNA mouse genomic DNA was amplified at low annealing stringency using the antisense cPRLr6 (5′-CTATATAGGC-CACATCCACC-3′) and sense cPRLr7 (5′-CT-ACGAGTGCCCAGATTACA-3′) primers. The 270 bp PCR product was purified by gel electrophoresis and cloned into pGEM-T Easy vector (Promega). The competitor RNA was synthesized from the resulting $pmCOMP_{270}$ using T7 RNA polymerase. Competitive RT-PCR reactions were carried out mixing total kidney RNA (2 μg) from summer- and winter-acclimatized carp with dilutions of competitor RNA (0.1–100 attomoles) as described by Vera et al. [2000].

Western Blot

An oligopeptide from the extracellular domain (YHLENSETVYECPDY) derived from the cPRLr sequence was synthesized (Invitrogene Corporation, Carlsbad, CA) and conjugated with hemocyanin to raise rabbit polyclonal antibody. To obtain membrane extracts winter-acclimatized carp kidney tissues (5 g) were homogenized on ice in 10 Vol. of homogenization buffer (0.25 M sucrose, 1 mM EGTA, 10 mM Tris pH 7.4) containing Complete Protein Inhibitor Mixture (Roche Diagnostics, Mannheim, Germany). The homogenate was first centrifuged at 1,000*g* for 15 min and the supernatant was subjected to higher centrifugation at 10,000*g* for 15 min. Finally the supernatant was centrifuged at 20,000*g* for 90 min. All procedures were performed at 4°C and the pellet was resuspended in the homogenization buffer. The membrane proteins were fractionated by SDS–PAGE and electro-transferred to Optitran membranes. These membranes were immuno-stained with anti-cPRLr sera and the detection was carried out using the chemiluminescence Western Lightning System (Perkin Elmer Life Science, Boston, MA).

Immunohistochemistry

Kidney and pituitary tissues from summer- and winter-acclimatized carp were fixed in Bouin and 5 μm sections were mounted on sylanized slides. Immunostaining was carried out using the anti-cPRLr sera and detected according to the supplier's instructions of the Tyramid Signal Amplification System (NEN Life Science Products, Boston). Quantification of the immunostaining was carried out as described [Alvarez et al., 2003] except that the automated image digitizing system was the UN-SCAN-IT (Silk Scientific, Inc., Orem, UT).

RESULTS

Analysis of Carp PRL Receptor cDNA

We constructed a full length cPRLr cDNA (GenBank accession no. AY044448) containing 204 bp corresponding to the 5′ UTR and ORF encoding for the receptor precursor protein comprising 604 amino acids (Fig. 1). The derived protein primary structure exhibits the same motifs that characterize the PRL and cytokine (class 1) receptors family that, as in other fish, correspond to the long form of the mammals' receptor protein.

Clearly, the 230 amino acids extracellular domain includes 22 residues that upon hydrophobicity assessment can be ascribed as the signal peptide. Four cysteine residues and the WS sequence are topologically conserved. Concurrently, the cDNA codes for five potential N-glycosylation sites (Fig. 1). Within the intracellular domain (350 amino acids) box 1, box 2, and three tyrosine residues are conserved (Fig. 1). We identified, in the carp, topological dileucines, found also in other fish, that in mammals correspond to motifs involved in the receptor's internalization [Lu et al., 2002].

cPRLr mRNA and the Seasonal Effect

Kidney cPRLr mRNAs from summer- and winter-acclimatized carp resolved in three transcripts containing approximately 4,500, 3,000, and 2,000 nt (Fig. 2). The level of these transcripts was clearly higher in the cold season-acclimatized fish when compared to the summer-adapted carp. While in the carp intestine only one size of transcript could be detected (4,500 nt), gills exhibited the other two carp mRNA forms found in kidney. Apart from these differences, which remained upon short and long periods of exposition of the hybridized blots, intestine and gill's cPRLr mRNAs were also higher in winter-acclimatized carp. Because Northern blotting was insufficient to track unequivocally cPRLr mRNAs in other tissues we used nested RT-PCR. As shown (Fig. 2) we could also detect the mRNAs in carp brain, liver, and pituitary gland tissues.

Using competitive RT-PCR to quantify cPRLr transcripts, kidney, followed by intestine, gills, and the pituitary gland, contained the higher

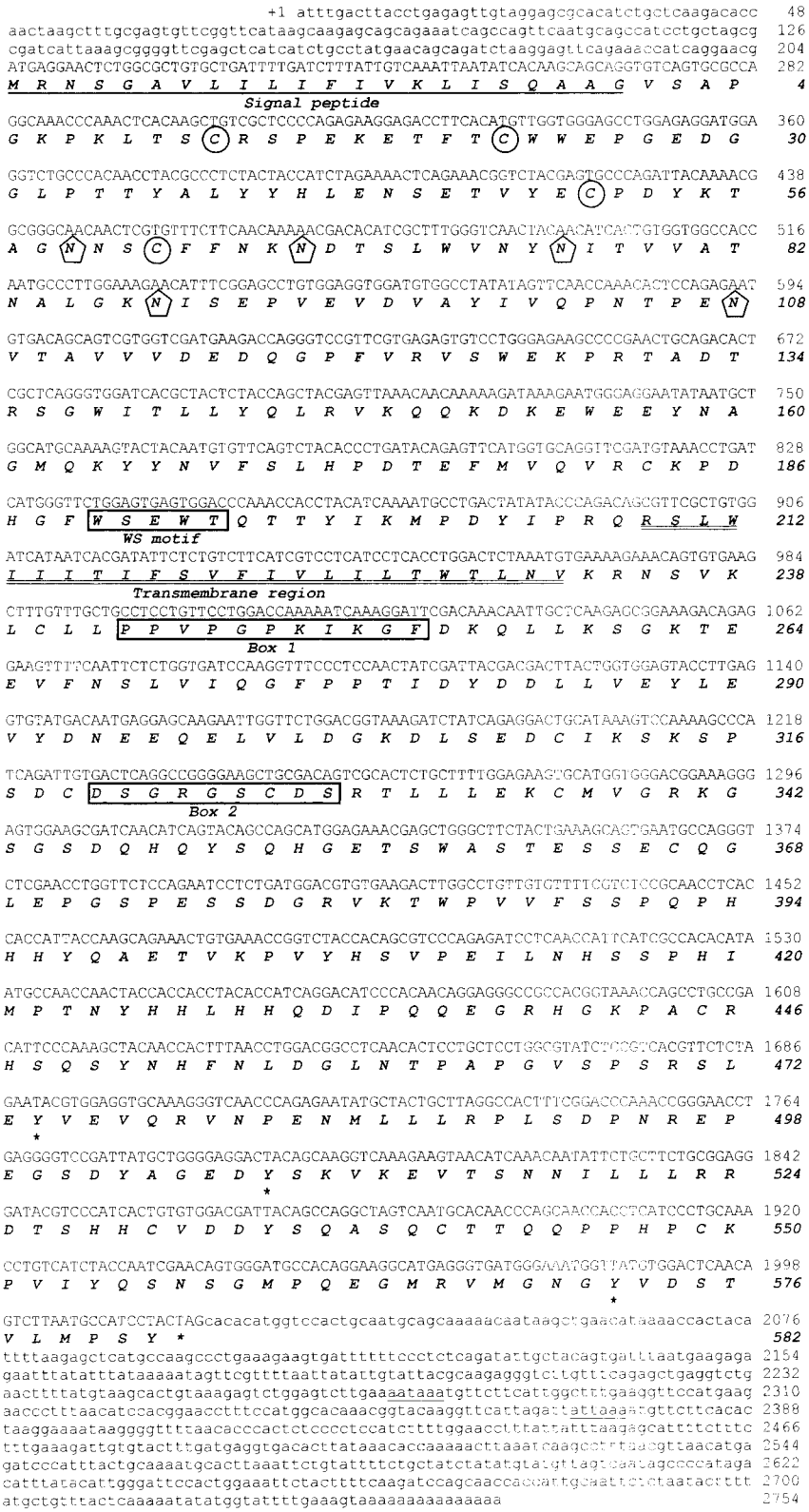

Fig. 1. Carp prolactin receptor (cPRLr) cDNA sequence and derived protein. Amino acids (capital letters) are numbered from the first residue after the potential cleavage site of the signal peptide. In the extracellular domain, the conserved cysteines residues are encircled and the potential N-glycosylation sites are in pentagons. The WS, Box 1, and Box 2 motif are in rectangles. The cytoplasmic domain contains three tyrosine residues (denoted by asterisk) present in all species. At the nucleotide level, the putative polyadenylation signals are underlined.

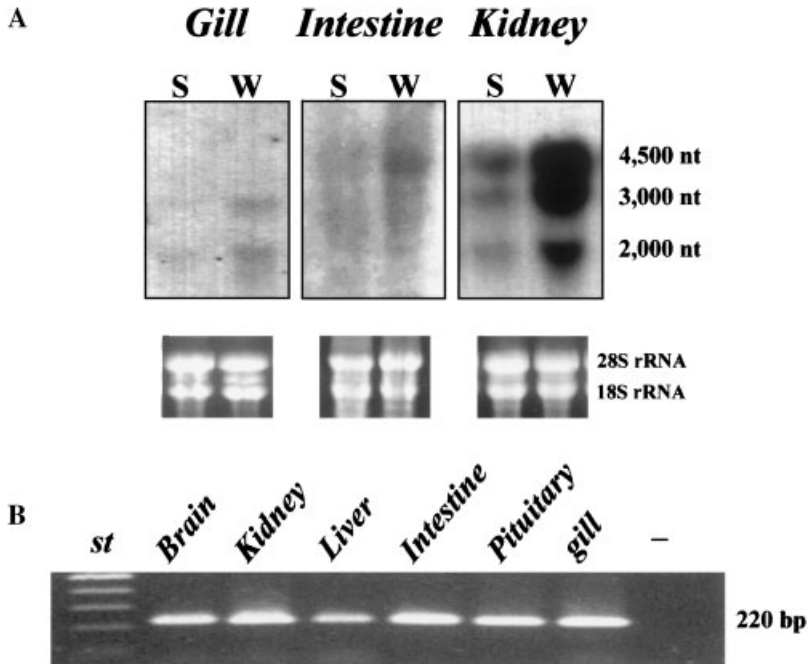

Fig. 2. Gene expression of cPRLr mRNA. **A**: **Upper panels** show Northern blot hybridization of total RNA from summer (S)- and winter (W)-acclimatized carp tissues. **Lower panels** show ethydium bromide stained RNA which were also used as loading controls. **B**: Nested RT-PCR detection of cPRLr transcript in W-acclimatized carp tissues.

amount compared to the other carp tissues examined. Furthermore, as shown in Table I, the amount of cPRLr mRNAs is significantly higher in winter-acclimatized carp kidney, intestine, and gills. No significant differences were observed in the pituitary gland of carp between summer and winter.

cPRLr Content is Cell and Season-Dependent

To asses the specificity of the polyclonal cPRLr antibody we used Western blotting. The antibody recognized an unique 76 kD band in membrane-protein extracts of carp kidney (Fig. 3). Because kidney and pituitary gland responded to acclimatization with two different cPRLr gene transcription behaviors we deemed important to localize and quantify the receptor protein in the fish cells from both tissues (Fig. 4). Immunohistochemistry on kidney sections showed strong staining in the epithelial cells of the renal distal tubes, although stromal cells were also labeled. Increased labeling was observed in winter-acclimatized carp compared to the summer-adapted fish in both cell types (Fig. 4A).

In the pituitary gland, cPRLr immunoreactivity is mainly restricted to the cells of the pars intermedia (PI). In summer, however, when the hormone receptor signals are clearly higher than in winter, we also observed signals in the RPD (Fig. 4B). When we quantified the distinctive expression of cPRLr in the immunostained tissues, kidney cells from winter-

TABLE I. Quantification of cPRLr mRNA by Competitive RT-PCR

	Summer cPRLr mRNA (attomoles/μgr total RNA)	Winter cPRLr mRNA (attomoles/μgr total RNA)	cPRLr mRNA winter/summer
Gill	1.9 ± 0.5 (5)	8.5 ± 0.8 (5)	4.5
Intestine	1.0 ± 0.05 (5)	15.5 ± 1.5 (5)	15.5
Kidney	10.2 ± 1.2 (7)	90.4 ± 7.5 (7)	8.9
Pituitary	0.9 ± 0.02 (6)	0.8 ± 0.1 (5)	n.s.

The mean cPRLr mRNA values were obtained from the number of individuals indicated in brackets. Significant differences were attained between summer- and winter-acclimatized carp. Student's t-test $P < 0.05$. n.s. indicate no significant differences.

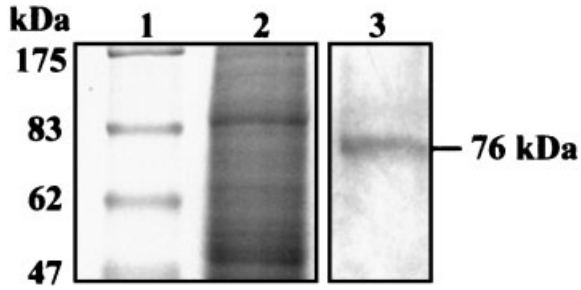

Fig. 3. Specificity of the polyclonal cPRLr antibody. Membrane protein extracts (10 μg) of kidney tissues from winter-acclimatized carp were fractionated (10% SDS–PAGE), blotted onto nitrocellulose membranes and immunolabeled with the antibody. **Left panel** shows coomassie blue staining of fractionated molecular weight standard (**1**) and kidney protein extract (**2**). **Right panel** shows immuno-detection of cPRLr (**3**).

acclimatized carp revealed a significantly higher level of hormone receptor when compared to the summer-adapted tissue (Fig. 5A). Conversely we observed in both, the PI and the RPD of pituitary glands a clear decrease of the cPRLr protein in the cold season. As shown (Fig. 5B,C) carp pituitary gland from the summer-acclimatized fish depicts a two-fold increase of the hormone receptor in the PI.

DISCUSSION

This study provides evidence that the PRL receptor expression, as is the case of its ligand, is seasonally regulated in an eurythermal fish. Furthermore, supports the notion that the pituitary gland may be acting as an important node in the production of the signal molecules that an ectotherm needs to maintain homeostasis upon natural temperature variations of its habitat [Kausel et al., 1999; Figueroa et al., 2000; Lopez et al., 2001]. The complex physiological adaptive mechanisms underlying fish acclimatization and acclimation has also been associated with the regulation of the expression of specific genes, among others, in muscle adaptation [Hirayama and Watabe, 1997; Watabe, 2002], in cell membrane fluidity responses [Tiku et al., 1996; Cossins et al., 2001], and in brain [Dietz and Somero, 1992; Tang et al., 1999]. Clearly, differential gene expression in ectotherms appears to be a fundamental mechanism for survival in variable thermal environments. Accordingly, preliminary findings [Pennisi, 2002] indicate that 10% of 14,000 carp genes examined using microarrays are altered by cold temperatures.

It has been well established that upon seasonal acclimatization of the carp a generalized nucleolar rearrangement occurs revealing profound changes in ribosomal biogenesis [Vera et al., 1993, 1997; Molina et al., 2002; Alvarez et al., 2003]. As a consequence of the nucleolar dynamics that environmental changes triggers, a dramatic segregation of the nucleolar components is induced during the cold season [Vera et al., 1993]. While nucleolar segregation with its functional effect, i.e., regulation of the rRNA synthesis, represents a unique cellular phenotypic expression of the complex mechanisms that conform the fish thermal adaptive response, an endocrine cascade of signals, involving the pituitary gland as a central node may be one of the mechanisms coordinating at the molecular level the systemic acclimatization process.

The pituitary gland from summer-acclimatized carp expresses a high amount of PRL mRNA in the RPD when compared to the negligible transcripts observed in the winter-acclimatized fish [Figueroa et al., 1994]. Further studies with acclimated carp revealed that in addition to temperature, photoperiod was a relevant modulator in the neuroendocrine cascade that induces PRL transcription [Figueroa et al., 1997]. Also, that in winter-acclimatized carp 17β-estradiol increases the reduced existing hormone protein in the PRL producing lactotrophs in pituitary gland [Figueroa et al., 2002].

Certainly, the effects of PRL are mediated by a specific cell membrane receptor. The PRLr belongs to the superfamily of the class 1 cytokine together with the receptors for growth hormone, erythropoietin, leptin, and the interleukins [Manzon, 2002]. Our results show that the 604 amino acids cPRLr precursor conserves the structural and signaling elements of the long isoform manifested in mammals and presents the higher homology with the goldfish receptor (91%). When compared to other fish, the receptor's amino acids identity is much lower (37–54%), similar to the 33–38% homology observed with regard to the mammalian and amphibian PRLr (Table II). Clearly, the greatest amino acids divergence occurs within the intracellular domain. Conversely, the extracellular domain is the most conserved, 53–92% identical between fish and 49–51% respect mammals. This domain, as in all species studied, contains four absolutely conserved

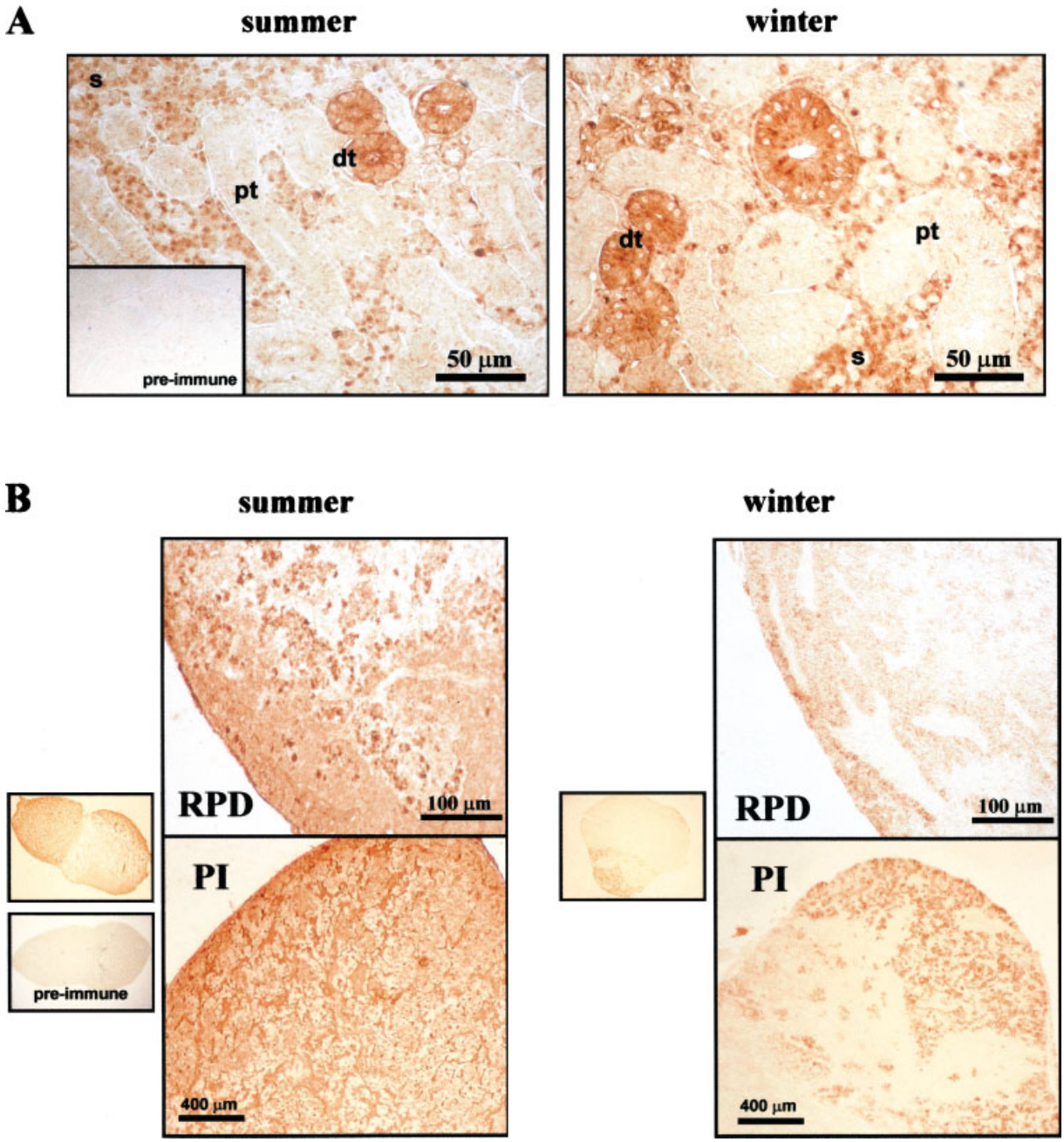

Fig. 4. Immunohistochemical detection of cPRLr in tissue sections from summer- and winter-acclimatized carp. **A**: In kidney sections a strong signal is observed in the distal tubules (dt) and the renal strome (s), while no signal is present in the proximal tubules (pt). **B**: Sagital sections of pituitary gland show signals mainly in the pars intermedia (PI) region and in the rostral pars distalis (RPD). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cysteine residues (positions 12, 22, 51, and 62), and a membrane-proximal pentapeptide, the WS motif of the mammalian PRLr, both of which, together with glycosylation in this domain [Buteau et al., 1998] are involved in the protein folding, trafficking, and probably in ligand binding [Rozakis-Adcock and Kelly, 1991; Somers et al., 1994; Freeman et al., 2000; Manzon, 2002]. cPRLr exhibits five putative N-glycosylation sites (residues 59, 67, 76, 88, and 108) (Fig. 1). Although this number of putative sites is higher compared to mammals, the degree of glycosylation seems to be lower according to our Western blots that reveal a 76 kD protein (Fig. 3) compared with the 85–90 kD observed in the human long isoform of the

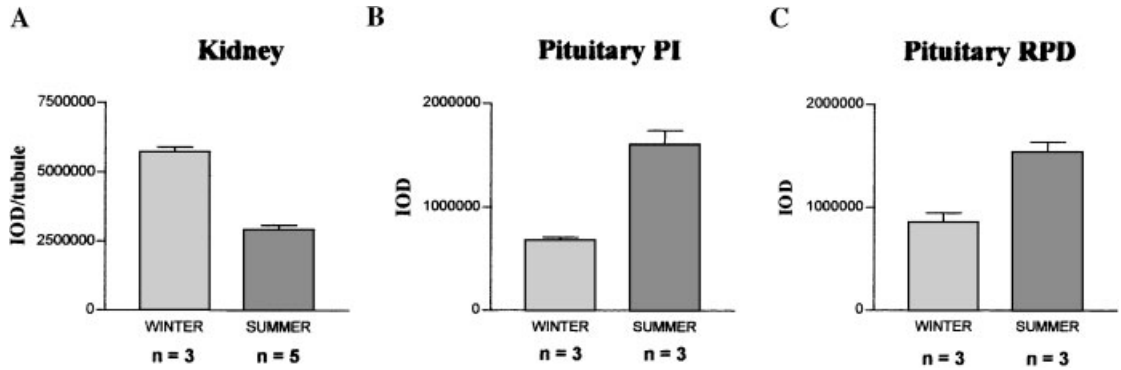

Fig. 5. Seasonal cPRLr content. The graphs depict the means of immuno-signals integrated optical densities in the winter- and summer- acclimatized carp tissue sections. n, represents the number of individuals used in tissue preparation.

receptor [Boutin et al., 1989; Kline et al., 2002]. Nevertheless, the role of glycosylation in fish PRLr remains to be elucidated. Box 1 (amino acids 243–253) and box 2 (resides 320–328) are highly conserved in cPRLr when compared to mammals, amphibians, and fish, with the exception of gilthead seabream, a protandrous hermaphrodite in that box 2 is not conserved. The intracellular domain contains, in all species studied three tyrosine residues (474, 508, and 572), which have been associated with signaling [Chang et al., 1998; Ali and Ali, 2000]. It has been documented that fish PRLr induces cellular proliferation in transfected cells suggesting that the receptor follow signaling patterns similar to those observed in mammals [Tse et al., 2000].

Northern blots analyses revealed three different cPRLr transcripts, the expression of which was distinctive in each of the tissues studied. In other fish, such as goldfish [Tse et al., 2000] and seabream [Santos et al., 2001] multiple PRLr transcripts have also been identified. In the latter, the cDNAs 3′ UTRs contain multiple polyadenylation signals that may explain the existence of transcripts with different sizes. Analogously, in spite of the various hormone receptor transcripts we detected,

TABLE II. Comparison of the Amino Acid Sequences of Vertebrate PRLr

	Human	Rat	Rabbit	Ovine	*Xenopus*	Tilapia	Goldfish	Carp	Trout	Sea bream	Flounder
A. Full-length											
Human	—	67	75	69	44	32	34	38	33	30	32
Rat	—	—	67	65	42	31	32	38	33	31	31
Rabbit	—	—	—	67	44	33	33	33	34	29	34
Ovine	—	—	—	—	44	35	33	38	35	27	36
Xenopus	—	—	—	—	—	33	33	35	32	29	32
Tilapia	—	—	—	—	—	—	44	46	50	34	64
Goldfish	—	—	—	—	—	—	—	91	54	34	46
Carp	—	—	—	—	—	—	—	—	54	37	46
Trout	—	—	—	—	—	—	—	—	—	38	52
Sea bream	—	—	—	—	—	—	—	—	—	—	34
B. Extracellular domain											
Human	—	69	78	70	50	45	44	50	48	43	47
Rat	—	—	75	71	52	47	47	50	51	44	47
Rabbit	—	—	—	78	56	51	48	49	52	45	50
Ovine	—	—	—	—	50	47	45	49	47	45	48
Xenopus	—	—	—	—	—	47	44	51	48	43	46
Tilapia	—	—	—	—	—	—	60	64	70	50	75
Goldfish	—	—	—	—	—	—	—	92	70	52	60
Carp	—	—	—	—	—	—	—	—	74	53	66
Trout	—	—	—	—	—	—	—	—	—	56	71
Sea bream	—	—	—	—	—	—	—	—	—	—	52

The percentages of amino acids identity was obtained by comparison of receptor primary structures present in GenBank with the following accession numbers: human (P16471), rat (P05710), rabbit (P14787), ovine (O46561), *Xenopus* (AF193800), tilapia (L34783), goldfish (AF144012), carp (AY044448), rainbow trout (AF229197), sea bream (AF253527), and flounder (AB047922).

Western blot analysis revealed only one cPRLr protein (Fig. 3). It is also possible, however, that more than one gene codes cPRLr. Figueroa et al. [2002], recently described two serum PRL isoforms in carp thus suggesting that more than one form of the receptor may exist in this fish.

The kidney showed the highest density of cPRLr, and the amount decreased in intestine and gills. In addition we assessed by nested RT-PCR the corresponding transcripts also in pituitary gland, brain, and liver. This wide distribution of the hormone receptor has been described in other fish [Sandra et al., 1995, 2000; Tse et al., 2000; Higashimoto et al., 2001; Santos et al., 2001]. It is consistent with the multiple functions that PRL fulfills and that have been phylogenetically conserved in vertebrates [Sandra et al., 2000].

Under seasonal acclimatization cPRLr expression depicts a tissue-specific response. As we show (Table I), cPRLr mRNAs content in winter-adapted carp is clearly higher in kidney, intestine, and gills. Interestingly, the cold-induced cPRLr transcription contrasts the up-regulation of the hormone expression that occurs in the pituitary gland of summer-acclimatized carp [Figueroa et al., 1994]. Thus, a down-regulation of the receptor transcription may result in certain tissues upon higher levels of the hormone. This homeostatic mechanism in response to a hyperosmotic environment has been observed in tilapia gills and intestine where while PRL, the fresh water-adapting hormone, decreases, the number of tiPRL receptors increases [Auperin et al., 1995; Sandra et al., 2001]. However, no increase in protein receptor was observed in kidney upon hyperosmotic adaptation in tilapia [Sandra et al., 2001]. There are evidences that in mammals high hormone levels may down regulate the PRLr favoring the internalization and degradation of the membrane receptor protein [Genty et al., 1994]. This may be the case in kidney from summer-acclimatized carp, a fresh water fish thus distinctive from the euryhaline tilapia, where the protein receptors content is significantly lower that in the cold-season adapted fish (Fig. 5A). A different response, however, occurred in the pituitary gland where, during the warm season, the receptor protein increases notoriously (Fig. 5B,C). The tissue-dependent cPRLr differential expression pattern can be understood in connection with the distinctive cell functions. While pituitary gland is the main locus of PRL, GH, and somatolactin in fish, kidney is involved in water balance as well as in ion balance, depending of the fish species [Manzon, 2002]. PRL provoke in freshwater fish urine output by increasing the glomerular filtration rate [Manzon, 2002].

As shown in Figure 5C and conversely with the kidney response during carp acclimatization, the lactotrophs of the anterior pituitary express higher levels of cPRLr during summer, season in that the hormone mRNA and the protein depicts a remarkable rise [Figueroa et al., 1994; Figueroa et al., 2002]. Although PRL receptors have been detected in the pituitary gland of several species, only in rodents and human they have been recognized in lactotrophs [Morel et al., 1994; Jin et al., 1997; Schuff et al., 2002].

We also found an increase of the hormone receptor protein in the PI of the warm-season-acclimatized carp (Fig. 5B). We previously reported that transcription factor Pit-1 expression in the PI is strongly up-regulated in summer-adapted carp [Kausel et al., 1999]. Somatolactin, a fish hormone partially regulated by Pit-1, is transcribed in this anterior pituitary region. However, when we quantified by in situ hybridization assays somatolactin transcripts in pituitary sections from seasonal-acclimatized carp we found no differences between summer and winter [Lopez et al., 2001]. It is well known that duplicated genes are common in the tetraploid carp [Ferris and Whitt, 1977]. Also, that there are two POMC genes that appears to be differentially responding to temperature stress [Arends et al., 1998]. Thus, we cannot rule out the differential expression of two or more mRNA.

Although, there are no significant differences in the pituitary cPRLr transcript levels between winter and summer adapted fish, we do observe a significant increase in the content of the protein in summer acclimatized carp. This apparent paradox may be explained by an increase in the half life of the receptor protein or in the efficiency of the mRNA translation in summer-adapted carp.

The fact that PRLr binds to different ligands including growth hormone obscures our understanding on the biological effects of PRL [Goffin et al., 2002]. Also, the few studies on this receptor in fish have been mainly focused to assess the role of PRL in osmoregulation [Manzon, 2002]. Thus, much more work is needed to gain

the knowledge we need to advance on the mechanisms by which PRL contributes to conform the seasonal acclimatization responses in eurythermal fish.

Our results suggest that, as occurs during osmoregulatory adaptations in fish [McCormick, 2001], the neuroendocrine system is fundamental in linking the environmental change and the physiological, cellular, and molecular adaptive responses during the cyclic seasonal acclimatization in fish. This is, to our knowledge, the first report documenting a cell- and temporal-specific expression of a PRL receptor in a teleost undergoing seasonal acclimatization. The distinctive pattern of expression that the cPRLr depicts in the pituitary gland supports its essential role in modulating the expression of PRL that appears to be, at least in the carp, a central coordinating molecule to build up the new homeostatic stage that the eurythermal fish needs to survive during the cyclical changes of its habitat.

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