Differential expression of two pro-opiomelanocortin mRNAs during temperature stress in common carp (*Cyprinus carpio* L.)

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Abstract

Pro-opiomelanocortin (POMC) is the precursor of a number of biologically active peptides, including adrenocorticotropic hormone, α-melanocyte-stimulating hormone and β-endorphin, which are released by the pituitary glands of fish as well as mammals. To quantify the levels of expression of the two POMC mRNAs relative to one another during the response of the common carp to temperature-induced stress, we used reverse transcriptase PCR combined with capillary electrophoresis and laser-induced fluorescence detection. The ratio of POMC-I mRNA to POMC-II mRNA determined in wild-type and four isogenic carp strains was found to be strain-dependent and influenced by temperature. In strain E20 × R8, the ratio had altered in favour of POMC-I from 1:3·2 (POMC-I:POMC-II) in fish adapted to 24 °C to 1:1·2 in fish adapted to a decrease of 9 °C in ambient temperature. A rapid drop in temperature from 24 to 15 °C decreased the POMC mRNA ratio at the expense of POMC-I from 1:1·9 in the control fish (strain E4 × R3R8) to 1:4·2 3 h after the temperature drop of 9 °C. We conclude that both POMC genes are expressed in the common carp and that their expression ratio is strain-dependent and changes in response to ambient temperature.

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Introduction

In fish, stressors activate the hypothalamic–pituitary–interrenal system, and the subsequent increased release of pro-opiomelanocortin (POMC)-derived peptides from the pituitary gland induces cortisol release from the corticosteroid-producing cells of the head kidney. A variety of external stimuli is known to induce a stress response in fish (for a review, see Wendelaar Bonga 1997). The common carp is a rather stenohaline fish (Gupta & Hanke 1982), and changes in water salinity increase plasma cortisol levels (Abo & Hanke 1984) which points to a stress response. In euryhaline teleosts also such as the Mozambique tilapia (*Oreochromis mossambicus*) (Balm *et al.* 1994) and the sea bream (*Sparus aurata*) (Mancera *et al.* 1994), rapid salinity changes can act as stressors. Moreover, stressors may also affect the integrity of the branchial epithelium, thereby increasing stress (Ultsch *et al.* 1981).

POMC is the precursor of the hormones adrenocorticotropic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH) and β-endorphin in all vertebrate classes. Genome duplication in the common carp resulted in a tetraploid animal (Uyeno & Smith 1972). In the common carp, the duplicate loci started to diverge from each other only some 16 million years ago (Larhammar & Risinger 1993). For this carp two POMC cDNAs (POMC-I and POMC-II) have been cloned; as the two POMC molecules show substitutions in their β-MSH and corticotropin-like intermediate peptide (CLIP) regions, different POMC end products with specific functions may be expected (Arends *et al.* 1998). The expression of two POMCs in tetraploid animals has been known for many years (Kawauchi 1983, Martens *et al.* 1985, Salbert *et al.* 1992), but there is as yet little evidence for the biological significance of the presence of two gene transcripts. Furthermore, no method for discriminating between the two hormone precursors at the mRNA level has been published.

We report here on the quantification of the two closely related carp POMC mRNAs. PCR, a sensitive method of quantifying mRNA expression (Murphy *et al.* 1990, Siebert & Larrick 1993), was combined with capillary electrophoresis (CE) and laser-induced fluorescence detection (LIF) (Fasco *et al.* 1995).

Changes in ambient temperature affect all kinds of biological processes. For carp it has been demonstrated that an increase in water temperature stimulates the basal release of pituitary gonadotropins (Lin *et al.* 1996); growth is also enhanced with rising temperature (Fine *et al.* 1996). In addition, low environmental temperatures have been

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shown to affect the immune system and suppress specific immune functions in carp (Le Morvan-Rocher *et al.* 1995, Won & Lin 1995). Whether changes in ambient temperature can activate the hypothalamic–pituitary–interrenal system and evoke a stress reponse in carp is not known. In the present study we quantified the expression of the two POMC mRNAs in different strains of isogenic carp, in response to sustained changes in ambient temperature and to a rapid fall in temperature.

Materials and Methods

Adult male and female carp, *Cyprinus carpio* L., weighing around 100 g, were obtained from the central fish culture facility ('de Haar vissen') of the Agricultural University of Wageningen, The Netherlands. The isogenic carp strains used for the experiments were E4, E4 × R3R8, E20 × R8 and R3 × R8; wild-type carp from our laboratory stock were also used (Bongers *et al.* 1997). The fish were held in 100 litre tanks with circulating, filtered and well-aerated tapwater at 24 °C and a photoperiod of 16 h light alternating with 8 h darkness. The fish were fed commercial fish food (Trouvit; Trouw, Putten, The Netherlands), at a ration of 1·5% of the body weight per day.

For CE, a P/ACE 5010 unit equipped with a 635 laser module and a double-stranded DNA 1000 coated capillary (47 cm × 100 μm; Beckman Instruments, Fullerton, CA, USA) was used. Restriction enzymes were from Boehringer-Mannheim (Mannheim, Germany), reagents for reverse transcriptase (RT)-PCR from Gibco-BRL (Grand Island, NY, USA), and primers from Biolegio (Malden, The Netherlands). DNA intercalators TO-PRO-3 and TOTO-3 for CE were from Molecular Probes Inc. (Eugene, OR, USA) and all other chemicals were from Pharmacia (Uppsala, Sweden). Sterilization filters (0·45 μm) were from Amicon (Danvers, MA, USA).

RT-PCR

RNA from pituitary glands was isolated according to the Gibco-BRL RNAzol protocol. Total RNA (5 μg in 100% ethanol) was precipitated, washed in 70% ethanol and air-dried. The pellet was dissolved in 15 μl double distilled water and 0·7 μl 5′-pd$(N)_6$ random hexamer oligonucleotide primer (10 μg/μl), incubated for 10 min at 65 °C, spun for 5 s and put on ice. Then 3 μl 0·1 M dithiothreitol, 1·5 μl 10 mM dNTPs, 7·5 μl 5 × RT buffer and 0·5 μl RNA inhibitor (20 U) were added. After incubation for 15 min at 65 °C, 0·5 μl RT-Superscript (100 U) was added and the mixture incubated for 60 min at 42 °C.

The primers used for PCR were: 5' CA GAA TTC CTA TGC AGG TCT GAT CTG 3' CCCAP (forward) and 5' TC CTC GAG TGC TCT TTG TTT ATG ACG TTT 3' CCCAP (reverse).

Template DNA (2·5 μl RT-mix) and 50 pmol of each primer were used in a final volume of 40 μl containing 1 × PCR buffer (Gibco), 1·5 mM $MgCl_2$, 375 μM dNTPs and 1 unit *Taq* DNA polymerase. After an initial denaturing step at 94 °C for 5 min, the subsequent cycles consisted of denaturing at 94 °C for 1 min, annealing at 51 °C for 1 min, and elongation at 72 °C for 1 min for 30 cycles. A final elongation step was carried out at 72 °C for 10 min.

After PCR amplification, 5 μl PCR-mix was digested in a final volume of 15 μl containing 1 × Sure cut buffer A+M (1:1) and 10 units restriction enzyme SacI, PvuII or both at 37 °C for 3 h. Two POMC mRNAs, POMC-I and POMC-II, were co-expressed in the pituitary gland of all carp studied. RT-PCR for detection of POMC in the pituitary gland resulted in a 515 bp product, in accordance with the primer set chosen. Proceeding from the sequence analysis of the two POMC cDNAs, PvuII was chosen to specifically digest POMC-I into two products of 227 and 288 bp, and SacI for digestion of POMC-II into two products of 171 and 344 bp respectively. It should be noted that the 515 bp band in lane s (see Fig. 1) contains POMC-I and possibly SacI-undigested POMC-II, and the 515 bp band in lane p contains POMC-II and possibly PvuII-undigested POMC-I. A small amount of undigested product POMC-I, POMC-II, or both was possibly present in lane p/s (515 bp band, Fig. 1).

In the RT-PCR the two POMC mRNAs were amplified by a single primer set. The ratio between the two POMC mRNAs was assessed using different numbers of PCR cycles in two strains, wild-type and E4 × R3R8. The ratio in wild-type and E4 × R3R8 carp proved to be independent of the number of PCR cycles. The POMC-I:POMC-II mRNA ratio was dependent on the strain used (see Fig. 2). The amount of RT-mix as template (3–17% of the total RT-mix) in the PCR was tested, and the POMC ratio was found to be independent of the amount of template (data not shown).

Capillary electrophoresis

The wash buffer was TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8·5) containing 50 nM TOTO-3 or TO-PRO-3. Separation medium contained 3% acrylamide, 0·04% ammonium persulphate and 0·1% *N,N,N′,N′*-tetramethylethylenediamine in wash buffer and was allowed to polymerize overnight at 4 °C. Buffers were filtered through a 0·45 μm sterilization filter before use. The column was high-pressure-washed in the reverse direction with fresh separation buffer for 5 min before each injection. Before injection of the sample, the capillary was loaded with TO-PRO-3 or TOTO-3 in wash buffer with high pressure for 12 s; next the sample was applied by either 25 s pressure loading or 60 s electrokinetic loading at 7·4 kV. Electrophoresis was performed at 200 V/cm, using a reversed polarity and separation medium.

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CE-LIF was used to quantify the PCR products. Integrated peaks corrected for retention times were calculated using Beckman 'GOLD' version 8·10 software. The sum of the integrated peak areas represents the amount of PCR product (see Fig. 3). The sum of the peak areas 2, 3, 4 and 5 represents the two POMC mRNAs and is designated 100%. Peaks 3 and 4 represent POMC-I and peaks 2 and 5 POMC-II. The POMC-I:POMC-II ratio was calculated by taking the ratio of the integrated peaks of POMC-I (peak 3 and 4) and POMC-II (peak 2 and 5). Primers for PCR appeared in peak 1 and the undigested fraction in peak 6; the latter was always less than 7%. CE-LIF analysis indicated that under the conditions chosen (30 PCR cycles and 3 h digestion by the restriction enzymes), the mean ± S.E.M. digestion of POMC cDNAs was 97·0 ± 0·4%. (n=61).

Temperature-induced stress

Strain E4 × R3R8 fish were kept in groups of 10 in 100 litre tanks (n=16). Each tank represented one experimental group. Eight groups were used as controls for each time point, and another eight groups were temperature-shocked by exposure to a 9 °C reduction in ambient temperature. The circulating water at 24 °C was changed by a flow through of water at 15 °C. Within 45 min the ambient temperature in the tank decreased from 24 to 15 °C. At time point t=0, one control and one experimental group were netted in one scoop using a net fitted to the aquarium. Fish were anesthetized in 0·3 g/l tetramethylsilane (Sigma, St Louis, MO, USA) and 0·6 g/l bicarbonate, and blood samples were taken within 5 min of capture (Weijts *et al.* 1997). This procedure allowed detection of basal cortisol levels in this species. After blood sampling, the fish were decapitated, and the pituitary glands removed and stored at − 20 °C. The temperature shock treatment started at t=0: the temperature (in the eight experimental tanks) was decreased within 45 min from 24 to 15 °C as described above, without handling of the fish. At time points 20, 40, 60, 90 and 180 min, one control and one experimental group were netted and sampled as described above. At 3 h after the onset of the temperature shock treatment, the temperature was increased again and within 45 min had reached 24 °C. At t=300 min, the last control and experimental groups were sampled.

In a separate experiment, long-term acclimation effects on POMC mRNA expression were studied. Three groups (strain E20 × R8, weight about 50 g) were adapted for 8 weeks to different environmental temperatures of 15, 24 or 29 °C. After 8 weeks, groups were sampled as described above.

Cortisol RIA

Cortisol was measured by RIA as described by Balm and colleagues (1998), using a commercial antiserum (Bioclinical Services Ltd, Cardiff, UK). All constituents were in phosphate–EDTA buffer (0·05 M Na_2HPO_4, 0·01 M Na_2EDTA and 0·003 M NaN_3). Standards or unknowns (10 µl) in RIA buffer (phosphate–EDTA buffer containing 0·1% 8-anilino-1-naphthalenesulfonic acid and 0·1% (w/v) bovine γ-globulin) were incubated with 100 µl antiserum (in RIA buffer containing 0·2% normal rabbit serum) for 4 h. Samples were incubated overnight with 100 µl iodinated cortisol (2000 d.p.m.; Amersham Nederland BV, 's Hertogenbosch, The Netherlands). Bound and free hormone were separated by adding 1 ml ice-cold precipitation buffer (phosphate–EDTA buffer containing 2% (w/v) BSA and 5% (w/v) polyethylene glycol). The tubes were centrifuged at 4 °C (2000 ***g***, 20 min), the supernatants removed, and counted in a gamma counter (1272 clinigamma; LKB Wallac, Turku, Finland).

Statistical analysis

In all experiments, differences among groups were assessed by one-way ANOVA. Subsequently, significance of differences between mean values was tested with the Tukey multiple comparison test. Statistical significance was accepted at P<0·05. Values are expressed as means ± S.E.M.

Interassay variation

The reproducibility of the CE-LIF method was assessed by determination of the interassay variation on nine consecutive experimental days. A 100 bp ladder was used as both standard and size control for the digested products. The integrated peak areas of this standard 100 bp ladder were calculated for six peaks as a percentage of the total (see Table 1). The S.E.M. was less then 6% of the mean peak area for all peaks. Proceeding from the smaller than 6% interassay error variation, we conclude that this procedure is valid.

Results

Using RT-PCR, we were able to demonstrate the expression of two POMC mRNAs in the pituitary gland of a single fish (Fig. 1). After cloning of the two POMC mRNAs, one single primer set was selected for the amplification of both mRNAs by RT-PCR. Digestion with the restriction enzymes PvuII and SacI allowed discrimination between POMC-I and POMC-II. We found a difference in the POMC-I:POMC-II mRNA ratio between strains. Furthermore, we showed that the ratio was independent of the number of PCR cycles (Fig. 2). PCR products were quantified by integrating the peak areas measured by CE-LIF (Fig. 3). The reproducibility of the CE-LIF method was assessed by interassay variation, and the S.E.M. was 1·8% or less (Table 1).

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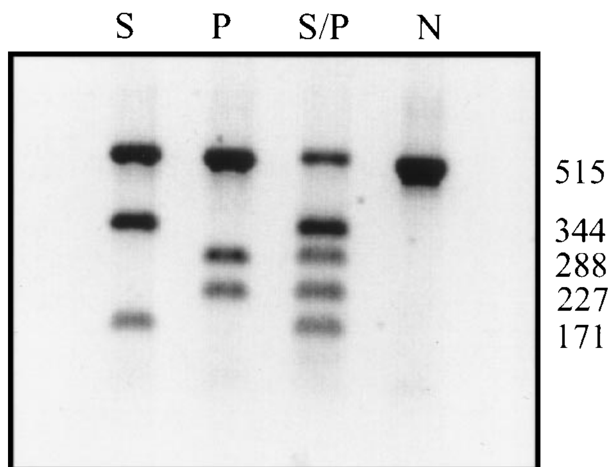

Figure 1 Digestion patterns of POMC-I and POMC-II RT-PCR products. RT-PCR products generated with carp POMC primers were digested for 3 h at 37 °C. All samples were derived from pituitary mRNA. Lane S, digested with SacI; lane P, digested with PvuII; lane S/P, digested with SacI and PvuII; lane N, not digested. cDNAs were fractionated on a 2% agarose gel and an inverted scan of the gel is shown. Molecular masses (Da) of the intact and digested products are given on the right.

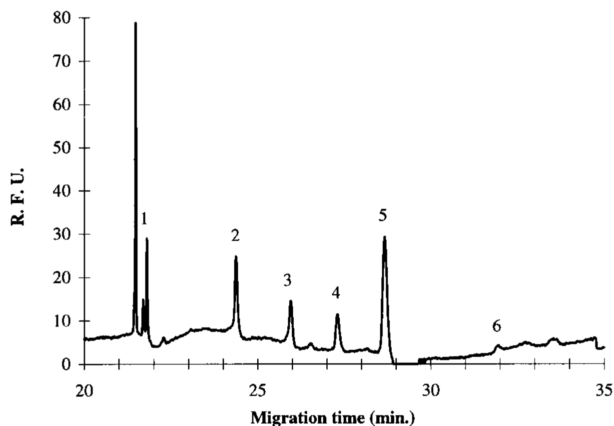

Figure 3 Quantification of digested RT-PCR products by CE-LIF. Separation of POMC SacI/PvuII digest by CE with the detection of TO-PRO-3 complexes by LIF. Relative fluorescence units (R.F.U.) were detected at excitation and emission wavelengths of 642 and 660 nm respectively. Peak 1, PCR primers; peaks 2 and 5, POMC-II; peaks 3 and 4, POMC-I; peak 6, undigested POMC. The POMC ratio was calculated by dividing the integrated areas of POMC-I (peaks 3 and 4) by POMC-II (peaks 2 and 5).

To test whether POMC mRNA expression is strain-dependent, we determined the POMC-I:POMC-II ratio in the isogenic carp strains E20 × R8, R3 × R8, E4 and E4 × R3R8, and the wild-type carp. Percentage POMC-I mRNA expression in these carp, all kept at 24 °C, is shown in Fig. 4. The POMC-I:POMC-II ratio was 1:1 for the wild type fish, 1:1·9 for strain E4 × R3R8, 1:3·2 for strain E20 × R8, 1:3·2 for strain E4, and 1:5·4 for strain R3 × R8.

Furthermore, POMC mRNA expression in fish acclimated to different ambient temperatures (15, 24 or 29 °C) was studied in strain E20 × R8. A significant increase in POMC-I mRNA expression was found in the fish acclimated to 15 °C ambient temperature, compared with those acclimated to 24 and 29 °C (Fig. 4). The POMC-I:POMC-II ratio changed in favour of POMC-I from 1:3·2 for fish kept at 24 °C to 1:1·2 in fish adapted to 15 °C.

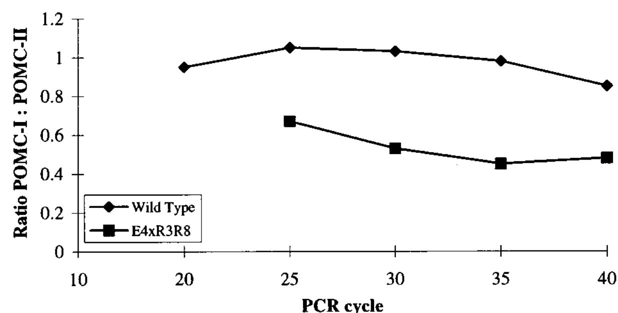

Figure 2 POMC mRNA expression during PCR amplification. Measurement of the POMC-I:POMC-II ratio during PCR amplification. PCR was performed in separate tubes for different numbers of cycles. PCR products were digested with SacI and PvuII and quantified by CE-LIF. The ratio was calculated by dividing the integrated peak areas of POMC-I by POMC-II.

Plasma cortisol levels in fish adapted to ambient temperatures of 15, 24 or 29 °C were significantly different (Fig. 5).

Next, short-term effects on POMC mRNA expression were studied in the isogenic strain E4 × R3R8. In these carp, a POMC-I:POMC-II ratio of 1:1·9 was found. At time points up to 5 h after the fish experienced a fall in temperature of 9 °C, POMC mRNA expression in the pituitary gland was measured. At 3 h after the rapid drop in temperature, percentage POMC-I had decreased significantly ($P<0{\cdot}02$), resulting in a change in the POMC-I:POMC-II mRNA ratio at the expense of POMC-I from 1:1·9 at t=0 to 1:4·2 at t=3 h, and increased in favour of POMC-I again to 1:1·7 at t=5 h (the temperature returned to 24 °C at between 3 and 5 h; Fig. 6).

Discussion

In the present study RT-PCR combined with CE-LIF allowed us to quantify differences in expression of closely related POMC genes (93·5% nucleotide sequence identity (Arends *et al.* 1998) from a tetraploid carp. The fact that no digestion of POMC-I by PvuII and no digestion of POMC-II by SacI occurred indicates the specificity of the enzymes used. As only one primer set was used in the PCR, irregular competition between competitive cDNAs was avoided and the suggested use of an internal standard in quantitative PCR (Siebert & Larrick 1993, Riedy *et al.*

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Table 1 Day to day (interassay) variation of a 100 bp cDNA ladder

	Day to day variation (n=9)							
Size (bp)	100	200	300	400	500	600	1400	2200
Mean peak area (%)	12·5	3·6	3·2	2·9	2·7	2·4	2·6	70·2
S.E.M.	1·1	0·3	0·2	0·2	0·2	0·2	0·1	1·8

Integrated peak areas were calculated as percentage of a total of six peaks of a 100 bp cDNA ladder. Mean ± S.E.M. peak area was calculated from nine experimental days.

1995) was thus not necessary. In PCR, exponential amplification of the cDNA takes place. For the PCR quantification, only the linear part of the reaction reflects the initial ratio of mRNAs. Indeed at between 20 and 40 cycles, the POMC-I:POMC-II ratio appeared to be independent of the number of PCR cycles. To avoid exhaustion of one of the PCR ingredients, 30 cycles were chosen to quantify the ratio of the two POMC mRNAs. With this method the ratio between the two POMC mRNAs could reproducibly be assessed and we consider the ratio to be an appropriate index of differential gene expression. Our method allows quantification of the ratio of differentially expressed genes and not of absolute amounts mRNA. For quantification of total mRNA expression a method using a control gene, e.g. that of β_2-microglobulin, should be used (Murphy *et al.* 1990).

The finding of a POMC-I:POMC-II mRNA ratio of 1:1 in wild-type carp points to an unbiased duplication of the POMC gene. However, to our surprise the ratio deviated significantly from 1 in the isogenic strains, from 1:5·4 for strain R3 × R8 to 1:1·9 for strain E4 × R3R8.

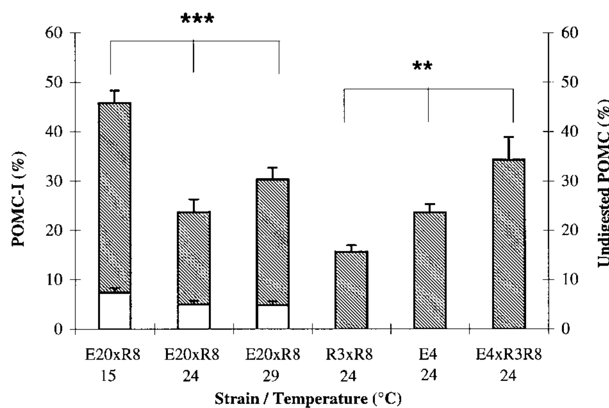

Figure 4 POMC mRNA expression in temperature-adapted carp and in different strains. Percentage POMC-I mRNA expression in the isogenic carp strains E20 × R8 (n=8), R3 × R8 (n=4), E4 (n=4) and E4 × R3R8 (n=5) is shown as closed bars. Open bars indicate the percentage of POMC not digested in strain E20 × R8; in the other groups the digestion was 100%. Values are expressed as means ± S.E.M. A significant decrease in POMC-I mRNA expression (***) was found between the 15 °C- and 24 °C- and 29 °C-adapted fish (P<0·001). Furthermore, the percentage POMC-I differed significantly (**) between the strains R3 × R8, E4 and E4 × R3R8 (P<0·01).

This may indicate that, as a result of polymorphism, annealing of one of the primers was inhibited, thereby introducing a strain-dependent POMC mRNA ratio. However, we think that polymorphism cannot be the cause of the ratio shift in carp. One could argue that strain-dependent differential POMC mRNA expression was introduced during the gynogenetic reproduction of these isogenic carp, as the result of embryonic damage caused by the chromosome manipulation treatment (Bongers *et al.* 1997). However, all these strains have been tested for microsatellites and proven to be homozygous, indicating that duplicate sets of all genes are present in these fish (Crooijmans *et al.* 1997).

We tested the hypothesis that ambient temperature may alter the POMC mRNA ratio. A significantly altered POMC mRNA ratio in favour of POMC-I was found in carp (strain E20 × R8) adapted to 15 °C compared with those adapted to 24 and 29 °C. The digestion of the PCR product in this experiment was not 100%, but the amount of undigested PCR product was less than 7% and could not be responsible for the shift in ratio. It is not known whether this ratio shift was caused by increased expression of POMC-I or decreased expression of POMC-II or a combination of the two. It is known that environmental temperature directly influences basal constitutive hormone release (Fine *et al.* 1996, Lin *et al.* 1996), but both the wild-type and isogenic strains R3 × R8 and E4 × R3R8 were kept at 24 °C, which would exclude upregulation of the release as a result of increased metabolic activity. We

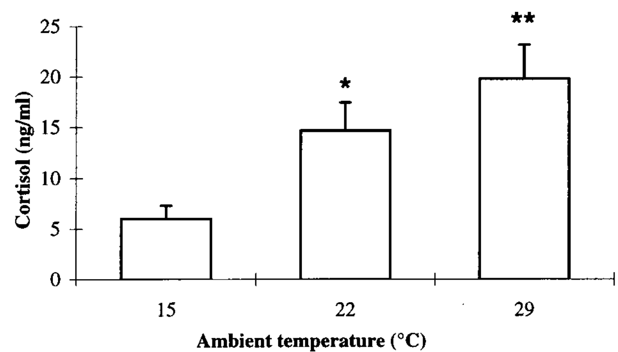

Figure 5 Cortisol levels in carp adapted to different ambient temperatures for at least 8 weeks. Plasma cortisol levels were significantly different (* P<0·05, ** P<0·01, n=17).

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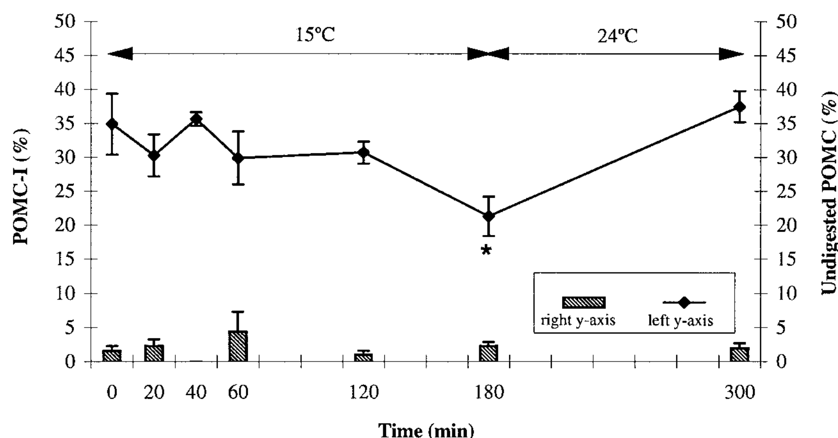

Figure 6 POMC-I mRNA expression during temperature-induced stress. Percentage POMC-I mRNA expression in the isogenic carp strain E4 × R3R8 after a temperature shock of − 9 °C (left y-axis, n=5). Bars represent percentage of POMC not digested (right y-axis). Values are expressed as means ± S.E.M. A significant decrease (*) in POMC-I mRNA expression was found at t=180 min (P<0·02).

measured plasma cortisol levels to check whether the environmental temperature had evoked a stress response, because in salmonid fishes temperature shock potentiates the stress response to handling and confinement (Sumpter *et al.* 1985, 1986). A significant effect of ambient temperature on basal cortisol secretion was found. However, plasma cortisol levels were less than 20 ng/ml, indicating that the fish were probably not stressed and were well adapted to the different ambient temperatures. These results therefore indicate that ambient temperature alters the quality (preference for either POMC-I mRNA or POMC-II mRNA) of the message rather than the quantity.

We then tested the hypothesis that a rapid drop in ambient temperature could evoke a stress response and induce differential expression of the the two POMC mRNAs in an experiment in which fish were exposed to a rapid drop in temperature of 9 °C, which evokes a time-dependent cortisol response. These fish were probably stressed by the treatment because, in a similar experiment, we found that plasma cortisol levels were elevated, up to 6 times the control level, 20 min after the start of the experiment and remained elevated until the end of the temperature shock treatment (t=3 h), indicating that this drop in temperature was a stressful event for the fish. At time point t=5 h (2 h after the temperature had returned to 24 °C), plasma cortisol levels were back to control levels (M Tanck, J Komen and S E Wendelaar Bonga, unpublished observations). We found a significantly altered POMC-I:POMC-II mRNA ratio at the expense of POMC-I 3 h after the onset of the rapid temperature drop, indicating that the fall in temperature increased POMC mRNA transcription and that this newly transcribed POMC mRNA was mainly POMC-II. This is in agreement with mammalian literature, in which a stress-induced increase in POMC mRNA expression has been demonstrated in the pituitary gland of rats (Hollt *et al.* 1986, Shiomi *et al.* 1986). Our results indicate that in carp a control mechanism for the regulation of POMC mRNA expression is activated during temperature-induced stress. However, as absolute amounts of mRNA were not measured, differences in POMC-I and POMC-II mRNA clearance could also explain the shift in ratio. The amount of undigested POMC mRNA in this experiment was less then 5% and could not be responsible for this ratio shift. We therefore conclude that the observed differential expression is induced by the temperature shock acting as a stressor. The mechanism for the activation of this stress-induced differential expression is not known, but differential affinity of transcription factors for the promoters of the two POMC genes is a possible candidate.

Although it has been known for many years that two POMCs are expressed in tetraploid animals (Kawauchi 1983, Martens *et al.* 1985, Salbert *et al.* 1992), there is still little evidence for any biological significance of the presence of two gene transcripts. In one report on rainbow trout, POMC-A and POMC-B cDNAs were cloned (Salbert *et al.* 1992), but the expression of POMC-A, but not POMC-B, in hypothalamic neurones of sexually immature fish was found. Furthermore, in the amphibian *Xenopus laevis*, differences in processing of end products of POMC-A and POMC-B could be detected in animals adapted to black and white backgrounds (Van Strien *et al.* 1996). Our results provide the first evidence for the differential expression of two POMC mRNAs during temperature-induced stress and in response to different ambient temperatures in the tetraploid carp.

It is still not known in which cells this POMC ratio shift occurs: the corticotropic cells of the pars distalis or the

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melanotropic cells of the pars intermedia. In carp, the two POMC molecules produce the same type of α-MSH and β-endorphin peptides, but two different types of ACTH and β-MSH (Arends *et al.* 1998). As the rapid drop in temperature described here induces an α-MSH-independent rise in cortisol (S E Wendelaar Bonga, unpublished observations), we hypothesize that the corticotropic cells are involved in this response. As amino acid substitutions occur in the ACTH peptide but not in α-MSH and endorphins, differential POMC expression in the corticotrophs may well be functional. RIAs are currently being set up to study these different POMC-derived peptides in plasma. Future studies will address the questions of whether the ratio of the POMC mRNAs affects the cortisol release, and whether cortisol feedback regulates the POMC gene and thereby affects the ratio of the POMC mRNAs.

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