Identification of β-endorphins in the pituitary gland and blood plasma of the common carp (Cyprinus carpio)

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Abstract

Carp β-endorphin is posttranslationally modified by N-terminal acetylation and C-terminal cleavage. These processes determine the biological activity of the β-endorphins. Forms of β-endorphin were identified in the pars intermedia and the pars distalis of the pituitary gland of the common carp (Cyprinus carpio), as well as the forms released in vitro and into the blood. After separation and quantitation by high performance liquid chromatography (HPLC) coupled with radioimmunoassay, the β-endorphin immunoreactive products were identified by electrospray ionisation mass spectrometry and peptide sequencing. The release of β-endorphins by the pituitary gland was studied after stimulation with corticotrophin–releasing factor (CRF) in vitro. In the pars intermedia, eight N-acetylated truncated forms were identified. Full length N-acetyl β-endorphin(1–33) coeluted with N-acetyl β-endorphin(1–29) and these forms together amounted to over 50% of total immunoreactivity. These products were partially processed to N-acetyl β-endorphin(1–15) (30·8% of total immunoreactivity) and N-acetyl β-endorphin(1–10) (3·1%) via two different cleavage pathways. The acetylated carp homologues of mammalian α- and γ-endorphin were also found. N-acetyl β-endorphin(1–15) and (1–29) and/or (1–33) were the major products to be released in vitro, and were the only acetylated β-endorphins found in blood plasma, although never together. CRF stimulated the release of opioid β-endorphin from the pars distalis. This non-acetylated β-endorphin represents the full length peptide and is the most abundant form in plasma.


Introduction

Beta-endorphin is a pro-opiomelanocortin (POMC)-derived peptide and is predominantly produced in the pituitary gland and the brain. Its bioactivity depends on C-terminal truncation and N-terminal acetylation. For instance, β-endorphin(1–31) is a very potent opiate, whereas its acetylated congener is essentially devoid of opioid activity (Akil et al. 1981). On the other hand, C-terminal cleavage produces the very effective opioid antagonist β-endorphin(1–27) (Nicolas & Li 1985).

In addition to its opioid function, β-endorphin was found to be involved in a variety of physiological processes (reviewed by Dalayeu et al. 1993), including regulation of the immune (Heijnen et al. 1987, Shahabi et al. 1991, 1996) and reproductive (e.g. Faletti et al. 1999) systems. Furthermore, β-endorphin plays a role in the vertebrate stress response (e.g. Akil et al. 1985, Kjaer et al. 1995, Wendelaar Bonga 1997, Mosconi et al. 1998).

Since forms of β-endorphin exert different biological effects, research so far has focused on the occurrence of β-endorphins that may each have different actions. Post-translationally modified β-endorphins have been described in a variety of vertebrates, including teleost fish (Takahashi et al. 1984) and mammals (Weber et al. 1981, Smyth 1984, Millington et al. 1992). However, data on the release and functions of β-endorphins are very scarce. In human blood plasma, the major form is β-endorphin(1–31) (Silberring et al. 1998). In fish, N-acetyl β-endorphin immunoreactivity has been reported in plasma (Rodrigues & Sumpter 1984, Sumpter et al. 1985, Mosconi et al. 1998), although the exact nature of the forms responsible for this immunoreactivity is unknown.

Recent cloning of carp POMC (Arends et al. 1998) showed that full length β-endorphin in this species consists of 33 amino acid residues, rather than 31 amino acids as in mammals and other vertebrates, and contains one potential dibasic and five potential monobasic cleavage sites. Therefore, identification of β-endorphins in the pituitary gland...
predicts how processing of carp $\beta$-endorphin takes place and how this relates to processing in mammals. In addition to this evolutionary perspective, it is also of great interest to study processing of $\beta$-endorphin isoforms from a physiological point of view, as N-acetyl $\beta$-endorphin may have corticotrophic activity in teleosts (Balm et al. 1995).

Thus, the present study aimed to identify post-translationally modified $\beta$-endorphin isoforms in the pars intermedia and the pars distalis of the pituitary gland of the common carp (Cyprinus carpio). Furthermore, we analysed which truncated forms are released in vitro and which are present in the blood plasma. Finally, the effects of corticotrophin-releasing factor (CRF) on $\beta$-endorphin release by the pituitary gland were assessed by means of in vitro superfusion. CRF stimulates the release of adrenocorticotropic hormones such as adrenocorticotropic hormone (ACTH) and $\alpha$-melanocyte-stimulating hormone ($\alpha$-MSH) during stress (Wendelaar Bonga 1997). The analysis of CRF-driven $\beta$-endorphin release may indicate whether any $\beta$-endorphins are secreted during stress.

Materials and Methods

Animals

Male carp (70–120 g) were obtained from laboratory stock. Fish were held in 100 litre tanks within a recirculating system supplied with UV-filtered and biologically filtered tap water. The water temperature was maintained at 22 °C and the daily photoperiod was 16 h. Fish were fed commercial fish food (Trouvit, Trouw, Putten, The Netherlands) at 1·5% of body weight per day.

Tissue preparation

Fish were anaesthetised in 0·1% 2-phenoxyethanol. One millilitre blood was collected from the caudal blood vessels using a 21-gauge syringe with Na$_2$EDTA as anti-antiglutamin. One trypsin inhibitor unit (TIU) aprotinin (Sigma, St Louis, MO, USA) per ml plasma was added to prevent proteolytic breakdown. After centrifugation (10 000 g, 10 min, 4 °C), plasma was collected and used immediately. To obtain the pituitary gland, the fish were placed on ice directly after the blood had been collected. The pituitary gland was then removed and the pars intermedia and pars distalis were separated with the aid of a dissection microscope. The tissue was homogenised mechanically on ice in 100 µl 10 mM HCl and 0·2 TIU aprotinin. After centrifugation (10 000 g, 10 min, 4 °C) to remove membranes and cellular debris, the supernatant was submitted to reversed-phase HPLC immediately or after storage at −20 °C.

Prepurification of plasma

A Pharmacia LKB Superdex-75 HR 10/30 column (Pharmacia LKB Biotechnology, Uppsala, Sweden) was used for initial purification of plasma. The eluent consisted of an 0·36 M acetate buffer (0·088 M ammonium acetate, 0·272 M acetic acid, 19% v/v ethanol, pH 4·4), as described by Sælsen et al. (1994). The flow was 0·5 ml/min, and 300 µl fractions were collected. One hundred microlitres of the fractions were vacuum-dried and redissolved in veronal acetate buffer: VAT-buffer, pH 8·6, 0·02 M sodium barbital, 0·2 g/l Na$_3$F, supplemented with 0·3% bovine serum albumin (Sigma), and 100 KIU/ml trypsin inhibitor (Trasylol; Bayer, Leverkusen, Germany), and analysed by radioimmunnoassay.

Radioimmunoassay

N-acetyl $\beta$-endorphin was quantitated in duplicate by radioimmunoassay with an antiserum against salmon N-acetyl $\beta$-endorphin (developed and characterised by Takahashi et al. 1984). This antibody has full cross-reactivity with acetylated forms of mammalian (Dores et al. 1991) and Xenopus (Van Strien et al. 1993) $\beta$-endorphin. Cross-reactivity of this antibody with non-acetylated forms is less than 0·1% (Dores et al. 1991). The final dilution of the antiserum was 1:250 000. Xenopus N-acetyl $\beta$-endorphin(1–8) used as tracer was iodinated using the iodogen method (Salacinsky et al. 1981) and purified through solid phase extraction (octadecyl Bakerbond column). Immunocomplexes were separated from free tracer by precipitation of the complexes with 15% polyethylene glycol and 2·4% ovalbumin.

Reversed-phase HPLC

Immunoreactive Superdex-75 fractions obtained from blood plasma samples were vacuum dried and resuspended in 100 µl 10 mM HCl. These samples, as well as the pars intermedia and pars distalis homogenates, were submitted to reversed-phase HPLC using a µRPC C2/C18 SC2-1/10 column (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Beta-endorphins were separated with an acetonitril gradient (22–36%) in water containing 0·1% trifluoroacetic acid (Merck, Darmstadt, Germany) at 150 µl/min. Fractions (300 µl) were dried under vacuum and redissolved in VAT-buffer for radioimmunoassay. Standards were human $\alpha$-endorphin, human $\gamma$-endorphin, N-acetyl $\beta$-endorphin(1–27) (Sigma) and Xenopus N-acetyl $\beta$-endorphin(1–8) (American Peptide Company, Santa Clara, CA, USA).

Affinity chromatography

To assess the presence of non-acetylated $\beta$-endorphin, homogenates of pars intermedia and pars distalis, as well as Superdex-75-fractions of plasma, were submitted to affinity chromatography to deplete the samples of N-acetyl $\beta$-endorphins. An affinity column was prepared according
to the manufacturer’s instructions (Pharmacia) using the anti-N-acetyl β-endorphin antibody. The samples were eluted with 2 ml 0.1 M Na₂CO₃, pH 8.0, and subsequently vacuum-dried. The pellets were redissolved in 10 mM HCl and submitted to reversed-phase HPLC. To measure non-acetylated β-endorphins, samples were chemically acetylated as described by Van Strien et al. (1993). Briefly, 100 µl of sample was dried under vacuum and resuspended in 100 µl 50 mM phosphate buffer, pH 8.0. Acetylation was started by adding 2 µl acetic anhydride (Sigma), followed by vigorous vortexing. Samples were dried and resuspended in VAT-buffer for radioimmunoassay for acetylated endorphins.

In vitro superfusion

The pars distalis and pars intermedia were separated and placed on a cheese-cloth filter in a superfusion chamber and superfused with a Heps-buffered (15 mM; pH 7.38) Ringer’s solution containing NaCl (128 mM), KCl (2 mM), CaCl₂, 2H₂O (2 mM), with 0.25% (w/v) glucose and 0.03% (w/v) bovine serum albumin. Medium was pumped through the chambers at 30 µl/min by a Watson-Marlow 503U multichannel peristaltic pump (Smith and Nephew Watson-Marlow, Falmouth, Cornwall, UK). After 180 min superfusion, when the release of endorphins had reached an apparent steady state, ovine CRF (Sigma) was added to the medium to a final concentration of 1 nM to test its effect on the release of β-endorphin isoforms. A 20-min fraction before and a 20-min fraction during the pulse were collected in 50 mM HCl and analysed for the presence of β-endorphin isoforms as described above.

Molecular weight determination by electrospray ionisation mass spectrometry (ESMS)

Partially purified β-endorphin immunoreactive peptides were analysed on a Micromass Q-TOF mass spectrometer (Wythenshawe, Manchester, UK), fitted with a nano-electrospray ion source. Lyophilised peptides were dissolved in 50% acetonitrile/1% formic acid in water; the final peptide concentration was approximately 1 µM (based on the theoretical molecular weight of the peptide). Four microlitre aliquots of sample solutions were loaded into borosilicate Nanoflow tips (Protana, Odense, Denmark). The mass spectrometer (MS) was operated in positive ion mode with a source temperature of 30 °C. A potential of 0.8–1.5 kV applied to the Nanoflow tip combined with a nitrogen backpressure of 5–10 psig produced a sample flow of 10–30 nl/min into the analyser. All data, both MS and MS/MS, were acquired with the TOF analyser. In MS/MS mode the quadrupole was used in resolving mode to select the precursor ion for fragmentation in the hexapole collision cell. MS/MS was performed with argon gas in the collision cell at a pressure of 6 × 10⁻⁵ mbar measured in the analyser. All data were processed using Masslynx software delivered with the instrument.

Peptide sequencing

Peptides were sequenced by automated Edman degradation in a model 476 pulsed-liquid sequenator (Applied Biosystems, Foster City, CA, USA).

Peptide synthesis

Carp N-acetyl β-endorphin(1–10) was synthesised by the Institute of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands. Carp β-endorphin(1–29) was synthesised by the Department of Immunohaematology and Bloodbank, Leiden, The Netherlands.

Immunohistochemistry

Brain–pituitary gland complexes were fixed in Bouin’s fixative. Sagittal sections (7 µm) were immunostained with the peroxidase-anti-peroxidase method, according to Van der Heijden et al. (1999) with minor modifications, using the salmon N-acetyl β-endorphin antibody as described above or a carp β-endorphin(20–29) antiserum (1:2000). The latter antiserum was produced in our laboratory by immunising rabbits with carp β-endorphin(20–29) coupled to keyhole limpet haemocyanin. The antiserum recognises acetylated as well as non-acetylated full length carp β-endorphin and carp β-endorphin(1–29). Serum specificity was assessed histochemically by omitting the antiserum and by replacing the antiseraum with normal rabbit serum. Immunostaining was fully abolished in these tests. The sections were subsequently incubated with this antibody (overnight), goat-anti-rabbit IgG (1:150; 1 h; Nordic Immunology, Tilburg, The Netherlands), rabbit peroxidase-anti-peroxidase (1:150; 1 h; Nordic) and 3,3-diaminobenzidine tetrahydrochloride (0.25 mg/ml) in the presence of 0.005% (v/v) H₂O₂ for 5 min.

Statistics

Six fish were sampled to quantify and identify the forms of β-endorphin in blood plasma, pars intermedia and pars distalis. Basal and CRF-stimulated release of β-endorphins was studied in quadruplicate, whereas the immunohistochemical analysis of distribution of β-endorphins in the brain–pituitary gland complexes was performed on three fish. As little variation between replicates occurred, results are presented as representative examples, unless stated otherwise.

Results

Seven immunoreactive fractions were observed in homogenates of the pars intermedia (Fig. 1) containing
8 N-acetyl β-endorphins. Peak VII contained 50.5% of the immunoreactivity and represented N-acetyl β-endorphin(1–29) plus (1–33), as determined by ESMS and peptide sequencing. The other predominant β-endorphin eluted in peak II (30.8%), and was found to be N-acetyl β-endorphin(1–15). N-acetyl β-endorphin(1–16) (peak III, 3.8%), N-acetyl β-endorphin(1–17) (peak V, 1.8%), N-acetyl β-endorphin(1–18) (peak IV, 9.4%), and N-acetyl β-endorphin(1–19) (peak VI, 0.6%) were the other constituents. The identity of the N-acetyl β-endorphin in peak I (3.1%) could not be assessed by ESMS. However, we observed the presence of β-endorphin(12–29) in fraction 14, indicating that N-acetyl β-endorphin (1–29) is cleaved at the single basic residue Arg11 to yield N-acetyl β-endorphin(1–10). Synthetic carp N-acetyl β-endorphin(1–10) was found to elute in fraction 6, indicating that peak I contains this peptide.

One peak reflecting non-acetylated β-endorphin was obtained (peak VIII, 1.7%, Fig. 2). We were unable to identify this peptide with ESMS. The immunoreactive product was found to coelute with synthetic carp β-endorphin(1–29) (data not shown), and, in parallel to the acetylated forms, probably also with β-endorphin(1–33). Beta-endorphin(1–29) and (1–33) are the most hydrophobic forms (as predicted from their amino acid composition) and thus the apparent immunoreactivity eluting in later fractions cannot represent β-endorphin. Immunoreactivity in these fractions (e.g. fractions 18 and 19 in Fig. 2), when present, is very low and therefore considered not specific. Fraction 5 always shows some immunoreactivity, but we were unable to identify its nature.

The pars intermedia released four forms in vitro (Fig. 3), of which N-acetyl β-endorphin(1–29) and/or (1–33), and N-acetyl β-endorphin(1–15) were predominant. The other secreted products were N-acetyl β-endorphin(1–10) and (1–18). The two former peptides were also found in plasma, but never simultaneously in the same fish (Fig. 4). Blood plasma from three fish contained N-acetyl β-endorphin(1–15), whereas plasma from the other three fish contained N-acetyl β-endorphin(1–29). The N-acetyl β-endorphin concentration in blood plasma was 3.7 ± 0.6 nmol/l (mean ± s.e.m., n = 6). The concentration of the non-acetylated β-endorphin in plasma (Fig. 5, peak VIII) varied considerably among individual fish and ranged from 7 to 84 nmol/l.

In vitro stimulation of the pars intermedia with 1 nM CRF did not stimulate release of any β-endorphin (data not shown). On the other hand, the secretion of...
non-acetylated β-endorphin was promoted in the pars distalis: basal release was almost undetectable but when stimulated with CRF the release increased to 57 fmol/min per pars distalis (Fig. 6).

Non-acetylated β-endorphin was localised in the nucleus preopticus (NPO) and in the hypothalamo-pituitary tract (Fig. 7). The β-endorphin-containing neurons spread throughout the pars intermedia (PI).
Non-acetylated β-endorphin was not detectable by immunohistochemistry in the pars distalis (PD). The immunostaining of the melanotroph cells in the PI reflects the presence of N-acetyl β-endorphin(1–33) and (1–29). Immunostaining with the antibody against acetylated β-endorphins only stained the melanotroph cells in the pars intermedia.

Discussion

We identified eight acetylated β-endorphins in the pars intermedia. N-acetyl β-endorphin(1–33) is the full length peptide (Arends et al. 1998), and its cleavage follows two pathways to yield either N-acetyl β-endorphin(1–10) or (1–15). The first pathway involves cleavage of N-acetyl
β-endorphin(1–33) to N-acetyl β-endorphin(1–29) at the dibasic cleavage site Lys30-Lys31 by prohormone converter 2 (PC2; Zhou et al. 1993). Next, this peptide is cleaved at Arg11 to N-acetyl β-endorphin(1–11) and β-endorphin(12–29). The former is further processed to N-acetyl β-endorphin(1–10) by a carboxypeptidase H-like enzyme. The second pathway includes cleavage of either N-acetyl β-endorphin(1–33) or (1–29) at Lys31 to N-acetyl β-endorphin(1–21). Processing at monobasic cleavage sites has been reported in the toad, *Bufo marinus* (Dores et al. 1994) and in rat prodynorphin processing (Day et al. 1998). N-acetyl β-endorphin is further truncated to N-acetyl β-endorphin(1–15) by carboxypeptidase activity. All of the predicted N-acetyl β-endorphin truncated forms were present in pars intermedia homogenates of the carp, except for N-acetyl β-endorphin(1–11), (1–20), and (1–21). Therefore, we predict the conversion of the latter isoforms to be immediate and complete. In fact, it is well known that carboxypeptidases effectively cleave off C-terminal basic amino acid residues. Takahashi et al. (1984) demonstrated the presence of N-acetyl β-endorphin(1–20) in chum salmon (*Oncorhynchus keta*) pituitary gland. However, the C-terminal amino acid residue in this species is Leu rather than Arg or Lys, which is less effectively removed by such enzymatic activity. The absence of carp N-acetyl β-endorphin(1–20) and (1–21) can also be explained when the full length peptide is immediately cleaved at the Leu19-Phe20 bond, as has been described in rats (Burbach et al. 1981).

The terminal products of the two processing routes are released by the carp pars intermedia in vitro, together with N-acetyl β-endorphin(1–29) and/or (1–33) and trace amounts of N-acetyl β-endorphin(1–18). In plasma, we found either N-acetyl β-endorphin(1–15) or N-acetyl β-endorphin(1–29)/(1–33). As the occurrence of these forms appeared to be individual-specific, we speculate that their release is under different hypothalamic control. The presence of N-acetyl β-endorphin(1–10) and (1–18) could not be demonstrated in plasma and their concentration may be under the detection limit of the assay (50 pM) or, alternatively, their secretion is under inhibitory hypothalamic control in vivo.

The processing pathway of N-acetyl β-endorphin appears to be similar to the mechanism in other vertebrates, since N-acetyl β-endorphin(1–29) (the homologue of mammalian N-acetyl β-endorphin(1–27)), as well as N-acetyl β-endorphin(1–18), (the homologue of N-acetyl α-endorphin), and N-acetyl β-endorphin(1–19) (the homologue of N-acetyl γ-endorphin) were found. On the other hand, we could not identify the carp homologue of β-endorphin(1–26), which may be due to the presence of Gln29 that is less susceptible to carboxypeptidase activity (Smyth et al. 1989) than is His27 in mammals. Cleavage to N-acetyl β-endorphin(1–10) resembles processing as observed in the amphibian *Xenopus laevis*, where the homologue of this carp β-endorphin, N-acetyl β-endorphin(1–8), is the terminal product of endorphin processing (Van Strien et al. 1993). Although a minor product in the carp pituitary gland (3%, N-acetyl

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**Figure 6** Reversed phase analysis of in vitro CRF-driven β-endorphin release from the pars distalis. The control consists of non-acetylated β-endorphin release during the 20 min preceding the 20-min pulse with CRF on the same tissue. Peak VIII coelutes with carp β-endorphin(1–29). (Conditions as in Fig. 1.)
\(\beta\)-endorphin(1–10) may be the most abundant \(\beta\)-endorphin in tilapia (Oreochromis mossambicus; Lee et al. 1999), indicating that utilisation of this pathway – which is not found in mammals – differs among fish species. Thus, in species where N-acetyl \(\beta\)-endorphin is preferentially truncated to N-acetyl \(\beta\)-endorphin(1–10), the \(\beta\)-endorphin may have a specific function not encountered in other species, or forms other than N-acetyl \(\beta\)-endorphin(1–10) exert similar effects.

The reason why mammalian \(\alpha\)-endorphin is not further processed to \(\beta\)-endorphin(1–13), similar to carp \(\beta\)-endorphin(1–15), may relate to species-specific processing. Indeed, Takahashi et al. (1984) found no cleavage of the salmon homologue of N-acetyl \(\alpha\)-endorphin. The C-terminal amino acid of \(\gamma\)-endorphin is Trp in all species examined, but the preceding amino acid varies. For instance, this amino acid is Leu in the carp (Arends et al. 1998), Ile in the chum salmon (Takahashi et al. 1984) and Val in humans (Takahashi et al. 1981). It appears that proteolytic activity depends not only on the nature of the C-terminal amino acid residue, but also on the neighbouring amino acids.

We were unable to identify non-acetylated \(\beta\)-endorphins by ESMS, although the presence of one such form was indicated by radioimmunoassay. It coeluted with synthetic carp \(\beta\)-endorphin(1–29) (not shown), which is likely to have the same hydrophobicity as carp \(\beta\)-endorphin(1–33). As measuring of these forms is possible only after chemical acetylation, it could be argued that an artefact induced by the acetylation procedure accounts for the occurrence of this immunoreactivity in the assay. However, this method has been shown to acetylate non-acetylated \(\beta\)-endorphins effectively (Lorenz et al. 1986, Van Strien et al. 1993). A more likely explanation is that the amount of the non-acetylated forms was too small for detection by ESMS, especially since the \(\beta\)-endorphins were only partially purified. In mammals, the non-acetylated \(\beta\)-endorphin present in the anterior pituitary corticotroph cells is the full length peptide, which is not further processed, because PC2 is hardly expressed in these cells (Benjamnet et al. 1991). Using 32 g chum salmon pituitary glands, Takahashi et al. (1984) were able to isolate and identify full length non-acetylated \(\beta\)-endorphin, which must have been produced by the corticotroph cells in the pars distalis. We assume that the same applies to carp, and therefore suggest the presence of \(\beta\)-endorphin(1–33) in the pars distalis homogenates. The release of this peptide would be under stimulatory control of CRF, as indicated by the strong induction of its release by this neuropeptide in vitro. The trace amounts of non-acetylated \(\beta\)-endorphin in the pars intermedia may originate from the hypothalamic–hypophyseal tract that we showed to contain non-acetylated \(\beta\)-endorphin(1–33) in carp. This peptide was not released by the melanotroph cells in the pars intermedia in vitro, not even in the presence of CRF (not shown). Thus, the non-acetylated \(\beta\)-endorphin in plasma must originate from the pars distalis and probably represents the full length peptide.

In human plasma, the most abundant \(\beta\)-endorphin isoform is the opioid \(\beta\)-endorphin(1–31) (Silberring et al. 1998). The existence of opiate active \(\beta\)-endorphins in teleosts is indicated by the localisation of \(\mu\)-opiod receptors in the coho salmon (Oncorhynchus kisutch) brain (Ebbesson et al. 1996).

In addition to their opioid activity, \(\beta\)-endorphins may be involved in the stress response. We showed that CRF, which has a pivotal role in stress in all vertebrates and is a potent stimulator of ACTH and MSH secretion in fish (Van Enckevort et al. 2000), induces \(\beta\)-endorphin release from the pars distalis in vitro. This indicates co-release with ACTH, being a well-known corticotrophic hormone in fish (Wendelaar Bonga 1997). In contrast, CRF failed to increase the release of acetylated \(\beta\)-endorphins from the pituitary gland in our study, and this does not support a role for these peptides in the stress response of carp.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure7}
\caption{Sagittal section of carp brain showing the hypothalamo–pituitary complex. (a) \(\beta\)-endorphin staining is observed in the nucleus preopticus (NPO), the hypothalamo–hypophyseal tract (arrow), and in the pars intermedia (PI). The antibody recognises \(\beta\)-endorphin(1–29) and (1–33) as well as their acetylated counterparts. (b) The antibody against acetylated \(\beta\)-endorphins only stains the melanotroph cells in the PI, demonstrating that acetylation does not occur in the NPO and the hypothalamo–hypophyseal tract. The sections shown are 35 \(\mu\)m apart, and both contain cells from the NPO as demonstrated by staining for CRF (not shown). ON, optic nerve; PD, pars distalis. Scale bar is 1 mm.}
\end{figure}
although increased plasma levels during stress have been reported for other fish species (Sumpter et al. 1985, Mosconi et al. 1998) as well as mammals (Akil et al. 1985). A factor other than CRF may be involved in the control of N-acetyl β-endorphin release in carp.

In summary, β-endorphin processing in carp, of which the main steps are emphasised in Fig. 8, displays the same pattern as reported for other vertebrates. α-N-acetylation of β-endorphin(1–33) and subsequent processing to truncated forms take place only in the melanotroph cells in the pars intermedia, whereas β-endorphin(1–33) becomes detectable in the corticotroph cells of the pars distalis when stimulated with CRF. The presence of at least three β-endorphins in the circulation suggests that post-translational modifications of β-endorphin expand the possibilities for multiple physiological effects. These findings warrant the search for regulatory mechanisms in the release of the different β-endorphins.

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