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Expression of tilapia prepro-melanin-concentrating hormone mRNA in hypothalamic and neurohypophysial cells

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ABSTRACT

Melanin-concentrating hormone (MCH) is a neuropeptide involved in background adaptation in teleost fish, and in multiple regulatory functions in mammals and fish. To study the expression of the MCH preprohormone (ppMCH) in teleosts, we first cloned a hypothalamic cDNA encoding the complete ppMCH of tilapia (Oreochromis mossambicus), and a cRNA probe derived from a 270 bp ppMCH cDNA fragment was used for the expression studies. The level of ppMCH mRNA expression in tilapia hypothalamus, measured by dot blot analysis, was significantly higher in fish adapted to a white background than in black-adapted animals, which is in accordance with the reported MCH plasma and tissue concentrations in fish. Northern blot analysis not only revealed a strong ppMCH

mRNA signal in the hypothalamus, but also the presence of ppMCH mRNA in the neurointermediate lobe (NIL) of the pituitary. In situ hybridization and immunocytochemistry showed that ppMCH mRNA as well as MCH immunoreactivity are located in perikarya of two hypothalamic regions, namely in the nucleus lateralis tuberis (NLT) and the nucleus recessus lateralis (NRL). Quantitative analysis by dot blot hybridization revealed about eight times more ppMCH mRNA in the NLT than in the NRL and NIL of mature tilapias. ppMCH mRNA in the NIL could be localized to cell bodies of the neurohypophysis, which were also MCH immunoreactive.

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INTRODUCTION

Melanin-concentrating hormone (MCH) is a cyclic neuropeptide first isolated from chum salmon pituitary extracts (Kawauchi et al. 1983). More recently the peptide has been identified in rat hypothalamus (Vaughan et al. 1989). Concerning MCH function and distribution, several differences exist between teleosts and mammals. First, some of the biological functions of MCH appear to be different in fish and mammals. In teleosts, but not in mammals, MCH is a hormone involved in background adaptation by acting at the skin and pituitary (Eberle 1988, Baker 1991). Another physiological function attributed to MCH is a role in the control of lactation and fluid homeostasis in mammals, an observation not yet documented in fish (Zamir et al. 1986, Knollema et al. 1992, Parkes & Vale 1993). In both teleosts and mammals, MCH could play a role in the stress response by

modulating the activity of the hypothalamic-pituitary-adrenal axis. The second difference between these vertebrate classes concerns the control mechanism and site of action in the stress response. It has been reported that in teleosts MCH acts at the pituitary level by inhibiting the release of adrenocorticotrophin (ACTH) (Baker et al. 1985, 1986). In mammals, it has been considered unlikely that MCH acts on the ACTH cells, since in vitro no effect of rat MCH was found on ACTH secretion from rat corticotrophs (Navarra et al. 1990). It has further been demonstrated in vivo that MCH can stimulate ACTH secretion from rat pituitaries indirectly by acting at the level of the central nervous system, primarily via a corticotrophin-releasing hormone (CRH)-dependent pathway (Jezova et al. 1992). In contrast, in teleost fish MCH inhibits CRH release from the hypothalamus in vitro (Baker 1991). The level of synthesis and secretion of MCH in response to stress also seem to

be different in teleosts and mammals. The secretion of MCH can be enhanced by repeated exposure to stressors in rainbow trout (Green & Baker 1991), whereas MCH gene expression in the rat appears to be decreased by chronic stress (Presse et al. 1992). Finally, the tissue distribution of MCH is different in teleosts and mammals. In teleost fishes, MCH is synthesized in perikarya of the ventral hypothalamus and most axons release the peptide in the neurohypophysis. Other fibres project into several regions of the brain. In mammals and other non-teleostean vertebrates, MCH-producing perikarya are located more centrally in the hypothalamus and the majority of axons project to various brain regions, whereas in general only few projections lead into the pituitary (Eberle 1988, Baker 1991).

Knowledge of the amino acid sequence of MCH has allowed several research groups to elucidate the sequences of cDNAs encoding MCH prepro-hormones (ppMCH) from salmon (Ono et al. 1988, Minth et al. 1989, Nahon et al. 1991), rat (Nahon et al. 1989), man (Presse et al. 1990) and mouse (Breton et al. 1993). In addition to the MCH peptide, a second potential cleavage product preceding MCH in the prohormone structure was postulated. This peptide was called MCH gene-related peptide (Mgrp) in fish (Bird et al. 1990, Baker 1991) and neuropeptide-E-I (NEI) in mammals (Nahon et al. 1989). Recently, evidence has been provided that this novel neuropeptide is actually processed from the ppMCH in mammals (Parkes & Vale 1992).

We recently reported the cloning of a partial hypothalamic ppMCH cDNA of the tilapia (*Oreochromis mossambicus*), an advanced teleost (Gröneveld et al. 1993). In the present study we cloned a hypothalamic cDNA encoding the complete structure of the tilapia ppMCH. To determine the synthetic activity of MCH perikarya in fish in response to changes in their environment, we measured the level of ppMCH mRNA expression in tilapias adapted to different backgrounds. Moreover, we examined the distribution of tilapia ppMCH mRNA. Surprisingly, we localized pp-MCH mRNA not only in the hypothalamus but also in the neurohypophysis.

MATERIALS AND METHODS

Animals

Freshwater tilapias, *Oreochromis mossambicus*, of both sexes were bred in our laboratory and fed on a commercial dried fish food (Tetramin; TetraWerke, Melle, Germany). The fish were kept in aquaria

with Nijmegen tap water at 28 °C, under a cycle of 12 h light:12 h darkness. Male tilapias weighing between 15 and 20 g were adapted to black and white backgrounds by transferring them from glass aquaria ('neutral background') to plastic black and white tanks respectively. The tanks contained 80 litres of tap water. The animals were kept for 2 weeks in these tanks, and were fed daily. They were killed by spinal transection and the hypothalami were dissected from the brain. In order to separate the nucleus lateralis tuberis (NLT) and nucleus recessus lateralis (NRL), a transverse incision was made from the ventral side of the hypothalamus just caudal of the pituitary to the dorsal side of the optic chiasm.

Construction and screening of tilapia hypothalamus cDNA library

A tilapia hypothalamus cDNA library was constructed with a λZAP cDNA synthesis kit (Stratagene, La Jolla, CA, USA) using about 4 μg poly(A)$^+$ RNA. RNA was isolated by the acid guanidinium thiocyanate–phenol–chloroform procedure (Chomczynski & Sacchi 1987), followed by purification of poly(A)$^+$ RNA with an oligo(dT) cellulose column (Stratagene), according to the manufacturer's instructions. cDNA was synthesized using an oligonucleotide that contained a poly(dT) sequence and a XhoI restriction site. EcoRI adaptors were ligated and the cDNA was directionally cloned into the EcoRI–XhoI sites of the Uni-ZAP XR vector. This library contained 2×10^5 clones. Replica nitrocellulose filters (Schleicher & Schuell, Dassel, Germany) of 60 000 clones of the primary hypothalamus cDNA library were made. The filters were screened at 45 °C in 5 × SSPE hybridization solution (5 × SSPE (1 × SSPE is 0·18 M NaCl, 0·01 M NaH_2PO_4, pH 7·4, 1 mM EDTA), 5 × Denhardt's solution (0·1% polyvinylpyrrolidine, 0·1% BSA, 0·1% Ficoll 400), 0·5% SDS, 50% formamide and 100 μg herring sperm DNA/ml). Washing was performed at room temperature (RT) in 1 × SSPE, 0·1% SDS, and subsequently for 20 min at 60 °C in 1 × SSPE, 0·1% SDS, 15 min at 65 °C in 0·25 × SSPE, 0·1% SDS and 15 min at 65 °C in 0·1 × SSPE, 0·1% SDS. The insert of the partial tilapia MCH cDNA clone TM16f (Gröneveld et al. 1993) was labelled with ^{32}P by random priming according to standard procedures (Sambrook et al. 1989). The labelled insert was used as a hybridization probe. Hybridization-positive phage plaques were purified, and pBluescript DNA was prepared by in vivo excision according to the manufacturer's protocol (Stratagene). DNA sequencing was performed with

T7 DNA polymerase and the dideoxy chain termination method (Sanger *et al.* 1977).

ppMCH mRNA analysis

For dot blot analysis, total RNA samples, isolated by the acid guanidinium thiocyanate–phenol–chloroform procedure, were resuspended in 300–1000 µl of a solution containing 5 × SSPE and 7·4% formaldehyde. After denaturing by heating for 10 min to 65 °C, and then cooling on ice, RNA was blotted on nitrocellulose filters using a dot blot apparatus (Bio Rad, Hercules, CA, USA). Filters were baked at 80 °C for 2 h and hybridized in 5 × SSPE hybridization solution with a ^{32}P-labelled cRNA probe of clone TMe58. This clone contains a 270 bp tilapia MCH cDNA encoding part of the prohormone and the 3′ non-translated region (Gröneveld *et al.* 1993). Washing was performed at RT for 5 min in 1 × SSPE, 0·1% SDS, and subseqently at 65 to 68 °C in 1 × SSPE, 0·1% SDS, 0·25 × SSPE, 0·1% SDS and 0·1 × SSPE, 0·1% SDS each for 20 min. Levels of ppMCH mRNA were quantified by densitometric scanning of the auto-radiograms. Prior to blotting, control samples were RNase-treated by resuspending RNA pellets in 30 µl RNase digestion mixture (10 mM Tris–HCl, pH 7·5, 5 mM EDTA, 300 mM NaCl, 0·8 µg RNase A (Pharmacia, Uppsala, Sweden) and 17·5 units RNase T1 (Boehringer, Mannheim, Germany)), followed by 1·5 h incubation at 37 °C.

For Northern blot analysis, total RNA was run on a horizontal 1% agarose gel in 2·2 M formaldehyde and morpholino-propanesulphonic (MOPS) buffer (0·02 M MOPS, 8 mM sodium acetate, pH 7·0 and 1 mM EDTA). RNA was transferred to a nitrocellulose filter and hybridized as described above. To control integrity of the RNA, hypothalamus and liver RNA samples of the same isolation were stained with ethidium bromide after gel electrophoresis.

Immunocytochemistry and *in situ* hybridization

Brains or pituitary glands, obtained from tilapias (20–80 g in weight) kept on a neutral background, were fixed overnight in Bouin's fluid, dehydrated and embedded in paraffin. Sections (5 µm) were mounted on poly-L-lysine-coated microscope slides. Alternating sections were used for either immunocytochemistry or *in situ* hybridization.

For immunocytochemistry, slides were incubated at RT (unless mentioned otherwise) with 0·05 M Tris-buffered saline (TBS), containing 0·3% Triton for 5 min (twice), and 20% normal goat serum in TBS/Triton for 10 min. This was followed by incubation with MCH antiserum (kindly provided by Dr B I Baker; Barber *et al.* 1987) diluted 1:4000 in TBS/Triton, for 16 h at 4 °C. The MCH antiserum also recognizes the MCH prohormone (Bird *et al.* 1990). Then the slides were incubated with goat anti-rabbit IgG (Nordic Immunology, Tilburg, The Netherlands), diluted 1:150 in TBS/Triton, for 60 min, and rabbit PAP (Nordic Immunology), diluted 1:1000 in TBS/Triton, for 60 min. Finally, slides were treated with 0·025% 3,3-diaminobenzidine, 0·25% nickel ammonium sulphate and 0·01% H_2O_2 in 0·05 M Tris–HCl, pH 7·6. For specificity control, the MCH antiserum was preadsorbed with 1 µM synthetic MCH (Peninsula, St Helens, Merseyside, UK) prior to incubation on the slides.

In situ hybridization was performed following a procedure slightly modified from the one described by Tensen *et al.* (1991). Briefly, sections were digested with 0·1% pepsine, post-fixed with 2% paraformaldehyde in PBS, and rinsed in 1% hydroxylammonium and PBS. Dehydrated slides were hybridized with 150 µl hybridization buffer containing the probe (see below), 4 × SSC, 5 × Denhardt's solution, 50% formamide, 10% dextran sulphate and 200 µg yeast tRNA/ml, at 50 °C for 16 h. After hybridization, slides were washed in 2 × SSC (twice for 30 min), 1 × SSC (30 min) and 0·5 × SSC (twice for 30 min) at 37 °C. Finally, sections were processed for detection of digoxigenin. Digoxigenin-labelled cRNA derived from the 270 bp tilapia ppMCH cDNA fragment of clone TMe58 was used as antisense probe. To assess the specificity of the hybridization signal, a 231 bases sense probe was used. Probes were labelled with digoxigenin, using a labelling and detection kit (Boehringer) according to the manufacturer's instructions. Briefly, probes were labelled by cRNA synthesis by incorporation of digoxigenin-labelled UTP (5 ng labelled cRNA/150 µl hybridization buffer). After hybridization to the target mRNA, hybrids were detected by enzyme-linked immunoassay using anti-digoxygenin/alkaline phosphate conjugate and subsequently enzyme-catalysed colour reaction with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium salt. Sections were examined with bright field microscopy. Nomenclature of brain areas was based on the work of Batten *et al.* (1990).

Data analysis

Data are presented as the means ± S.E.M. For statistical analysis Student's *t*-test was used. Significance was accepted at *P*<0·05.

RESULTS

Analysis of hypothalamic cDNA encoding tilapia ppMCH

A primary tilapia hypothalamus cDNA library (60 000 clones) was screened under high-stringency hybridization conditions with TM16f, a partial tilapia ppMCH cDNA encoding proMCH and a portion of the signal peptide (Gröneveld *et al.* 1993). Ten hybridization-positive clones were isolated. Three of them were selected for further analysis. Two clones appeared to contain a full-length tilapia ppMCH cDNA encoding a 136 amino acid precursor with a calculated M_r of 15 410. Both full-length tilapia ppMCH cDNA clones were identical to the corresponding part of TM16f. The complete tilapia ppMCH cDNA sequence has been assigned accession number X81144 *O. mossambicus* mRNA in the EMBL Data Library. The full amino acid sequence of the putative tilapia ppMCH signal peptide is MRQS RLSIIFAAALFFKCYALTVA. Note that Ala[20] in this sequence also follows accepted criteria (von Heijne 1986) for signal peptide cleavage.

Quantification of hypothalamic ppMCH mRNA expression

In order to measure relative ppMCH mRNA levels in tilapia hypothalami in response to environmental changes, a dot blot analysis assay was developed. ppMCH mRNA hybridization signals of dot blotted hypothalamic RNA samples from individual fish were measurable after 1–3 days of autoradiography. In RNase-treated hypothalamus RNA samples, no ppMCH mRNA signal was detectable. Specificity of the proMCH cRNA probe was tested by Northern blot analysis at the same hybridization conditions. Only one band of 0·9 kb was obtained (not shown, see also Fig. 2). Analysis of hypothalamic RNA of tilapias adapted for 2 weeks to black or white backgrounds revealed significantly more ppMCH mRNA in the hypothalamus of white-adapted fish than in the hypothalamus of black-adapted animals. Tilapias kept in glass aquaria ('N' in Fig. 1) were found to contain intermediate ppMCH mRNA levels relative to black- and white-adapted fish.

Tissue distribution

Northern blot analysis of total RNA isolated from a number of tissues revealed in the hypothalamus a band of 0·9 kb, whereas for liver and rostral pars distalis (RPD) of the pituitary no signal was obtained. Surprisingly, the neurointermediate lobe
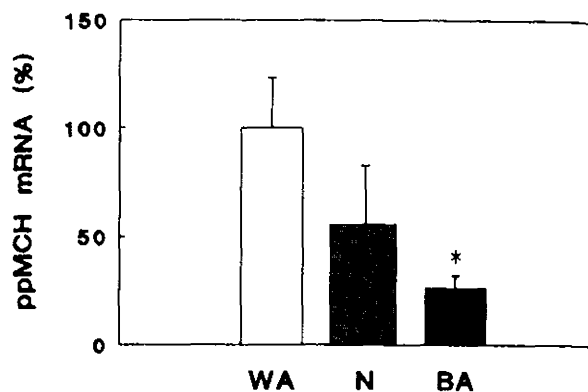
FIGURE 1. Levels of ppMCH mRNA in the hypothalamus of tilapia adapted to a white background (WA, open bar, $n=7$), a neutral background (N, shaded bar, $n=4$), and a black background (BA, solid bar, $n=8$). ppMCH mRNA levels were determined by dot blot analysis. Signals were quantified by densitometric scanning of the autoradiogram. The relative ppMCH mRNA levels are expressed as percentage of the level in white-adapted tilapia. The experiment was performed three times with comparable results. *$P<0.01$ compared with white-adapted animals (Student's t-test).

(NIL) of the tilapia pituitary also contained ppMCH mRNA, although per animal the amount of MCH mRNA in this tissue was much less than in the hypothalamus (Fig. 2). Note that different quantities of RNA derived from different numbers of animals were loaded in each lane.

Immunocytochemical analysis revealed MCH-containing perikarya in the ventral hypothalamus. Two groups of cells could be distinguished. The biggest group of large cell bodies, 12–20 μm in diameter, was located ventrally in the hypothalamus in the NLT (Fig. 3A). A second population of scattered small cell bodies (4–6 μm in diameter) was found near the lateral ventricle in the NRL (Fig. 3C). A few MCH-containing cell bodies (about 12 μm in diameter) were observed in the neurohypophysis (Fig. 3E). However, the majority of staining in the neurohypophysis was found in axonal nerves derived from MCH-containing hypothalamic neurones (Fig. 3E). MCH-containing fibres were also observed in several parts of the brain. Most were found in the hypothalamus and in the ventral telencephalon, whereas some others were detected in the optic tectum (OT). When antiserum preadsorbed with synthetic MCH was used, no staining was found in brain and pituitary (not shown).

The anatomical distribution of ppMCH mRNA was investigated by *in situ* hybridization using an anti-sense proMCH cRNA probe. The sections used for *in situ* hybridization alternated with
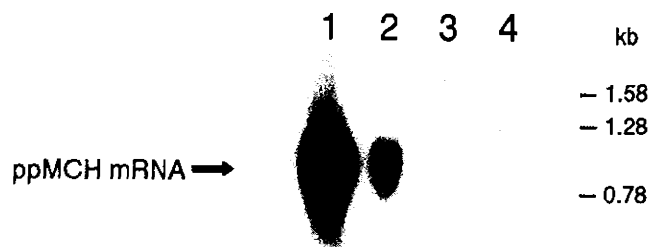
FIGURE 2. Northern blot analysis of tilapia RNA. Total RNA was subjected to electrophoresis on an 1% agarose gel, transferred to a nitrocellulose filter and hybridized with a tilapia proMCH (TMe58) cRNA probe. The blot was exposed for 40 h with intensifying screens. RNA was extracted from: lane 1, hypothalamus ($\sim$20 µg, corresponding to two hypothalami); lane 2, neurointermediate lobe of pituitary ($\sim$7 µg, four NILs); lane 3, RPD ($\sim$4 µg, four RPDs); lane 4, liver ($\sim$25 µg).

sections used for immunocytochemistry. ppMCH mRNA was detected in hypothalamic cell groups corresponding to areas stained by MCH antiserum. In neurones of the NLT both MCH immunostaining and ppMCH mRNA were present in the same cell bodies (Fig. 3A and B). Because of the small size of the cells in the NRL, it was not possible to stain the same cells of this region in alternating sections. However, as shown in Fig. 3C and D, small MCH-producing cells were detected in the NRL with MCH immunostaining as well as with *in situ* hybridization. In accordance with the Northern blot results, ppMCH mRNA was observed in some regions of the neurohypophysis. Neurohypophysial ppMCH mRNA could be located in the same cell bodies that were immunostained for MCH (Fig. 3E and F). These cells were often located in the dorsal part of the neurohypophysis, as shown in Fig. 3E and F, but occasionally they were also observed in the part of the neurohypophysis that penetrates deeply into the intermediate lobe. No staining for ppMCH mRNA was observed in axonal fibres in the brain and pituitary gland, or when a sense control probe was used (not shown).

In order to study the relative amounts of ppMCH mRNA in the NLT and the NRL, tilapia hypothalami were divided into two parts by a transverse incision. The actual separation of the cell groups was confirmed by MCH immunocytochemistry on sagittal brain sections of three animals (not shown). To be able to measure ppMCH mRNA levels in individual hypothalamic and hypophysial regions, the above mentioned dot blot analysis

assay had to be refined. By using a sense MCH cRNA standard curve, the detection limit was found to be about 1 pg ppMCH mRNA/dot. The signal in a negative control of OT tissue of equal weight to NRL tissue was significantly lower (five times) than in NRL ($P<0.01$) and NIL samples ($P<0.025$). Furthermore, hybridization signals of RNase-treated samples were around the detection limit (Fig. 4). Using this assay, the majority of ppMCH mRNA was measured in the NLT region (400 pg/fish, Fig. 4), about eight times more than in the NRL and NIL.

DISCUSSION

In this study we cloned a hypothalamic cDNA encoding the complete structure of tilapia ppMCH. Comparison of the tilapia MCH prohormone with its salmon and mammalian counterparts showed that only the MCH peptide is well conserved during evolution, while Mgrp and NEI are remarkably poorly conserved among the species examined (Gröneveld *et al.* 1993). Cloning of the complete tilapia ppMCH mRNA allowed us to compare the signal peptide sequences. The amino acid sequence identity between the tilapia and salmon (MCH-1; Minth *et al.* 1989) signal peptides is considerable (58%), whereas the identity between the tilapia and human (Presse *et al.* 1990) signal peptides is much lower (17%).

For quantification of ppMCH mRNA levels, we developed a dot blot assay because of its sensitivity and simplicity (van Tol & Burbach 1989). With this
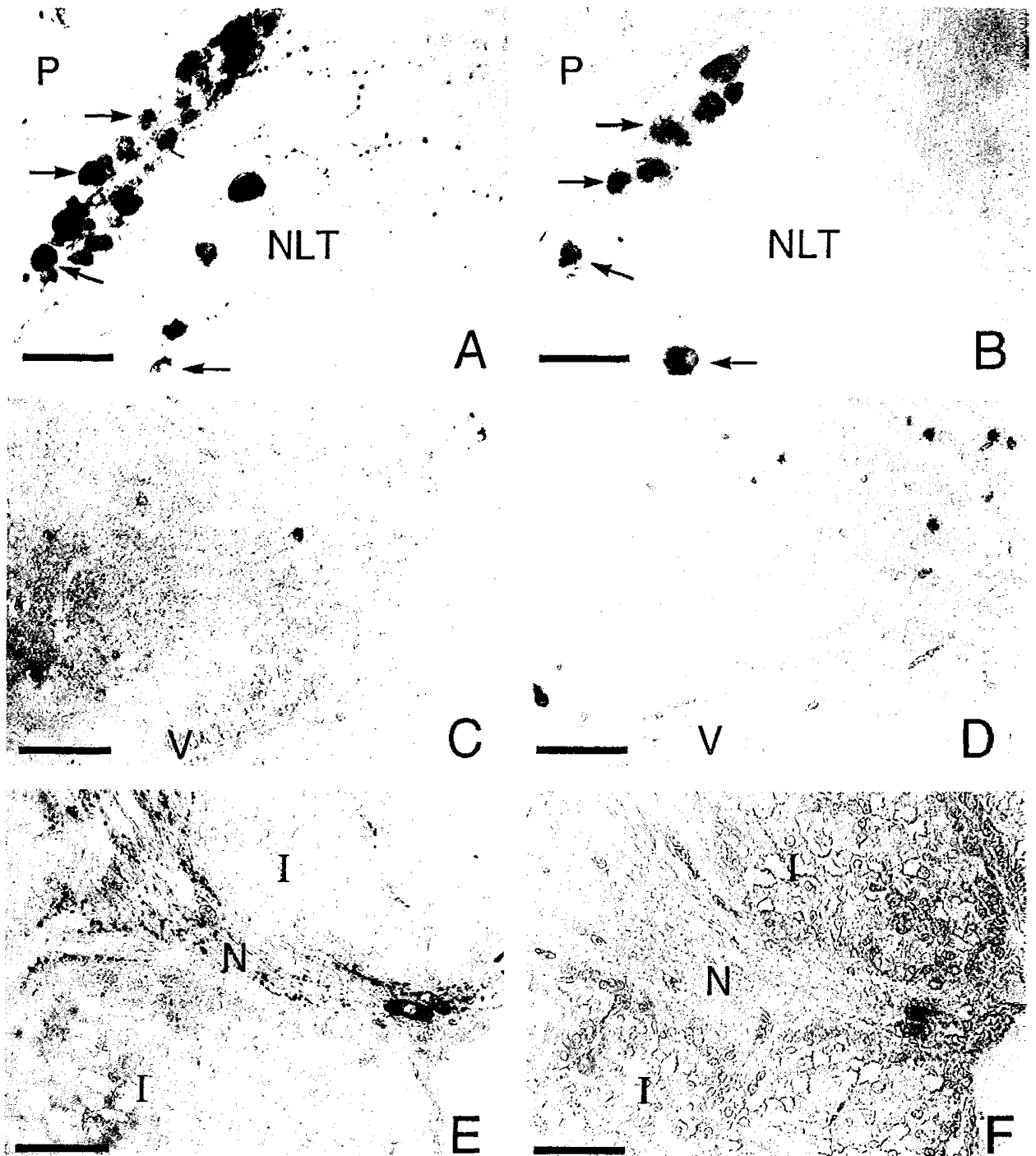
FIGURE 3.   Immunocytochemical and *in situ* localization of tilapia MCH and ppMCH mRNA. Scale bars are 50 μm. A and B, C and D, E and F are alternating sections. A, C and E are stained with MCH antiserum; in B, D and F, ppMCH mRNA is shown by non-radioactive *in situ* hybridization. A and B, bottom of ventral hypothalamus; magnocellular neurones (arrows) containing MCH and ppMCH mRNA are found in the NLT. Note that cells immunostained with MCH antiserum (A) are stained for ppMCH mRNA as well (B; indicated by arrows). C and D, smaller cells occur near the lateral ventricle. E and F, pituitary; MCH immunostaining is visible in the neurohypophysis, predominantly in axons and also in a cell body. Only the cell body is also labelled for ppMCH mRNA (F), whereas no ppMCH mRNA is detectable in axons. Abbreviations: NLT, nucleus lateralis tuberis; P, pituitary; V, lateral ventricle; N, neurohypophysis; I, intermediate lobe.
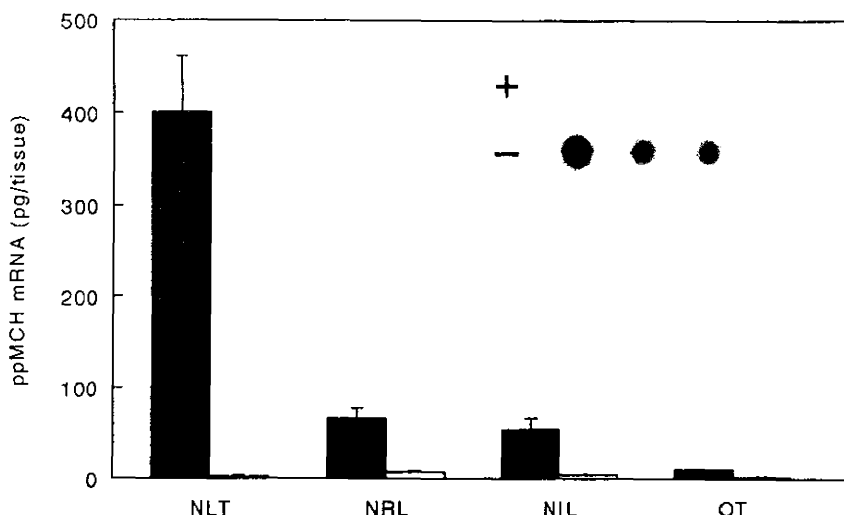
FIGURE 4. Dot blot quantification of ppMCH mRNA in different brain and pituitary regions. Total RNA samples of tissues of individual mature male tilapias (108 ± 11 g in weight, $n=5$) from a neutral background were divided into two portions. One was blotted without further treatment (solid bars), the other was RNase-treated before blotting (open bars). The amount of total RNA on the blot was 1 µg for NLT (derived from a quarter of the total tissue of one animal), 1 µg for NIL and 5 µg for NRL (both derived from half of the tissue of one fish) and 5 µg for OT as a negative control. The blot, containing a sense proMCH cRNA standard dilution series, was hybridized with a proMCH cRNA probe. Hybridization signals were quantified by densitometric scanning of the autoradiogram. The values were converted by a sense proMCH cRNA standard curve to pg ppMCH mRNA per tissue of one animal. A representative autoradiograph showing ppMCH mRNA in respectively NLT, NRL, NIL and OT is displayed in the inset. The row of RNase-treated samples is indicated with +, the untreated samples with −. Note that in the case of NLT only half of the sample was loaded on the blot.

method, significantly more ppMCH mRNA was found in hypothalami of tilapia adapted to a white background than in black background-adapted fish. The difference in ppMCH mRNA level supplements the reported differences in synthetic activity of MCH neurones of teleosts kept on black and white backgrounds. Morphological studies in carp indicate that MCH-producing cells are more active in white-adapted fish. These cells have larger cytoplasmic and nuclear areas and more prominent nucleoli than in black-adapted carps (Bird & Baker 1989). Recently, it was shown that hypothalamic *de novo* MCH synthesis of trout adapted to a white background is doubled if compared with black background-adapted fish (Baker & Bird 1992). The levels of hypothalamic ppMCH mRNA and MCH synthesis are in accordance with MCH secretion. It is known that more MCH is present in the circulation of white-adapted trout than in black-adapted animals, whereas the rate of MCH secretion in eel and carp also differs in response to changes of background coloration, as judged by

the MCH content of the pituitary gland (Baker 1991).

The distribution of abundant MCH-containing perikarya in the NLT and some in the NRL of the tilapia hypothalamus, as well as their projections to the pituitary and several brain areas is in line with findings on other teleosts (Naito *et al.* 1985, Batten & Baker 1988, Bird *et al.* 1989, Baker 1991). The difference in neuronal size, i.e. magnocellular MCH-containing neurones in the NLT, and smaller cells in the NRL, as was demonstrated here for the tilapia, has only been reported before for the molly *Poecilia latipinna* (Batten & Baker 1988). In contrast with the immunological staining, ppMCH mRNA was only found in perikarya of the NLT and NRL of the hypothalamus, but not in MCH-immunoreactive fibres in the brain. In this report we also explored the possibility of measuring ppMCH mRNA levels in the NLT and NRL by dissecting these regions out of the brain separately. Using the dot blot assay we showed high levels of ppMCH mRNA in the NLT (hundreds of

picograms) and much lower levels in the NRL (tens of picograms). The quantitative distribution fits with the *in situ* hybridization results showing the majority of ppMCH mRNA-containing perikarya in the NLT. This assay will enable us to investigate differential effects of environmental changes on the two cell populations.

In this study we demonstrated the presence of ppMCH mRNA-containing cell bodies, that were also MCH immunoreactive, in the neurohypophysis of tilapia. Although the anatomical distribution of MCH has been studied in a number of other teleosts and in some mammalian species, the occurrence of MCH cell bodies in the pituitary has never been reported before (Baker 1991). In elasmobranchs, MCH-containing cells were localized, by immuno-cytochemistry, in the caudal part of the median lobe of the pars distalis of the pituitary. However, this immunoreactivity was not located in the neural tissue of the lobe (Vallarino *et al.* 1989). Tilapia ppMCH mRNA has never been detected in axons in the neurohypophysis, where MCH immunostaining is very abundant, which is in accordance with the rule that axons lack mRNA (Gordon-Weeks 1988).

It remains to be established what type of cell in the tilapia neurohypophysis contains MCH and what its biological function is. The only cell bodies that have been reported for the neurohypophysis of teleosts are pituicytes, a kind of specialized astrocytes (Holmes & Ball 1974). Perhaps, tilapia neurohypophysial ppMCH mRNA is produced in a subpopulation of these pituicytes. In rats, Schäfer *et al.* (1990) detected mRNA of the neuropeptide enkephalin in pituicytes of the neural lobe by *in situ* hybridization. However, pro-enkephalin mRNA-containing cells seem to be more widely distributed through the rat neurohypophysis than MCH-synthesizing cells in the neurohypophysis of tilapia. Nevertheless, we investigated the possibility that neurohypophysial ppMCH mRNA is produced in pituicytes. We incubated pituitary sections with antiserum to mammalian glial fibrillary acidic protein, a known marker for pituicytes (Salm *et al.* 1982), but we were unable to stain pituicytes in tilapia with this antiserum. Hence, at this stage there is no evidence that the MCH-producing cells in the neurohypophysis are pituicytes. Another explanation for the presence of MCH-producing cells in the neurohypophysis is that they represent perikarya of hypothalamic origin that migrated along axonal tracks to the pituitary. Migration of neurones along axonal tracks has been described before for invertebates (Wendelaar Bonga 1970), but never for vertebrates. An observation supporting this explanation is that the few MCH-

containing cells present in the pituitary are usually located in the dorsal part of the neurohypophysis, and less frequently in the parts penetrating deeper into the intermediate lobe. The function of these neurohypophysial MCH cells is as yet unclear. They might have a neuroendocrine function although, in that case, quantitatively, their contribution to MCH release into the blood seems to be limited, since overall the MCH immunoreactivity in the neurohypophysial MCH cells is much lower than in the surrounding axons derived from the NLT, and the level of ppMCH mRNA in the NIL is very low if compared with the level in the NLT. Alternatively, the neurohypophysial MCH cells might have a local regulatory function.

ACKNOWLEDGEMENTS

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