Melanin-concentrating hormone gene-related peptide stimulates ACTH, but not α-MSH, release from the tilapia pituitary
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abstract
Tilapia (Oreochromis mossambicus; teleostei) melanin-concentrating hormone gene-related peptide (tMgrp) was tested for tropic actions on adenocorticotropin hormone (ACTH) and α-melanocyte stimulating hormone (α-MSH) producing cells in the tilapia pituitary gland in vitro. Up to 100 μM synthetic tilapia Mgrp (tMgrp) had no effect on α-MSH release from tilapia neuro-intermediate lobes in a superfusion set up. However, at concentrations above 1 μM, tMgrp concentration dependently stimulated ACTH release from tilapia anterior lobes. This is the first evidence that Mgrp modulates ACTH release from teleost corticotropes, and this might implicate the peptide in the regulation of the pituitary-interrenal axis of fish.

introduction
Melanin-concentrating hormone (MCH) was first discovered in teleostean fishes as a neurohypophysial hormone involved in the regulation of background adaptation. More recently a function in the regulation of the stress response has been attributed to this peptide in teleosts (Baker 1991, baker & bird 1992, groneveld et al. 1995a), and mammals (baker 1994). Sequence analysis of MCH preprohormones of teleosts and mammals indicated that processing of the prohormone may yield more functional peptides besides MCH (baker 1991). Indeed, the actual processing of mammalian neuropeptide glutamic acid-isoleucine amide (NEI) (Parkes & Vale 1992) and of tilapia MCH gene-related peptide (tMgrp) (Groneveld et al. 1995c) from the MCH preprohormone has been demonstrated. Both peptides directly precede MCH in the prohormone. Tilapia Mgrp is very different from NEI in length (22 and 13 amino acids respectively), as well as in amino acid sequence (groneveld et al. 1993, 1995c), in contrast to MCH itself, which has been highly conserved during evolution. MCH and Mgrp are colocated in the hypothalamus and pituitary of tilapia (Groneveld et al. 1995c).

In teleosts, a modulatory action of MCH on pituitary melanotropes and corticotropes has been described [baker 1991]. At physiological concentrations MCH inhibits α-MSH release from the pituitary of trout and tilapia, whereas at high concentrations MCH in vitro stimulates α-MSH release of tilapia (Barber et al. 1987, Groneveld et al. 1995b). For trout an inhibitory action of MCH on CRH stimulated ACTH release has been reported (Baker et al. 1985, 1986), which is assumed to result from an inhibitory action of MCH on the release of CRH (baker 1994).

In mammals, the actions attributed to NEI are partly similar to, and partly different from that of MCH (Parkes & Vale 1993, Baker 1994). However, the function of tMgrp is still unclear. In a previous study it was shown that tMgrp has no effect on pigment dispersion of tilapia scale melanophores in vitro, and that it does not interfere with the pigment concentrating action of MCH (Groneveld et al. 1995c). The question arises whether tMgrp, like MCH, exhibits effects at the pituitary level. Therefore, in the present study effects of synthetic tMgrp on α-MSH and ACTH release from the tilapia pituitary gland were investigated using an in vitro superfusion set up.

materials & methods

animals
Male tilapia (Oreochromis mossambicus), ranging from 15 to 25 g, were bred in the aquarium facility of the Dept. of Animal Physiology of the University of Nijmegen. They were kept in fresh water at 28°C and were fed a commercial dried fish food
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(Tetramin). The fish were kept in glass aquaria, illuminated by overhead TL tubes, with a day-night rhythm of 12 h light and 12 h darkness. Immediately after removal from the tank (9.00 h; 2 h after light on), the animals were sacrificed by spinal transection, pituitary glands were dissected from the brain, and neurointermediate lobes (NILs) and anterior lobes (ALs) were separated.

Superfusion of pituitary tissue

Freshly dissected NILs or ALs were superfused as described before (Groneveld et al. 1995b). 100 μl chambers were superfused at 30 μl/min. Pulses (30 min) were given with 1 nM to 100 μM synthetic tMgrp (Groneveld et al. 1995c). Synthetic tMgrp was synthesized at the Department of Organic Chemistry of our university by solid phase peptide synthesis using the Fmoc strategy for cleavage (see Chang & Meienhofer, 1978 for details). Controls, which were superfused simultaneously, received incubation medium (142 mM NaCl, 2 mM KCl, 2 mM CaCl2, 15 mM HEPES (pH 7.38), 0.3 mg/ml bovine serum albumin (Sigma), 2.5 mg/ml glucose) without tMgrp. In a typical experiment, 3 groups (controls and 2 Mgrp doses; usually n=6 each) were incubated. The collected superfusion fractions were immediately frozen and stored at -20°C until α-MSH or ACTH radioimmunoassay.

Radioimmunoassays

The α-MSH radioimmunoassay (RIA) with L9 α-MSH antiserum, which shows negligible crossreactivity with ACTH1–39, has been described previously (Balm et al. 1995). The RIA for ACTH using an antiserum against ACTH1–24 also has been described previously (Balm et al. 1994). α-MSH is not detected by this assay; the α-MSH and ACTH antisera did not crossreact with tMgrp.

Processing of data and statistics

For the concentration response relationship of tMgrp-induced effects on ACTH and α-MSH release, values for maximum stimulation of ACTH or α-MSH release during the tMgrp pulse were calculated by subtracting the basal release value from the maximum pulse values for each incubation. Basal release was defined as the average hormone release in 20 to 30 min prior to addition of secretagogue. The presented values for maximum stimulation were corrected for the increasing in vitro unstimulated release of ACTH (Balm et al. 1994) by subtraction of the maximum increase of the controls. The difference between maximum stimulation in the tMgrp pulsed lobes and in controls during the same period was statistically analyzed. Statistical analyses were performed using the unpaired Student’s t test. Significance was accepted at P < 0.05.

RESULTS

Effects of tMgrp on ACTH and α-MSH release

The stimulatory effect of tMgrp on the ACTH release from tilapia ALs during in vitro superfusion lasted for the duration of the pulse; after the pulse ACTH release returned to control levels (Fig. 1). At a concentration of 1 μM tMgrp tended to stimulate ACTH release, although at this concentration the stimulation was not statistically significant (P > 0.1). At 10 and 100 μM tMgrp caused a significant stimulation of ACTH release, which was most
The observed stimulatory effect of synthetic tMgrp on ACTH release is the first demonstration that a teleost peptide derived from the MCH preprohormone, other than MCH itself, regulates ACTH release at the level of the pituitary. In the course of this study it was reported that in rats i.c.v. NEI abolished the MCH-induced inhibition of ACTH plasma levels in response to an ether stress (Bluet-Pajot et al. 1995). Whether tMgrp exerted a direct effect on the corticotropes, or a presynaptic effect on nerve terminals, which in teleosts innervate the endocrine cells, remains to be established. At least two lines of evidence can be advanced that native tMgrp will show the same bioactivity as synthetic tMgrp. Firstly, the amino acid sequence of synthetic tMgrp was based on the sequence deduced from native preproMCH mRNA, and secondly the HPLC retention times of native tMgrp in pituitary and hypothalamus extracts and of the synthetic peptide were identical (Groneveld et al. 1995c). The influence of tMgrp on tilapia corticotropes implies the involvement of a novel component of the MCH-system in stress response. By preproMCH mRNA expression studies (Groneveld et al. 1995a), studies on newly synthesized MCH (Baker & Bird 1992) and plasma MCH measurements (Green et al. 1991, Green & Baker 1991) it was previously demonstrated that the MCH system is responsive to stress. However, knowledge on the sites of action of MCH preprohormone-derived peptides on the hypothalamus-pituitary-interrenal (HPI) axis is limited. In trout MCH has, besides its effect on CRH-induced ACTH release (Baker et al. 1985, 1986), no effect on cortisol release from trout interrenal tissue (Green et al. 1991). The effect of MCH on melanotropes in both trout (Baker et al. 1986, Barber et al. 1987) and tilapia (Groneveld et al. 1995b) may be an additional mechanism of action of the peptide on the HPI axis (Lamers et al. 1992, Balm et al. 1995).

The doses of tMgrp required for the modulation of ACTH release may be indicative for a paracrine or synaptic action. Micromolar concentrations of tMgrp can only be expected in the vicinity of the tMgrp nerve terminals, where the peptide is released. Evidence for the contention that high peptide concentrations may actually arise, comes from structural studies (Batten & Baker...
1988). This would be consistent with dose response curves of other secretagogues, such as MCH (Gröneveld et al. 1995b), and GABA (Verburg-van Kemenade et al. 1986) on α-MSH release in lower vertebrates. However, comparison of cellular sensitivity of tilapia melanotropes in vitro to various secretagogues is hampered by the lack of homologous peptides, such as CRH, and the fact that information regarding the site of release for several of these compounds is not available.

The effects of Mgrp on ACTH release in tilapia are opposite to the inhibitory action of MCH on CRH stimulated ACTH release from trout pituitaries (Baker et al. 1985). The action in trout is consistent with the idea that in this species MCH forms part of a feedback loop, modulating CRH secretion and the HPI axis (Baker 1994). Whether these differences relate to species (trout-tilapia) or peptide (MCH-Mgrp) differences cannot be resolved at present. In addition, the route of administration and the time of day may also be a major determinant of the effect of MCH and Mgrp on the parameters studied (Bluet-Pajot et al. 1995). Since MCH gene expression in teleosts also seems to be subject to diurnal rhythmicity (Suzuki et al. 1995), it may well be that tilapia corticotrope Mgrp sensitivity varies with the time of day.

The lack of effect of 1 nM to 100 μM tMgrp on α-MSH release contrasts with the inhibition of α-MSH induced by MCH at low concentrations, and the stimulation at high concentrations of MCH (Barber et al. 1987, Gröneveld et al. 1995b). A possible explanation for this difference would be that pituitary melanotropes of tilapia have receptors for MCH but not for Mgrp. These findings together with the previously reported lack of effect of tMgrp on tilapia scale melanophores (Gröneveld et al. 1995c) indicate that in tilapia Mgrp by itself does not exert a direct, nor an indirect function in the regulation of background adaptation. However, it cannot be ruled out that tMgrp, which is co-released with MCH (Gröneveld et al. 1995c), influences the action of MCH on pituitary targets.

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