

Regulation of differential release of α -melanocyte-stimulating hormone forms from the pituitary of a teleost fish, *Oreochromis mossambicus*

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ABSTRACT

Using high-performance liquid chromatography (HPLC) in combination with radioimmunoassay, three forms of α -MSH (des-acetyl, mono-acetyl and di-acetyl α -MSH) were separated and identified in tilapia neurointermediate lobes and plasma, and in medium from lobes superfused *in vitro*. The presence of acetylated forms in lobe extracts indicated that the peptides are acetylated intracellularly. Di-acetyl α -MSH was, especially in comparison with mono-acetyl α -MSH, relatively more abundant in lobe extracts than in plasma. This suggests that the three forms of α -MSH are not released according to their relative intracellular abundances. The possibility of

regulation of this differential release by dopamine and TRH was investigated, using a microsuperfusion system. Dopamine was a potent inhibitor of α -MSH release, but did not modulate the relative abundance of the different forms of α -MSH released from the MSH cells. TRH was a potent stimulator of α -MSH release. It enhanced *in vitro* the release of di-acetyl α -MSH more than the release of mono-acetyl α -MSH. Thus tilapia may be able to modulate not only the quantitative but also the qualitative signal from the MSH cells. This might enhance the flexibility of the animals to respond to environmental challenges.

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INTRODUCTION

In vertebrates, α -melanocyte-stimulating hormone (α -MSH), synthesized in pituitary pars intermedia cells, is cleaved from the multifunctional precursor pro-opiomelanocortin (POMC). In addition to α -MSH, a number of other peptide hormones are cleaved from this precursor. They can be divided into three groups: adrenocorticotrophic hormone (ACTH)-like, endorphin-like and MSH-like products. Post-translationally, POMC-derived peptides can be modified, for example by glycosylation or acetylation. Three forms of α -MSH are known: des-, mono- and di-acetylated α -MSH (des-ac, mono-ac and di-ac α -MSH). Acetylation of α -MSH may be of functional significance, because the different forms of α -MSH have different melanotrophic and lipolytic potencies in mammals and fish (Rudman, Hollins, Kutner *et al.* 1983; Kishida, Baker & Bird, 1988). Our group has reported a higher corticotrophic effect for di-ac α -MSH than for des- and mono-ac α -MSH on tilapia

interrenal tissue *in vitro* (Balm, Lamers, Jenks & Wendelaar Bonga, 1987).

Acetylation of α -MSH apparently takes place intracellularly in mammals and fish, because all three forms of α -MSH are found not only in the blood plasma but also in extracts of the neurointermediate lobe (NIL) (Rudman *et al.* 1983; Follenius, Van Drosselaer & Meunier, 1986a; Kishida *et al.* 1988). If the degree of acetylation is indeed of functional significance, differential release of the three forms of α -MSH may be anticipated under various circumstances. We were therefore interested in the control of α -MSH release in fish, particularly in the possibility of modulation of the differential release of the three forms of α -MSH by two regulatory factors, dopamine and thyrotrophin-releasing hormone (TRH). In mammals, amphibians and fish there are biochemical data that dopamine inhibits α -MSH secretion from melanotropes *in vivo* as well as *in vitro* (e.g. Tilders, Berkenbosch & Smelik, 1985; Verburg van Kemenade, Tonon, Jenks & Vaudry, 1986; Omeljaniuk & Peter,

1989). Direct innervation of α -MSH cells by dopaminergic fibers of hypothalamic origin has been described for the lower vertebrates, including fish (Kah, Dubourg, Chambolle & Calas, 1984). Another hypothalamic factor known as an α -MSH secretagogue in the lower vertebrates is TRH. Tonon, Leroux, Leboulenger *et al.* (1980) and Verburg-van Kemenade, Jenks, Visser *et al.* (1987b) demonstrated a strong stimulatory effect of TRH on α -MSH release from *Rana ridibunda* and *Xenopus laevis* NIL. In contrast, TRH has no α -MSH releasing activity in rats (Kraicer, 1977) and newts (Danger, Perroteau, Franzoni *et al.* 1989). A regulatory role for TRH in α -MSH release in teleost fish has been indicated by Omeljaniuk, Tonon & Peter (1989). These authors found a dose-related stimulation of α -MSH release by TRH from goldfish NIL *in vitro*.

The species investigated in this study is the tilapia *Oreochromis mossambicus*. In this species Van Eys (1981) showed three peaks with melanocyte-stimulating activity. In the present study we report that these peaks represent des-, mono- and diacetylated α -MSH. To study the control of the release of the different α -MSH forms, a superfusion system was developed in which the effects of dopamine and TRH on the kinetics of hormone release were analysed. We further determined blood plasma levels of the three forms of α -MSH and of TRH.

MATERIALS AND METHODS

Experimental animals

In all experiments mature female tilapia, *Oreochromis mossambicus* (formerly *Tilapia mossambica* and *Sarotherodon mossambicus*), bred in the laboratory, were used. The fish were kept in tanks (120 litres) with tap water at 28 °C on a neutral coloured background. The photoperiod was 12 h of direct illumination alternated with 12 h of darkness. The body weight of the fish ranged from 10 to 25 g. The fish were fed on a commercial dried fish food (Tetramin). Feeding was stopped 24 h before the experiments. Immediately after removal from the tank the fish were killed by spinal transection and pituitary glands were dissected from the brain.

In-vitro superfusion

After separation from the pars distalis, NILs were placed on a filter on the bottom of a superfusion chamber (volume 10 μ l). One superfusion unit consisted of four superfusion chambers. Three units were available, so a maximum of 12 chambers could be superfused simultaneously. Two NILs per chamber were superfused with a solution of Hepes (15 mmol/l),

NaCl (132 mmol/l), KCl (2 mmol/l), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (2 mmol/l) (pH 7.38), with 0.25% (w/v) glucose and 0.03% (w/v) bovine serum albumin. The superfusion medium was pumped through the chambers by a 16 channel peristaltic pump (Watson Marlow) at a rate of 30 μ l/min. Fractions were collected over varying periods, using an Isco fraction collector. After reaching a basal level of release, secretagogues were administered, diluted to the desired concentration in superfusion medium just before use. At the time of administration the superfusion medium reservoir was changed for the reservoir containing the secretagogue. After a 30-min pulse, the reservoirs were changed again. The fractions collected just before and during the pulse were split in two. One half of the fraction was used for determination of the immunoreactive (ir) α -MSH in a radioimmunoassay (RIA), the other halves of the fractions were pooled and used for analysis by high-performance liquid chromatography (HPLC). The fractions were immediately stored at -20 °C. Concentrations of α -MSH were determined by means of an RIA and release was expressed as fmol ir- α -MSH/min per NIL or as percentage of basal release. 100% was calculated as the average release during the last 30 min before the pulse.

Preparation of NIL extracts

Freshly dissected NILs were homogenized in a Potter homogenizer in 500 μ l ice-cold HCl (0.1 mol/l). The homogenate was centrifuged for 5 min at 10 000 g in an Eppendorf centrifuge. The supernatant was submitted to HPLC.

Collection of blood plasma

The blood of nine fish was collected from the caudal artery and diluted 1:1 in 0.9% (w/v) NaCl containing 2 mmol EDTA/l and 100 000 KIU aprotinin/ml (Sigma, St Louis, MO, U.S.A.). After centrifugation the plasma was put onto an equilibrated octadecyl Bakerbond column (J. T. Baker, Phillipsburg, NJ, U.S.A.). α -MSH was eluted with 40% propanol in formic acid/pyridin buffer. After drying in a Savant Speedvac concentrator, α -MSH was dissolved in 0.05 mol HCl/l and subjected to HPLC. The recovery of α -MSH after the Bakerbond column was 30–40% and equal for all three forms of α -MSH.

Characterization of peak C

Six NILs were incubated for 3.5 h with 10 μ Ci [^3H]lysine, [^3H]serine, [^3H]proline and [^3H]tryptophane (75, 28, 22 and 25 Ci/mmol respectively), in 100 μ l incubation medium. After rinsing, the NILs were chased for 3 h with medium containing 2 mmol lysine/l, 2 mmol serine/l, 2 mmol proline/l and 2 mmol tryptophane/l. Newly synthesized products in

NIL extracts were separated on HPLC and the material coeluting with di-ac α -MSH, peak C, was isolated, dried and dissolved in 50 μ l demineralized water; 25 μ l NaOH (0.02 mol/l) was added to 25 μ l peak C and incubated at 37 °C for 30 min. As a control 25 μ l demineralized water was added to the other 25 μ l peak C. Both control and NaOH-treated peaks C were submitted to HPLC.

HPLC

HPLC was performed on a Spherisorb ODS column (Martens, Jenks & Van Overbeeke, 1981). α -MSH was eluted with a propanol-1 gradient in a buffer consisting of 0.5 mol formic acid/l, plus 0.14 mol pyridin/l (pH 3), with a flow rate of 2 ml/min. The eluate was collected in 0.3-min fractions. The fractions were dried in a Savant Speedvac concentrator and stored at -20 °C. Shortly before the MSH assay fractions were dissolved in HCl (0.1 mol/l)/methanol (1:1, v/v). The recovery of α -MSH after HPLC was 45–55% and equal for all three forms of α -MSH.

Radioimmunoassay for α -MSH

Concentrations of α -MSH were measured in duplicate in an RIA with an antiserum against mono-ac α -MSH, produced and characterized by Vaudry, Tonon, Delarue *et al.* (1978) and used at a final dilution of 1:60 000. α -MSH was labelled with 125 I (Amersham International plc, Amersham, Bucks, U.K.) using the iodogen method (Salacinsky, McLean, Sykes *et al.* 1981) and purified through solid-phase extraction (octadecyl Bakerbond column). Bound and unbound radioactivity were separated by polyethylene glycol precipitation. The sensitivity of the assay was 6 fmol. Cross-reactivity with des-ac and di-ac α -MSH was 100%. The interassay variation was $6 \pm 1\%$ and the intra-assay variation $2 \pm 1\%$.

Determination of TRH

Circulating TRH levels were determined by means of a specific RIA for TRH (Visser & Klootwijk, 1981). To 1 vol. blood, 4 vol. ice-cold 100% ethanol were added and the mixture was shaken and centrifuged. The supernatant was dried in a Savant Speedvac concentrator and stored at -80 °C.

Chemicals

Des-ac α -MSH, mono-ac α -MSH, di-ac α -MSH (1–13, synthetic bovine) and 3-hydroxytyramine (dopamine) were from Sigma Chemical Company. Before use dopamine was dissolved in ascorbic acid (1 mg/ml; Merck, Darmstadt, F.R.G.). TRH was a gift from H. Vaudry (Rouen, France). Tritiated amino acids were purchased from Amersham International plc. All other chemicals were of analytical grade.

Statistics

Results are presented as means \pm S.E.M. Statistical analyses were performed using Student's *t*-test or Mann–Whitney U test where appropriate.

RESULTS

Separation and identification of the forms of α -MSH

α -MSH in NIL cells

The HPLC of NIL extracts showed predominant α -MSH immunoreactivity only between 10 and 16 min of elution. Within this period three major α -MSH

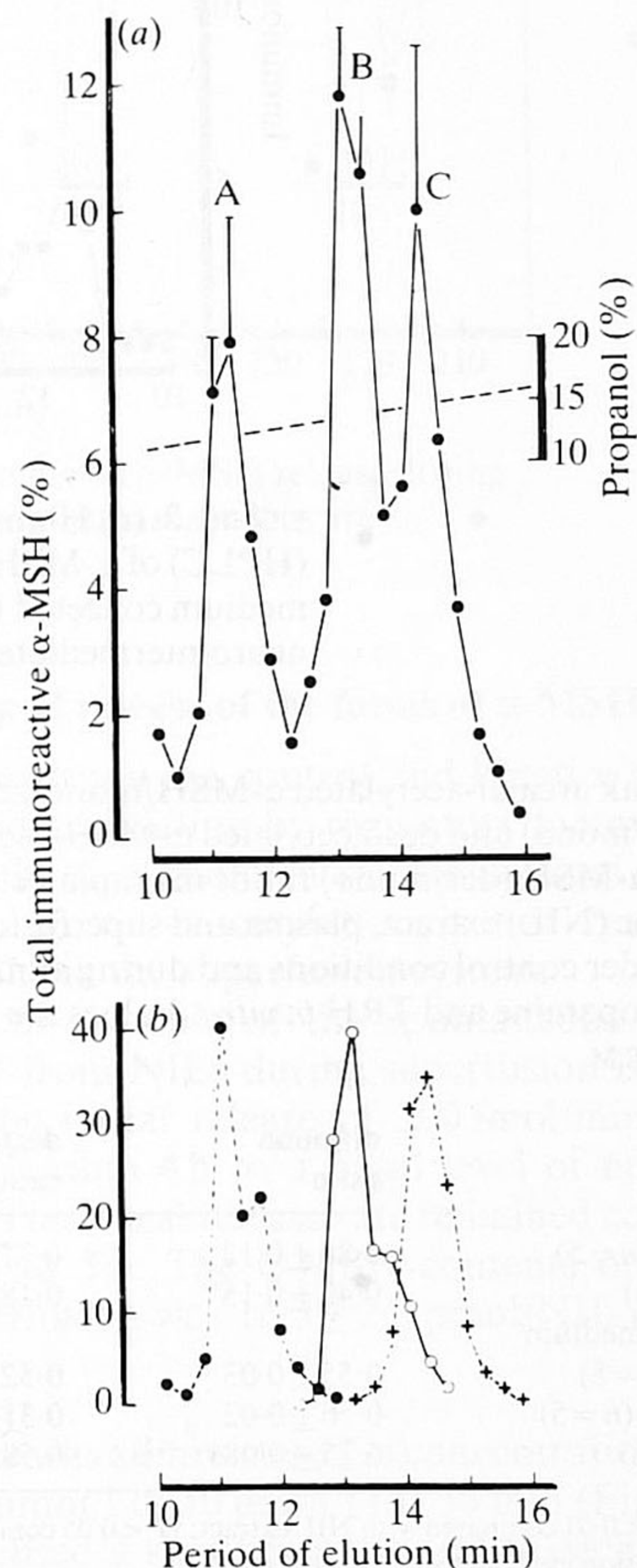


FIGURE 1. (a) High-performance liquid chromatography (HPLC) of extract of tilapia neurointermediate lobe (NIL) between 10 and 16 min of elution, expressed as a percentage of total α -MSH immunoreactivity. The broken line represents the propanol gradient. Values are means \pm S.E.M., $n=4$. A, B and C are the three major α -MSH immunoreactive peaks. (b) HPLC of synthetic des-acetyl α -MSH (\bullet), mono-acetyl α -MSH (\circ) and di-acetyl α -MSH ($+$) standards, subjected separately to HPLC.

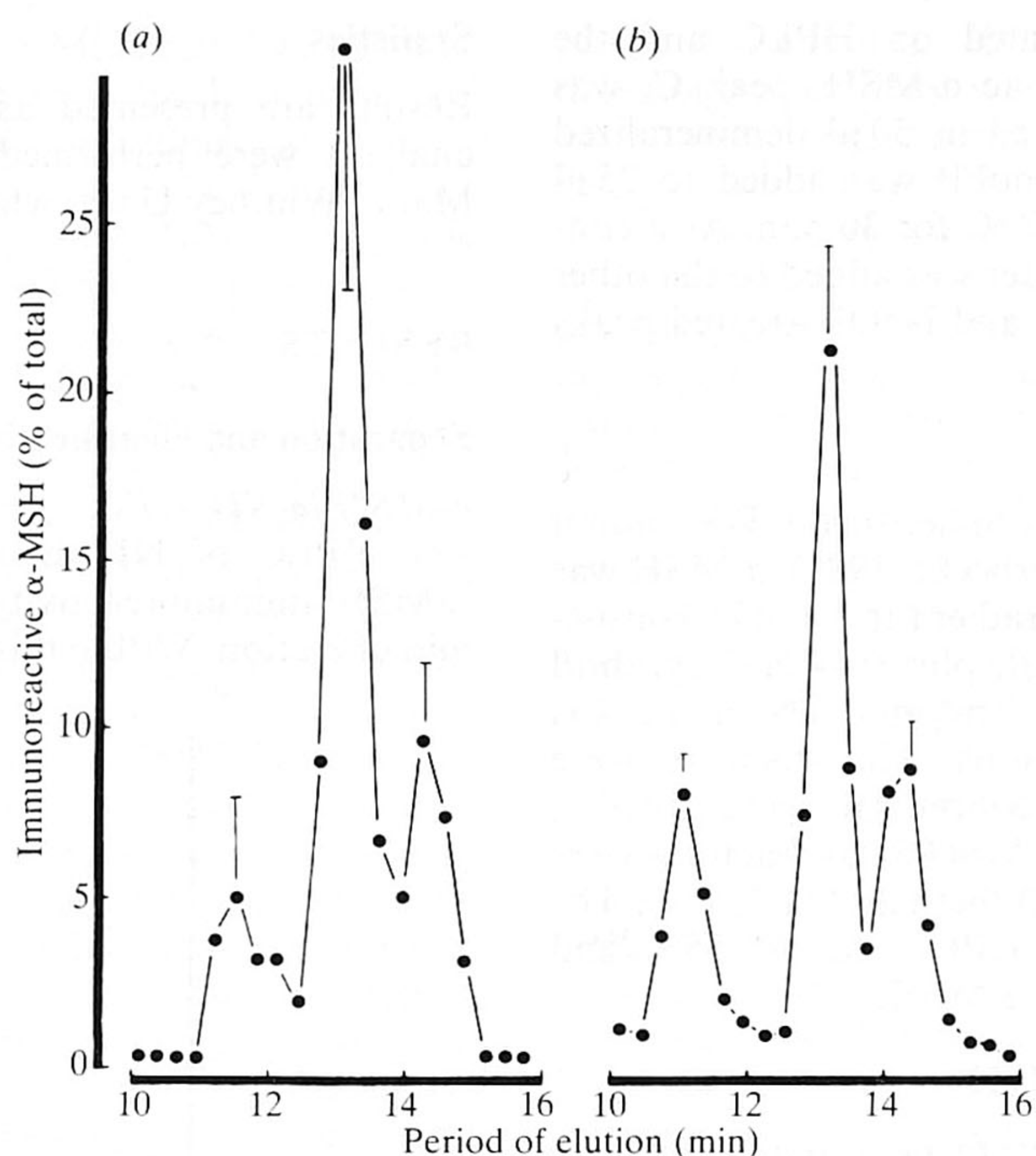


FIGURE 2. (a) High-performance liquid chromatography (HPLC) of α -MSH in plasma ($n=4$). (b) HPLC of α -MSH in medium collected between 30 and 60 min of superfusion of neurointermediate lobes ($n=8$). Values are means \pm S.E.M.

TABLE 1. Peak area di-acetylated α -MSH/mono-acetylated α -MSH (di/mono) and des-acetylated α -MSH/mono-acetylated α -MSH (des/mono) ratios in tilapia neurointermediate lobe (NIL) extract, plasma and superfusion medium under control conditions and during administration of dopamine and TRH *in vitro*. Values are means \pm S.E.M.

| | di/mono ratio | des/mono ratio |
|-----------------------|-------------------------|----------------------|
| NIL extract ($n=5$) | 0.86 ± 0.12 | 0.77 ± 0.06 |
| Plasma ($n=4$) | $0.40 \pm 0.15^*$ | $0.18 \pm 0.06^{**}$ |
| Superfusion medium | | |
| Control ($n=8$) | 0.55 ± 0.03 | 0.52 ± 0.07 |
| Dopamine ($n=5$) | 0.56 ± 0.02 | 0.31 ± 0.06 |
| TRH ($n=6$) | $0.75 \pm 0.08^\dagger$ | 0.58 ± 0.07 |

* $P < 0.05$, ** $P < 0.01$ compared with NIL extract; $^\dagger P < 0.05$ compared with control superfusion medium (Student's *t*-test).

immunoreactive peaks eluted at 11.5, 13.2 and 14.5 min (Fig. 1a). These three peaks represent at least 90% of the total α -MSH immunoreactivity. There were a few small peaks which contained less than 10% of the ir- α -MSH before 10 min of elution (data not shown).

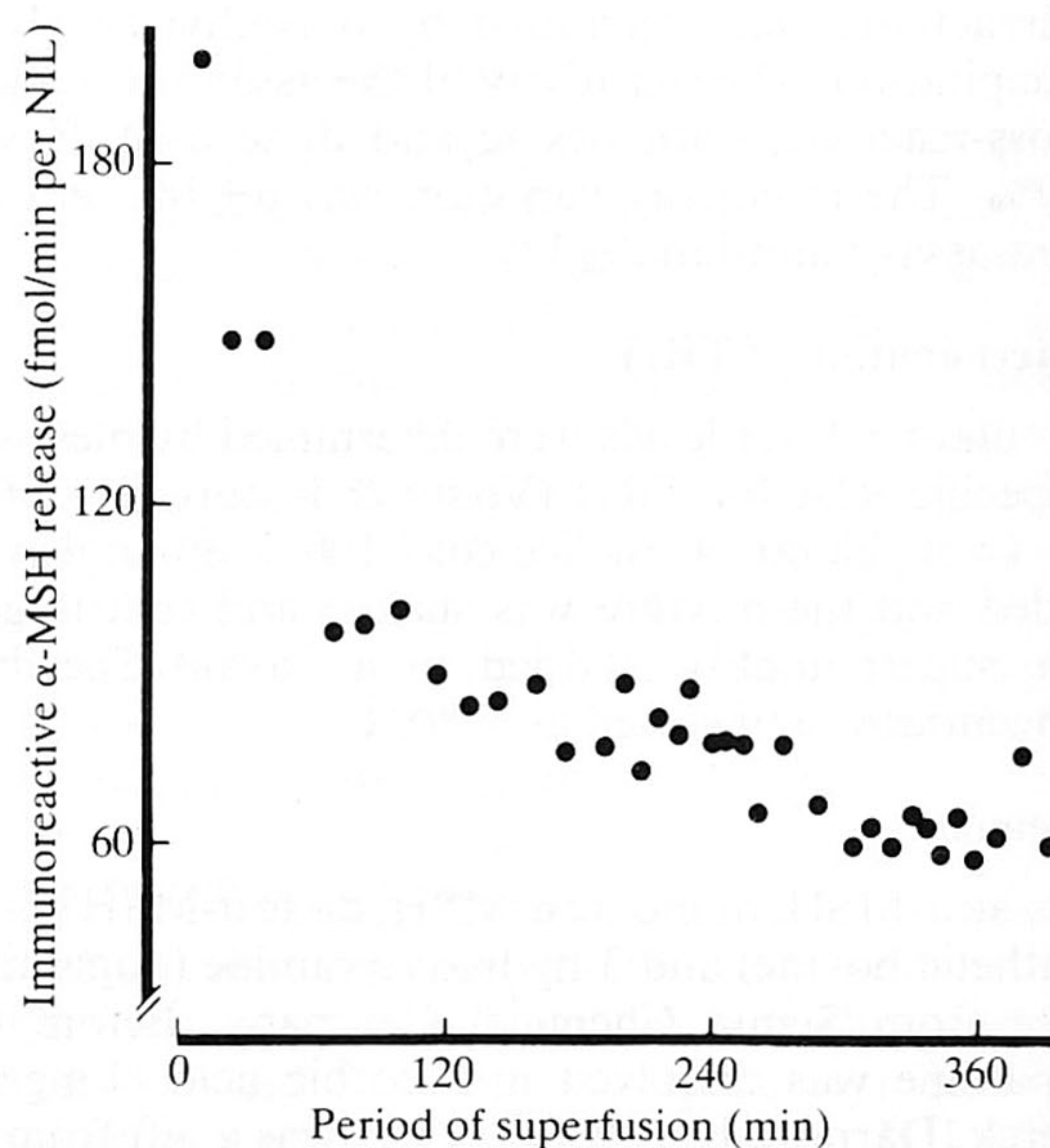


FIGURE 3. Typical pattern of the unstimulated in-vitro α -MSH release during superfusion of tilapia neurointermediate lobes (NIL) for 400 min.

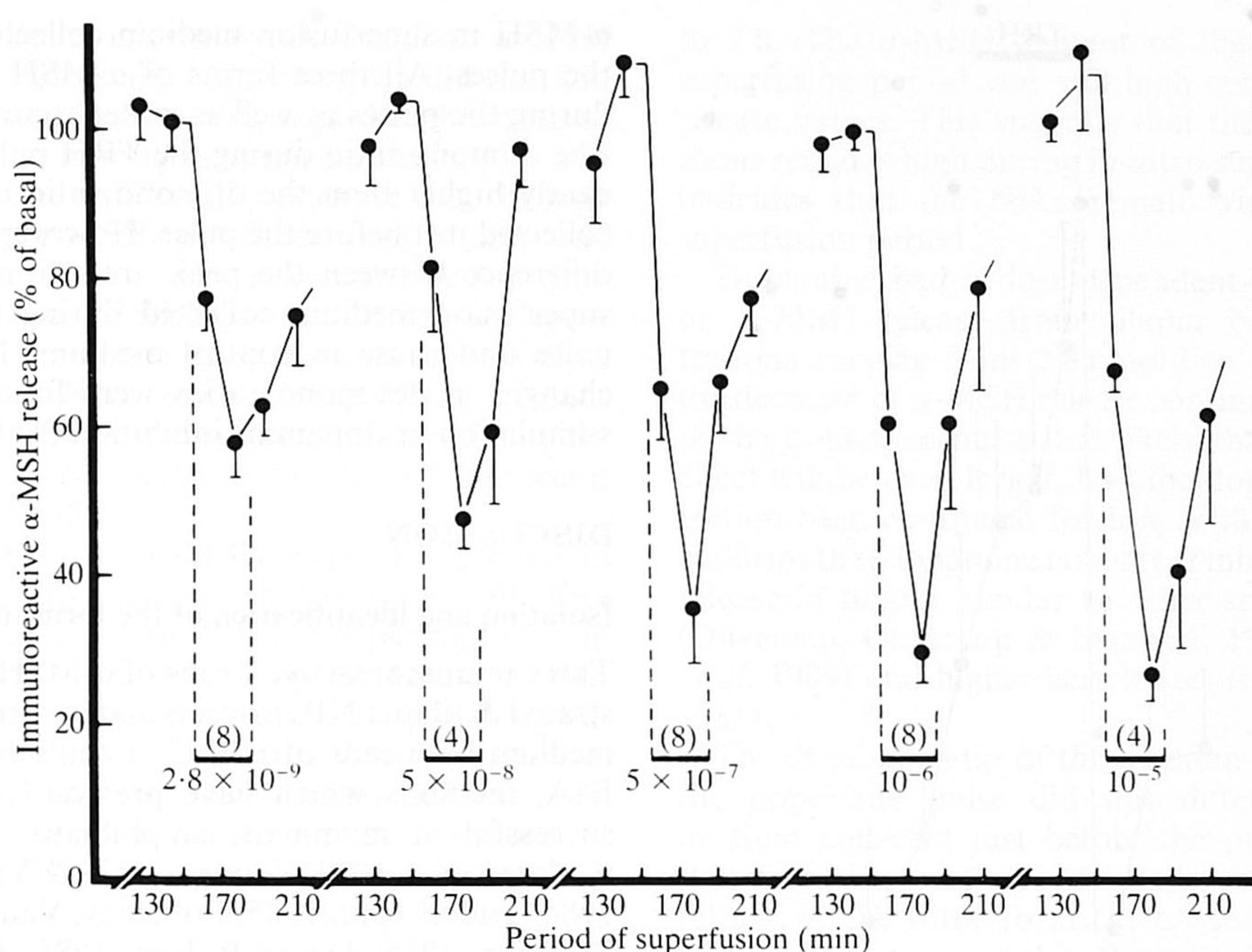


FIGURE 4. Effects of different concentrations (mol/l) of dopamine on α -MSH release during superfusion of neurointermediate lobes as a percentage of basal release. Values are means \pm S.E.M.; numbers are shown in parentheses.

To determine the identity of the major peaks, elution times were compared with those of synthetic des-, mono- and di-ac α -MSH standards, which were subjected to HPLC separately (Fig. 1b). Peak A coeluted with des-ac α -MSH, peak B with mono-ac α -MSH and peak C with di-ac α -MSH. Further characterization of peak C was performed by treating the material in peak C collected from radioactive labelled NILs with 0.01 mol NaOH/l for 30 min. This high pH treatment converts di-ac α -MSH to mono-ac α -MSH by removing the 0-acetyl group. The elution time of the treated product was earlier and corresponded to that of mono-ac α -MSH.

α -MSH forms released in plasma and in superfusion medium

The three forms of α -MSH were also found in plasma and superfusion medium (Fig. 2). However, when compared with the intracellular situation, relatively more mono-ac α -MSH than di-ac α -MSH was measured. In the NIL homogenates the ratio of the peak areas of di-ac α -MSH to mono-ac α -MSH (di/mono ratio) was significantly higher than in the superfusion medium and in the plasma ($P < 0.01$ and $P < 0.05$ respectively; Table 1).

The total ir- α -MSH in the blood plasma was 294.2 ± 50.9 pmol/l ($n = 6$).

Regulation of release of the forms of α -MSH

In order to study the control and kinetics of α -MSH release and acetylation by regulatory factors, we first analysed the unstimulated release of total ir- α -MSH (i.e. the degree of acetylation is not specified) by tilapia NILs in the superfusion system.

A typical example of the spontaneous release of ir- α -MSH from NILs during superfusion is shown in Fig. 3. The initial release of 200 fmol/min per NIL decreased within 4 h to a basal level of 66 fmol/min per NIL. This basal release rate remained constant for at least 4 to 5 h. The α -MSH contents of the NILs after superfusion was 16.3 ± 2.3 pmol/NIL ($n = 6$).

Dopamine

Dopamine was administered in concentrations ranging from 2.8 nmol/l to 10 μ mol/l for 30 min (Fig. 4). Each dosage caused a significant ($P < 0.01$) decline in total ir- α -MSH release into the medium within 7.5 min. The decline continued until the end of the pulse. After changing to dopamine-free medium, the release was restored to 61–97% of the basal release within 30 min. The effect of dopamine was dose-dependent.

TRH

The TRH concentration in blood plasma was 0.3 ± 0.02 nmol/l.

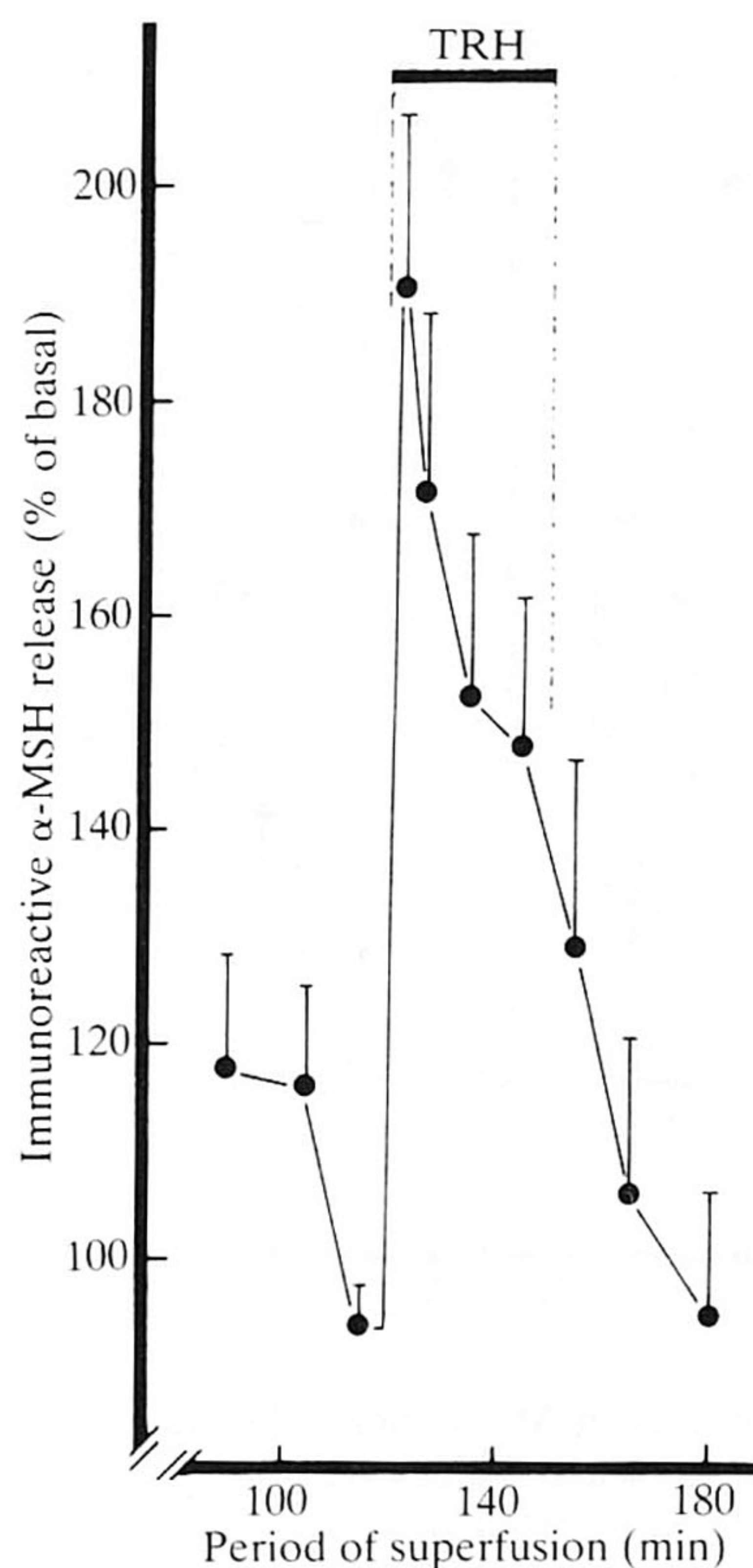


FIGURE 5. Effect of a 30-min TRH pulse (10 nmol/l) on α -MSH release from tilapia neurointermediate lobes (NIL). Values are means \pm S.E.M. of the fractions of 12 superfusion chambers as a percentage of the basal release. This unstimulated release was 50.4 ± 6.0 fmol α -MSH/min per NIL and was defined as 100%.

Release of α -MSH increased during the TRH pulse (Fig. 5) and reached its peak value during the first or second 5-min fraction. Because of this, the mean maximal stimulation was calculated as the mean of the highest α -MSH value of every individual superfusion chamber during the TRH pulse. This mean maximal stimulation by 10 nmol TRH/l was $100.4 \pm 15.6\%$ higher than basal values ($P < 0.01$). In general, the maximal stimulation was reached within 5 min ($190.2 \pm 15.7\%$ of basal release, $P < 0.01$), and tended to decline to a stable release rate, approximately 50% higher than basal. After changing to TRH-free medium, the release immediately returned to the basal level.

Effects of dopamine and TRH on the differential release of α -MSH

The ir- α -MSH that was released during the administration of both dopamine (10 μ mol/l) and TRH (10 nmol/l) pulses was collected and analysed by HPLC. The profiles were compared with the HPLC of

α -MSH in superfusion medium collected just before the pulses. All three forms of α -MSH were released during the pulses as well as under control conditions. The di/mono ratio during the TRH pulse was significantly higher than the di/mono ratio of the α -MSH collected just before the pulse. However, there was no difference between the peak area di/mono ratios in superfusion medium collected during the dopamine pulse and those in control medium. No significant changes in des/mono ratios were found after TRH stimulation or dopamine inhibition (Table 1).

DISCUSSION

Isolation and identification of the forms of α -MSH

Three immunoreactive forms of α -MSH were demonstrated in tilapia NIL extracts and in-vitro superfusion medium by means of HPLC in combination with an RIA, methods which have previously been proven successful in mammals, amphibians and fish (e.g. Rudman *et al.* 1983; Vaudry, Jenks & Van Overbeeke, 1984; Tilders *et al.* 1985; Follenius, Van Drosselaer & Meunier, 1985; Dorez & Joss, 1988; Kishida *et al.* 1988). Peaks A, B and C coincided with standard des-ac, mono-ac and di-ac α -MSH respectively. Peak C appeared to be unstable in high pH conditions and its converted form coeluted with mono-ac α -MSH. This phenomenon has previously been described for di-ac α -MSH (Rudman *et al.* 1983; Vaudry *et al.* 1984; Follenius *et al.* 1985; Dorez & Joss, 1988). Consequently, we concluded that peaks A, B and C are des-ac, mono-ac and di-ac α -MSH respectively. The three peaks represent about 90% of all α -MSH immunoreactivity. A further 10% was found as a few small peaks eluting before 10 min of elution and might be sulphoxide forms of α -MSH (Leenders, Janssen, Theunissen *et al.* 1986).

The three α -MSH forms were found in NIL extracts as well as in in-vitro superfusion medium. This is in line with observations in other species of fish and in mammals (Evans, Lorenz, Weber & Barchas, 1982; Follenius *et al.* 1985; Dorez & Joss, 1988), but in contrast with findings in two amphibians (Martens *et al.* 1981; Vaudry, Jenks & Van Overbeeke, 1983), where acetylation is associated with release of α -MSH. We conclude that acetylation of α -MSH in tilapia occurs before release, perhaps within the granules of melanotrope.

The di/mono ratio that we found in tissue extracts of tilapia NILs was lower than those for NIL homogenates of carp and goldfish (Follenius *et al.* 1986a; Kishida *et al.* 1988) and higher vertebrates (Tilders *et al.* 1985), as deduced from the data presented. Follenius *et al.* (1985) suggested that their high di-ac α -MSH values were due to homogenization of

the NILs in acetic acid in which the di-ac α -MSH would be far more stable. However, we did not find any quantitative difference with regard to the forms of α -MSH between homogenization in acetic acid and HCl (data not shown). Further evidence that the high values of di-ac α -MSH in NIL extracts of carp and goldfish are not due to procedural artefacts is indicated by the results of Kishida *et al.* (1988). Using the same isolation procedure, they found much less di-ac α -MSH in trout NILs than in carp NILs, whereas mono-ac α -MSH values were comparable. Thus, the di/mono ratio in NIL extracts of teleost fishes seems to be species-specific.

The three forms of α -MSH were also present in tilapia blood. This indicates that in tilapia all three forms are released *in vivo*. When comparing the ratio of di-ac to mono-ac α -MSH peak areas in NIL extracts and in plasma, we found that it was significantly lower in plasma. Follenius, Van Drosselaer & Meunier (1986b) reported even larger differences in di/mono ratios between plasma and NIL extracts of carp and goldfish. The ratio differences between plasma and NIL cells were explained by these authors as a conversion of di-ac α -MSH to mono-ac α -MSH. However, conversion in plasma is unlikely as an explanation for this difference, since the half-life of di-ac α -MSH in plasma appeared to be longer than that of mono-ac α -MSH *in vivo* as well as *in vitro* (Rudman *et al.* 1983). Furthermore, di-ac α -MSH is a more energetically expensive form of α -MSH than mono-ac α -MSH and is therefore unlikely to be an intermediate product in the synthesis of mono-ac α -MSH. Because this difference in di/mono ratio was also found in in-vitro medium we suggest that di-ac and mono-ac α -MSH are not released in proportion to their intracellular ratio, but in variable ratios that are under physiological control.

The fact that the des/mono ratio in plasma is much lower than in in-vitro medium might be explained by the possibility that the half-life of des-ac α -MSH is shorter in plasma than in in-vitro medium. This high ratio in in-vitro analysis might therefore be considered a non-physiological phenomenon.

The total amount of ir- α -MSH in tilapia blood plasma is in the range of values reported for other unstressed fish adapted to a neutral background (Rodrigues & Sumpter, 1984; Sumpter, Dye & Bentley, 1986; Follenius *et al.* 1986b). Blood values seem to be species-dependent and can be influenced by background colour and some types of stress (Baker, Wilson & Bowley, 1984; Rodrigues & Sumpter, 1984; Sumpter, Pickering & Pottinger, 1985).

Control of release of α -MSH *in vitro*

In the superfusion system used in this study, the unstimulated α -MSH release was stable for at least 6

to 7 h. The α -MSH content of the NILs after the superfusion period was still high compared with the release values. This suggests that the rate of biosynthesis remains high during in-vitro superfusion, which indicates that the NILs remain viable during the superfusion period.

Dopamine had a dose-dependent inhibitory effect on α -MSH release from tilapia NILs in concentrations varying from 2.8 nmol/l to 10 μ mol/l. Since the decrease of α -MSH release continued until the end of the dopamine pulse it is likely that the inhibitory effect will be even larger, had the dopamine administration been continued for longer than 30 min. This confirms that dopamine is a potent inhibitor of α -MSH release in tilapia, similar to other species of teleosts (Olivereau, Olivereau & Lambert, 1987; Omeljaniuk *et al.* 1989) and higher vertebrates (e.g. Tilders *et al.* 1985).

The di/mono ratio of the medium collected during the dopamine pulse did not differ from that of medium collected just before the pulse. Dopamine, therefore, does not seem to modulate the differential release of the three forms of α -MSH. Tilders *et al.* (1985) did not report the effects of dopamine on the spectrum of α -MSH forms in rats. In contrast, in some amphibians the release of des-ac α -MSH was preferred over mono-ac α -MSH under dopamine treatment (Jenks, Verburg van Kemenade, Tonon & Vaudry, 1985; Verburg-van Kemenade, Jenks & Smits, 1987a).

TRH induced a twofold increase in α -MSH release in a 10 nmol/l concentration, a concentration within the dose-response curve of TRH in goldfish (Omeljaniuk *et al.* 1989). Because the presence of TRH has been demonstrated in the hypothalamus and pituitary of teleost fish (Jackson & Reichlin, 1980), we suggest that TRH is a physiologically relevant stimulatory factor for α -MSH release in tilapia. Verburg van Kemenade *et al.* (1987b) found a stimulatory effect of 0.1 and 1 μ mol TRH/l on α -MSH release *in vitro* in the clawed toad, but they questioned a physiological role for this peptide in the regulation of α -MSH secretion because plasma TRH levels were as high as 0.10 μ mol/l. In this animal, the poison glands of the skin are another source of TRH (Richter, Kawashima, Egger & Kreil, 1984), and they can increase plasma TRH to levels within the range for stimulation of α -MSH release. In tilapia, however, the plasma TRH concentration was as low as 0.3 nmol/l, a concentration that lies below the lowest effective concentration in goldfish (Omeljaniuk *et al.* 1989). We conclude that circulating TRH levels do not interfere with the effects of TRH secreted into the NIL by neurones of hypothalamic origin.

In mammals, where TRH is known to stimulate thyrotrophin release from the pars distalis of the

pituitary, it failed to stimulate α -MSH and ACTH secretion from the pituitary (Kraicer, 1977). In fish and amphibians, TRH is thought to be of less importance in regulating thyrotrophin release (Jackson, 1978), although Garcia-Navarro, Malagon, Garcia-Navarro *et al.* (1990) demonstrated stimulation of the TSH cells of frogs by TRH. Apparently, the targets for this peptide have changed during evolution, although in all vertebrates TRH seems to be involved in the regulation of prolactin release.

The kinetics of α -MSH release during 30 min of TRH administration, showing a transient peak after 5 min and a subsequent decline to a stable level at approximately 150% of the basal release, are very similar to the findings of Lamacz, Tonon, Danger *et al.* (1987) who described this as a biphasic effect.

Our observation that the di/mono ratio in TRH-stimulated medium was higher than that observed just before the TRH pulse indicates that TRH not only increased the total amount of released α -MSH, but also stimulated the release of di-ac α -MSH more than that of mono-ac α -MSH. Thus TRH seemed to shift the differential release of α -MSH forms in favour of the more potent α -MSH form. A comparable differential effect of TRH on the acetylation of α -MSH was found in the frog (Tonon, Leroux, Jenks *et al.* 1985), where the mono-acetylated form was favoured over des-ac α -MSH. Our findings suggest that, in tilapia, di-ac α -MSH is kept in storage under control conditions and is released in relatively high quantities during stimulation by TRH. In this respect TRH differs from dopamine which indiscriminately inhibited the release of all α -MSH forms in tilapia. Since di-ac α -MSH has a higher melanotrophic activity, as suggested by Kishida *et al.* (1988), the control of differential release of di-ac α -MSH may have physiological implications. This mechanism may enhance the flexibility of the animals to respond to environmental challenges.

Preliminary results from our group (Balm *et al.* 1987) suggest a corticotrophic function for α -MSH, since it stimulates the cortisol release from tilapia interrenal tissue. On the other hand, several authors have suggested a corticotrophic function for α -MSH in mammals (Szalay & Stark, 1982; Kawauchi, Kawazoe, Adachi *et al.* 1984). In some fish, α -MSH secreting cells have been shown to be activated during stress (Malo-Michele, 1980; Sumpter *et al.* 1985) and might be involved in the stimulation of adrenal tissue during stress. The function of acetylation of α -MSH with regard to its corticotrophic effect will receive special attention in our further studies.

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