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MELANOTROPIN POTENTIATING FACTOR INHIBITS LIPOLYTIC ACTIVITY OF β-LIPOTROPIN BUT NOT OF MELANOCYTE STIMULATING HORMONES

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ABSTRACT

Melanotropin potentiating factor (MPF) potentiates the melanotropic activity of melanocyte stimulating hormones. Although the message sequence for the melanotropic and lipolytic activity are identical for β-lipotropin, α- and β-MSH, MPF was not able to affect the lipolytic response to α- and β-MSH in rabbit adipocytes. However, MPF at concentrations of 10^{-5} and 10^{-6} mol/l inhibited the lipolytic activity of β-lipotropin. This suggests that the inhibition of the lipolytic response to β-lipotropin is not connected with the common lipolytic message sequence (β-LPH 47-53). Since β-lipotropin has a second lipolytic sequence in its C-terminal part this second lipolytic core of β-lipotropin might interact with MPF which has no intrinsic lipolytic activity.

INTRODUCTION

The C-terminal tetrapeptide Lys-Lys-Gly-Glu-OH of human β-lipotropin (β-LPH 86-89) has been identified as melanotropin potentiating factor (MPF) because it potentiates the melanotropic activity of melanocyte stimulating hormones (MSH) (1). The molar pigmentary potency of β-LPH is greater than that of other melanocyte-stimulating hormones on Anolis skin which is due to a potentiation of the MSH sequence of β-LPH (β-LPH 47-53) by MPF.

As β-LPH and the melanocyte stimulating hormones β-MSH (β-LPH 41-58) and α-MSH are also well known stimulators of lipolysis we investigated the effect of MPF on the lipolytic activity of these hormones in rabbit adipocytes.

MATERIAL AND METHODS

β-lipotropin was extracted from porcine pituitaries and purified by high performance liquid chromatography as previously described (2). Synthetic α- and β-MSH as well as MPF were purchased from Bachem, Bubendorf, Switzerland. Perirenal adipose tissue was obtained from female rabbits (white Neuseeländer, body weight 3.5 - 4.5 kg) fed ad libitum. Adipocytes were prepared according to Rodbell (3) by digestion of adipose tissue with purified collagenase (CLSPA), Lot 3 D 553, Worthington, Berlin, 13.3 U/ml) which are free of unspecific proteolytic activity. Bovine serum albumin fraction V (Roth, Karlsruhe, W-Germany) was defatted by the method of Chen (4).

The lipolytic assay was performed by incubation of about 6o ooo adipocytes in 1 ml Krebs-Ringer-bicarbonate buffer pH 7.4 containing 3 mmol/l glucose and 4 % bovine serum albumin fraction V as free fatty acid acceptor for 1 hour under continuous shaking. The incubation was terminated by adding o.2 ml of a semisynthetic oil, dinonylphtalate and centrifugation at 12 ooo g for 4 minutes. The infranatant was collected and frozen at 35°C for glycerol analysis. Glycerol was determined enzymatically with an automated device as described recently (5). Glycerol release is expressed as nmol per 1o mg dry weight of the cells (mean of six determinations). α- and β-MSH and β-LPH were tested in triplicate in the range of $1o^{-7}$ to $1o^{-9}$ mol/l, MPF was added in concentrations of $1o^{-11}$ to $1o^{-5}$ mol/l. Each experiment was performed with adipocytes from one individual animal. Statistical evaluation was done by the Wilcoxon matched-pair rank test.

RESULTS

β-lipotropin ($1o^{-7}$ to $1o^{-9}$ mol/l) stimulated glycerol release from adipocytes of 8 rabbits in a dose dependent manner while MPF alone was lipolytically inactive in concentrations from $1o^{-5}$ to $1o^{-11}$ mol/l (28 rabbits). However, MPF inhibited β-LPH stimulated glycerol release dose dependently at concentrations of $1o^{-5}$ and $1o^{-6}$ mol/l (table 1): The addition of $1o^{-5}$ mol/l MPF resulted in a mean decrease of $1o^{-8}$ mol/l β-LPH stimulated glycerol release by 48.6 % respectively by 27.1 % when only $1o^{-6}$ mol/l MPF were added. Lipolysis induced by the higher β-LPH concentrations of $1o^{-7}$ mol/l was inhibited less by the addition of $1o^{-5}$ mol/l MPF (15.6 %) and $1o^{-6}$ mol/l MPF (14.7 %). All these differences were significant ($p < o.o1$).

TABLE 1

Glycerol release (nmol/1o mg dry weight) by β-lipotropin and melano tropin potentiating factor (MPF) from rabbit adipocytes. Determination in triplicates. Mean values ± SEM.

		plus MPF (mol/l)	
		$1o^{-5}$	$1o^{-6}$
Basal	2.8 ± o.5	2.o ± o.2	2.2 ± o.5
β-LPH (mol/l)			
$1o^{-7}$	3o.2 ± o.3	27.1 ± o.6	29.1 ± o.3
$1o^{-8}$	27.5 ± o.2	17.6 ± o.5	19.3 ± o.7
$1o^{-9}$	1.8 ± o.6	1.9 ± o.5	2.o ± o.4

At lower concentrations ($1o^{-7}$ to $1o^{-11}$ mol/l) MPF had no effect on lipolysis.

α- and β-MSH were tested under identical conditions in adipocytes from 2o animals. In no case an inhibition or stimulation of glycerol release caused by these peptides was observed after the addition of MPF in concentrations of $1o^{-5}$ to $1o^{-11}$ mol/l (table 2). Even at submaximal stimulation of glycerol release by α- and β-MSH the addition of MPF was ineffective.

TABLE 2

Glycerol release (nmol/1o mg dry weight) by α-MSH and β-MSH alone and in combination with melanocyte potentiating factor (MPF) from rabbit adipocytes. Determination in triplicates, mean values ± SEM.

		plus MPF (mol/l)	
		$1o^{-5}$	$1o^{-6}$
Basal	3.o ± o.2	2.7 ± o.1	2.6 ± o.3
α-MSH (mol/l)			
$1o^{-7}$	13.4 ± o.2	14.1 ± 1.2	13.9 ± o.3
$1o^{-8}$	13.4 ± 1.2	12.9 ± o.7	14.1 ± 1.9
$1o^{-9}$	8.1 ± 1.3	8.9 ± 1.4	8.4 ± o.2
β-MSH (mol/l)			
$1o^{-7}$	14.8 ± o.1	14.1 ± o.4	14.3 ± o.5
$1o^{-8}$	14.3 ± o.5	14.5 ± o.2	14.2 ± o.5
$1o^{-9}$	2.4 ± o.5	2.5 ± o.5	2.4 ± o.1

DISCUSSION

MPF, the C-terminal tetrapeptide of β-lipotropin, potentiates the melanotropic activity of the melanocyte stimulating hormones α-MSH, β-MSH and β-lipotropin (1). It was of special interest to investigate the influence of MPF on the lipolytic activity of these hormones because the message sequences for the lipolytic and melanotropic activity are identical. This so-called common heptapeptide is located in the residues 4-1o of α-MSH, 7-13 of β-MSH and 47-53 of β-LPH.

Though all the three peptides contain this message sequence, MPF only did affect the lipolytic effect of β-LPH but not of α-MSH and β-MSH. This suggests that the inhibitory effect of MPF on β-LPH stimulated lipolysis is not due to an interaction with the common heptapeptide.

However, β-LPH contains a second lipolytic sequence in its C-terminal portion (6) which might be inhibited by MPF. This is supported by the finding that the lipolytic activity of human β-endorphin (the 31 amino acid residues C-terminal part of β-LPH) could be blocked by the addition of MPF in a concentration of $1o^{-4}$ mol/l (glycerol release by β-endorphin $1o^{-5}$ mol/l: 6.2 ± o.6 nmol, after the addition of MPF $1o^{-4}$ mol/l: 3.4 ± o.6 nmol, unpublished data).

The importance of the C-terminal amino acid residue or of C-terminal amino acid sequences for the lipolytic activity has already been shown: partial sequences of β-LPH 59-89 (β-endorphin) without the last two amino acid residues are lipolytically inactive (6,7) while the complete sequence of β-endorphin stimulates glycerol release from rabbit adipocytes (6,8). Furthermore, the modification of the carboxyl group of β-LPH by glycine methyl ester and taurine resulted in a partial loss of the lipolytic activity (9). As the residues 86-89 (MPF) and 85-89 were not lipolytically active it is suggested that they interact with the receptor for β-LPH. The importance of the C-terminal part for binding to receptors has recently been shown for lymphocytes (1o)and human complement (11).

In conclusion, β-LPH contains two lipolytic message sequences and a further sequence which inhibits the lipolytic activity of this polypeptide. A similar effect has recently been shown for the analgesic effects of β-LPH 59-89 (β-endorphin) which could be inhibited by β-LPH 59-86 and β-LPH 59-85 while shorter amino acid sequences were without any effect (12). The lipolytic activity of α- and β-MSH was not affected by MPF although the message sequences for lipolysis and melanotropic activity were identical.

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REFERENCES

1. Carter,R.J., Shuster,S. and Morley,J.S. (1979). Melanotropin potentiating factor is the C-terminal tetrapeptide of human β-lipotropin. Nature 279: 74-75.

2. Richter,W.O. and Schwandt,P. (1985). Separation of neuropeptides by HPLC: Evaluation of different supports, with analytical and preparative applications to human and porcine neurophysins, β-lipotropin, adrenocorticotropic hormone, and β-endorphin. J.Neurochem. 44: 1697-17o3.

3. Rodbell,M. (1964). Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J.Biol.Chem. 239: 375-38o.

4. Chen,R.F. (1967). Removal of fatty acids from serum albumin by charcoal treatment. J.Biol.Chem. 242: 173-181.

5. Schwandt,P., Richter,W.O. and Kriegisch,N. (1981). A simple automated system for the continuous determination of glycerol. Clin.Chem. 27: 512-513.

6. Richter,W.O. and Schwandt,P. (1983). In vitro lipolysis of proopiocortin peptides. Life Sci. 33: 747-75o, Supl.I

8. Jean-Baptiste,E. and Rizack,M.A. (198o). In vitro cyclo AMP-mediated lipolytic activity of endorphins, enkephalin and naloxone. Life Sci. 27: 135-144.

9. Gilardeau,C., Chretien,M. and Lis,M. (1974). Lipotropic and adrenocorticotropic hormones.Biological activities of the carboxyl-modified hormones. Can.J.Physiol.Pharmacol. 52: 723-726.

1o. Hazum,E., Chang,K.J. and Cuatrecasas,P. (1979). Specific nonopiate receptors for β-endorphin. Science 2o5: 1o33-1o35.

11. Schweigerer,L., Teschemacher,H., Bhakdi,S. and Lederle,M. (1983). Interaction of human β-endorphin with nonopiate binding sites on the terminal SC5b-9 complex of human complement. J.Biol.Chem. 258: 12287-12292.

12. Nicolas,P. and Li,C.H. (1985). Inhibition of analgesia by C-terminal deletion analogs of human β-endorphin. Biochem. Biophys.Res.Comm. 127: 649-655.

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