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In Vitro Lipolytic Activity of β-Endorphin and Its Partial Sequences*

W. O. RICHTER, R. J. NAUDÉ, W. OELOFSEN, AND P. SCHWANDT

Department of Internal Medicine II, Klinikum Grosshadern, University of Munich, D-8000 Munich 70, Federal Republic of Germany; and Department of Biochemistry (R.J.N., W.O.), University of Port Elizabeth, Port Elizabeth 6000, Republic of South Africa

ABSTRACT. β-Endorphin stimulates glycerol release from adipose tissue *in vitro* in the rabbit. Thirty different amino acid sequences of this peptide were tested for lipolytic activity. Four turned out to be active: porcine and human β-endorphin-(1–31), human β-endorphin-(6–31), and human β-endorphin-(1–5)-(16–31). Structure-activity investigations showed that for the lipolytic action of β-endorphin the C-terminal part [longer than β-endorphin-(27–31)] is relatively important. Of special importance seems to be the C-terminal amino acid residue, because none of the sequences lacking the last two amino acid residues was lipolytically active. Furthermore, a different lipolytic response to β-endorphin was obtained in starved, *ad libitum*-fed, and starved-refed animals, showing that the regulation of the lipolytic potency is not only mediated by peptide concentrations in the medium. (*Endocrinology* **120:** 1472–1476, 1987)

OF 50 highly purified peptide hormones tested for lipolytic activity in rabbit adipocytes, only sequences derived from the precursor hormone proopiomelanocortin stimulated glycerol release from fat cells (1). One of these sequences was the C-terminal part of β-lipotropin, β-endorphin, which is commonly known as an endogenous opiate agonist. β-Endorphin is not only a stimulator of glycerol release from rabbit adipocytes *in vitro* (2, 3), but also *in vivo* after iv injection (4). Furthermore, there is evidence that β-endorphin may be involved in physiological adaption to fasting (5), and overeating of genetically obese (ob/ob) mice and rats is abolished by small doses of the opiate antagonist naloxone (6). Elevated β-endorphin levels were reported also in obese hirsute women (7) and in genetically obese rats (6). These findings suggest a role of β-endorphin in the regulation of adipose tissue metabolism either by a direct effect on adipose tissue or via mediators.

To obtain further information on the direct effect of β-endorphin on the adipocyte, we investigated 30 different amino acid sequences of β-endorphin as stimulators of lipolysis in isolated rabbit fat cells. Furthermore, investigations were performed with adipocytes from starved, *ad libitum*-fed and starved-refed rabbits.

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Address requests for reprints to: Dr. P. Schwandt, Department of Internal Medicine II, Klinikum Grosshadern, University of Munich, Marchionistrasse 15, Munich 70, D-8000 West Germany.

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Materials and Methods

Synthetic human β-endorphin-(1 - 31),-(1 - 27),-(1 - 26), and-(1 - 5)-(16 - 31) were purchased from Peninsula (San Carlos, CA), β-endorphin-(1 - 17) from Bachem (Bubendorf, Switzerland), β-endorphin-(1 - 16) and D-met^2-pro^5-enkephalinamide from Serva (Heidelberg, FRG), and leu-enkephalin from Sigma (Munich, FRG). Synthetic porcine β-endorphin was obtained from Serva (Heidelberg, FRG) and also extracted and purified from porcine pituitary glands as described previously (3). β-Endorphin-(1–5) and (2–5) were a gift of Ciba-Geigy (Basel, Switzerland). β-Endorphin D-ala^2-leu^5-(1–5)-(28–29), -(25–29), -(21–29), -(16–29), and -(10–29), as well as human β-endorphin-(6–31), -(6–29), -(27–31), -(28–31), and porcine β-endorphin-(28–31) were synthesized by the Peptide Group of ICI, Pharmaceutical Division (Cheshire, UK) and were a gift of Dr. J. S. Morley. β-Endorphin-(2–4), -(15–18), and the ostrich β-endorphin sequences-(5–14), -(10–11), -(12–19), -(19–24), -(20–24), and -(25–28) were obtained by tryptic and chymotryptic digestion of ostrich β-lipotropin, as described previously (8).

Perirenal adipose tissue was obtained from female rabbits (body weight between 3 and 6.5 kg) either fed *ad libitum*, starved for 12 or 72 h, or starved for 48 h and refed for 24 h. All rabbits were fed a commercially available diet (altromin 2023, containing 17% protein and 3.75% fat). For the investigations in different nutritional states, animals with identical body weight and age (6–7 months) were chosen. Isolated fat cells were prepared according to Rodbell (9) by digestion of adipose tissue by collagenases which have been shown to be essentially free of contaminating unspecific proteases (collagenase CLSPA,

TABLE 1. Amino acid sequences of β-endorphin and partial sequences tested for lipolytic activity in rabbit adipocytes

	1 10 20 30
1	Tyr-Gly-Gly-Phe- Met-Thr-Ser-Glu-Lys- Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys- Asn-Ala-Ile-Ile-Lys- Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH
2	Tyr-Gly-Gly-Phe- Met-Thr-Ser-Glu-Lys- Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys- Asn-Ala-Ile-Val-Lys- Asn-Ala-His- Lys-Lys-Gly-Gln-OH
3	Tyr-Gly-Gly-Phe- Met-Thr-Ser-Glu-Lys- Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys- Asn-Ala-Ile-Ile-Lys- Asn-Ala-Tyr-OH
4	Tyr-Gly-Gly-Phe- Met-Thr-Ser-Glu-Lys- Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys- Asn-Ala-Ile-Ile-Lys- Asn-Ala-OH
5	Tyr-Gly-Gly-Phe- Met——————————————————Thr-Leu-Phe-Lys- Asn-Ala-Ile-Ile-Lys- Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH
6	Tyr-Ala-Gly-Phe- Pro——————————————————————————————————Lys-Lys-OH
7	Tyr-Ala-Gly-Phe- Pro——————————————————————————————Asn-Ala-Tyr-Lys-Lys-OH
8	Tyr-Ala-Gly-Phe- Pro——————————————————————Ala-Ile-Ile-Lys- Asn-Ala-Tyr-Lys-Lys-OH
9	Tyr-Ala-Gly-Phe- Pro——————————————Thr-Leu-Phe-Lys- Asn-Ala-Ile-Ile-Lys- Asn-Ala-Tyr-Lys-Lys-OH
10	Tyr-Ala-Gly-Phe- Pro——————Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys- Asn-Ala-Ile-Ile-Lys- Asn-Ala-Tyr-Lys-Lys-OH
11	Tyr-Gly-Gly-Phe- Met-Thr-Ser-Glu-Lys- Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH
12	Tyr-Gly-Gly-Phe- Met-Thr-Ser-Glu-Lys- Ser-Gln-Thr-Pro-Leu-Val-Thr-OH
13	Tyr-Gly-Gly-Phe- Met-Ser-Ser-Glu-Arg- OH
14	Tyr-Gly-Gly-Phe- Met-OH
15	Tyr-Gly-Gly-Phe- Leu- OH
16	Tyr-Met-Gly-Phe-Pro- amide
17	Gly-Gly-Phe- Met-OH
18	Gly-Gly-Phe- OH
19	Met-Ser-Ser-Glu-Arg- Gly-Arg-Ala-Pro-Leu- OH
20	Thr-Ser-Glu-Lys- Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys- Asn-Ala-Ile-Ile-Lys- Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH
21	Thr-Ser-Glu-Lys- Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys- Asn-Ala-Ile-Ile-Lys- Asn-Ala-Tyr-Lys-Lys-OH
22	Gly-Arg-OH
23	Ala-Pro-Leu- Val-Thr-Leu-Phe-Lys- OH
24	Val-Thr-Leu-Phe-OH
25	Lys- Asn-Ala-Ile-Val-Lys-OH
26	Asn-Ala-Ile-Val-Lys-OH
27	Ser-Ala- Tyr-Lys-OH
28	Tyr-Lys-Lys-Gly-Glu-OH
29	Lys-Lys-Gly-Glu-OH
30	Lys-Lys-Gly-Gln-OH

Underlined amino acid residues are different from the human sequence.

TABLE 2. Lipolysis by β-endorphin and partial sequences in rabbit adipocytes

		n	Active	Inactive
1	β-Endorphin-(1–31)	18	×	
2	p-β-Endorphin-(1–31)	10	×	
3	β-Endorphin-(1–27)	6		×
4	β-Endorphin-(1–26)	6		×
5	β-Endorphin-(1–5)-(16–31)	6	×	
6	β-Endorphin D-ala^2-leu^5-(1–5)-(28–29)	4		×
7	-(25–29)	4		×
8	-(21–29)	4		×
9	-(16–29)	4		×
10	-(10–29)	4		×
11	β-Endorphin-(1–17)	10		×
12	β-Endorphin-(1–16)	14		×
13	os-β-Endorphin-(1–9)	4		×
14	β-Endorphin-(1–5) = met-enkephalin	15		×
15	leu-enkephalin	15		×
16	D-Met^2-pro^5-enkephalinamide	10		×
17	β-Endorphin-(2–5)	10		×
18	β-Endorphin-(2–4)	4		×
19	os-β-Endorphin-(5–14)	4		×
20	β-Endorphin-(6–31)	6	×	
21	β-Endorphin-(6–29)	6		×
22	os-β-Endorphin-(10–11)	4		×
23	os-β-Endorphin-(12–19)	4		×
24	β-Endorphin-(15–18)	4		×
25	os/p-β-Endorphin-(19–24)	4		×
26	os/p-β-Endorphin-(20–24)	4		×
27	os-β-Endorphin-(25–28)	4		×
28	β-Endorphin-(27–31)	12		×
29	β-Endorphin-(28–31)	20		×
30	p-β-Endorphin-(28–31)	20		×

Tested concentration 10^{-6} M. Amino acid sequences different from human β-endorphin are marked with p (porcine) or os (ostrich). n, Number of animals. Activity is defined as stimulation of glycerol release above basal lipolysis in all animals (see Table 3).

Worthington, Seromed, Munich, FRG). This proteolytic activity could lead to a rapid unspecific degradation of peptides. For the lipolytic assay about 70,000 adipocytes (cell number determined in a Fuchs-Rosenthal chamber) were incubated in 1 ml Krebs-Ringer-bicarbonate buffer (pH 7.40) containing 4% defatted BSA (fraction V) and 3 mM glucose, at 37 C for 1 h under continuous shaking. The peptides were added in concentrations of 10^{-6} to 10^{-9} M. Each concentration was tested in triplicate, with cells prepared from a specific animal. The viability of the adipocytes was evaluated by microscopical appearance and by their ability to respond to β-lipotropin. The cell diameter was determined microscopically, and the mean value ± SD of 50 determinations is reported. The incubation was terminated by ice-cooling and centrifugation at 12,000 × *g* for 4 min. The resulting infranatant was deep-frozen at −35 C for determination of glycerol. Glycerol was measured by an enzymatic automated procedure described recently (10).

Results

Thirty amino acid sequences related to β-endorphin were tested for their lipolytic activity in isolated rabbit fat cells (Table 1). Four of the fractions turned out to possess the ability to stimulate glycerol release from the adipocytes: human and porcine β-endorphin, human β-endorphin-(1–5)-(16–31), and human β-endorphin-(6–31) (Table 2). The minimal effective concentration was lowest for porcine β-endorphin (10^{-8} M; in some assays 10^{-9} M), followed by human β-endorphin (10^{-7} M; in some assays 10^{-8} M). β-Endorphin-(6–31) and β-endorphin-(1–5)-(16–31) were active in all animals at a concentration of 10^{-6} M, in three cases also at 10^{-7} M (Table 3). The order of potency was porcine β-endorphin > human β-endorphin > human β-endorphin-(6–31) > human β-endorphin-(1–5)-(16–31) at 10^{-6} and 10^{-7} M (Table 3). The lipolytic activity of human β-endorphin was dependent upon the nutritional status of the rabbit. A starvation period of 72 h led to a diminished responsiveness of the adipocytes to β-endorphin: glycerol release in adipocytes of *ad libitum*-fed animals was significantly higher at β-endorphin concentrations of 10^{-6} and 10^{-7} M; there was also a significant difference in basal lipolysis (Table 4). An even stronger lipolytic response to β-endorphin was obtained with fat cells from 48-h starved-24-h refed animals (statistically significant at β-endorphin concentrations of 10^{-6} and 10^{-7} M); basal lipolysis was significantly higher than in adipocytes of *ad libitum*-fed animals.

Discussion

Human as well as porcine β-endorphin stimulated glycerol release from rabbit adipocytes in a dose-depend-

TABLE 3. Lipolytic response to human and porcine β-endorphin, human β-endorphin-(1–5)-(16–31) and -(6–31) in adipocytes from 12-h starved rabbits (body weight 3.5–4.0 kg)

	Basal lipolysis	10^{-6} M	10^{-7} M	10^{-8} M
β-Endorphin-(1–31)	2.9 ± 0.3	13.9 ± 1.3[a]	4.6 ± 0.2[a]	3.0 ± 0.4
p-β-Endorphin-(1–31)	4.2 ± 0.7	18.1 ± 0.3[a]	10.4 ± 1.4[a]	5.8 ± 0.5[a]
β-Endorphin-(1–5)-(16–31)	3.4 ± 0.5	6.2 ± 0.6[a]	4.0 ± 0.6	3.3 ± 0.7
β-Endorphin-(6–31)	2.9 ± 0.4	7.1 ± 0.9[a]	4.3 ± 0.8	2.9 ± 0.5
p-β-Lipotropin	3.3 ± 0.4	20.2 ± 1.8[a]	16.6 ± 1.9[a]	6.3 ± 0.9[a]

Glycerol release in nanomoles per 10 mg dry weight of the cells. Mean values ± SEM of the results in six rabbits.
[a] $P \leq 0.05$, Wilcoxon matched pair signed rank statistic, in comparison to basal lipolysis in respect to number of tested animals.

TABLE 4. Lipolytic response to human β-endorphin in adipocytes from *ad libitum*-fed, 72-h starved, and 48-h starved/24-h refed rabbits

β-Endorphin	72-h Starved	*Ad libitum*-fed	48-h Starved/ 24-h refed	
	n = 7	n = 7	n = 5	
	3.68 ± 1.25 kg	3.84 ± 1.32 kg	3.52 ± 1.27 kg	body weight
	44.4 ± 11.5 μm	59.7 ± 3.9 μm	61.4 ± 11.9 μm	cell diameter
10^{-6} M	9.7 ± 1.7	17.3 ± 1.5[a]	26.9 ± 5.7[b]	
10^{-7} M	5.2 ± 0.9	8.2 ± 1.1[c]	11.7 ± 2.2[b]	
10^{-8} M	3.3 ± 0.5	6.5 ± 1.1[d]	9.2 ± 1.1[e]	
Basal lipolysis	3.2 ± 0.6	6.6 ± 1.1[d]	9.0 ± 1.3[e]	

Glycerol release in nanomoles per 10 mg dry weight of the cells. Mean values ± SEM.
[a] $P \leq 0.0001$, Student's t test, in comparison to 72-h starved rabbits.
[b] $P \leq 0.01$, Student's t test, in comparison to *ad libitum*-fed rabbits.
[c] $P \leq 0.001$, Student's t test, in comparison to 72-h starved rabbits.
[d] $P \leq 0.05$, Student's t test, in comparison to 72-h starved rabbits.
[e] $P \leq 0.001$, Student's t test, in comparison to *ad libitum*-fed rabbits.

TABLE 5. Inhibition of the lipolytic activity of β-endorphin by its C-terminal tetrapeptide [β-endorphin-(28–31)]

β-Endorphin		+β-Endorphin-(28–31) (10^{-4} M)
10^{-6} M	6.1 ± 0.7	3.4 ± 0.7[a]
10^{-7} M	1.9 ± 0.4	0.7 ± 0.3[a]

Glycerol release above basal lipolysis in nanomoles per 10 mg dry weight of cells. Mean values ± SEM. Twelve determinations with adipocytes from four animals. (3.52 ± 0.5 kg body weight, fed *ad libitum*).
[a] $P \leq 0.01$, Wilcoxon matched pair signed rank statistic.

ent manner, but the lipolytic potency of the porcine sequence, expressed on a molar basis, was greater than that of human β-endorphin. Porcine β-endorphin differs from the human sequence in three amino acid residues: valine instead of isoleucine at position 23, histidine instead of tyrosine at position 27, and glutamine instead of glutamic acid at position 31, the C-terminal amino acid residue. These amino acid differences are responsible for the lower lipolytic potency of human β-endorphin, which is reflected not only in the decreased lipolytic potency but in the lower minimal effective concentration (10^{-7} to 10^{-8} M).

Of the other 28 amino acid sequences of β-endorphin tested, lipolytic activity could only be shown for two further sequences, β-endorphin-(6–31) and β-endorphin-(1–5)-(16–31). Both peptides had a lower lipolytic potency on a molar basis than the complete β-endorphin sequence, but the minimal effective concentrations were identical. A characteristic feature of all four sequences capable of stimulating lipolysis is the complete C-terminus. Omission of the last two amino acid residues, as in β-endorphin-(6–29), or the last four and five amino acid residues, as in β-endorphin-(1–27) and β-endorphin-(1–26), respectively, resulted in a total loss of lipolytic activity. The N-terminal amino acid sequence appears to be less important for stimulation of lipolysis, as could be seen for β-endorphin-(6–31). N-Terminal amino acid sequences from β-endorphin-(1–5) (met-enkephalin) up to β-endorphin-(1–17) (γ-endorphin) were without any lipolytic effect, which was also true for the modified peptides leu-enkephalin, D-met^2-pro^5-enkephalinamide and the ostrich sequence β-endorphin-(1–9). As β-endorphin-(1–5)-(16–31) was lipolytically active, but β-endorphin-(1–5) and β-endorphin D-ala^2-leu^5-(1–5)-(16–29) were ineffective, it can be concluded that for the lipolytic action of β-endorphin the amino acid sequence 16–31 is important. From this sequence region several portions were tested: β-endorphin-(15–18), -(19–24), -(20–24), -(27–31), and -(28–31), but none of these displayed lipolytic activity.

From these data it can be concluded that the minimum amino acid sequence needed for mediating the lipolytic activity is longer than β-endorphin-(27–31), with special emphasis on residues 30 and 31. A role for the C-terminal amino acid residues in binding to the fat cell receptor can be envisaged and has recently been shown for the binding to human complement (11) or to lymphocytes (12). This is also supported by our finding that preincubation of adipocytes with β-endorphin-(28–31) led to an inhibition of the lipolytic activity of β-endorphin. Moreover, β-endorphin-(28–31) was able to inhibit the lipolytic activity of β-lipotropin (β-endorphin is its C-terminal amino acid sequence) but not of other strong stimulators of lipolysis such as α-MSH and β-MSH [β-lipotropin-(41–58)] (13).

The investigations with adipocytes from rabbits starved for 72 h, fed *ad libitum*, or starved for 48 h and refed for 24 h showed a greatly reduced lipolytic activity in starved animals, whereas the strongest response was observed in starved-refed rabbits. The difference between the three groups remained evident also when the lipolytic

response was correlated with the adipocyte number, because the dry weights of the adipocytes differed by no more than 15%. These data show that in rabbit adipose tissue lipolysis by β-endorphin is dependent upon the nutritional status of the animal. This is also true for other peptide hormones such as β-lipotropin or α-MSH (14).

In conclusion, the lipolytic activity of β-endorphin appears to be mediated by its C-terminal part. Furthermore, the lipolytic response to this peptide is dependent on the nutritional status, showing that its lipolytic activity is regulated not only by the extracellular peptide concentration.

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