

# Influence of amphipathic peptides on the HIV-1 production in persistently infected T lymphoma cells

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The effects of several amphipathic peptides on HIV-1 production in persistently infected cells are described. Melittin, a 26 amino acid  $\alpha$ -helical amphipathic peptide, reduces HIV-1 production dose-dependently, whereas other amphipathic peptides do not. Six melittin derivatives which retain the  $\alpha$ -helical portion have similar effects as melittin. The reduction of viral infectivity is not due to an effect of melittin on the virus particles but to an intracellular action of the peptide, which is readily taken up into cells, as shown by quantitative ELISA. Western blots of cells from melittin-treated cultures suggest that the processing of the gag/pol precursor is impaired.

Amphipathic peptide;  $\alpha$ -Helix; Melittin; HIV-1; Assembly

## 1. INTRODUCTION

In controlling the development of the acquired immunodeficiency syndrome (AIDS) in persons infected with the human immunodeficiency virus (HIV), it has become a major aim to interfere with the production of infectious HIV particles in the infected cells. One possible strategy is to impair the budding and/or the assembly of the virus particle, steps that involve interactions of viral proteins with the cellular membrane: the N-terminus of the gag precursor appears to be responsible for its membrane association [1], and the myristoylation of the glycin at position 2 from the N-terminus is thought to play a major role in membrane anchoring [2]. However, other mechanisms of targeting the gag precursor to the cell membrane have also been discussed such as electrostatic interaction between gag and gp41 [3] and formation of a coiled coil of the p17 gag with the intracellular portion of gp41 [4]. Because of this wide range of observed and postulated interactions between HIV proteins and the cellular membrane we investigated whether substances with known membrane affinity, such as amphipathic peptides, were able to reduce virus production.

Because of its known amphiphilicity, which is combined with an  $\alpha$ -helical conformation [5,6], and its membrane affinity [7,8], melittin was an interesting candidate for testing the effect of a membrane-active substance on HIV production. Furthermore, it has been

postulated that melittin exhibits structural homology to the transmembrane part of the HIV glycoprotein gp41 [9]. Thus it seemed possible that melittin might hinder the formation of infectious HIV particles by affecting the correct formation of the viral envelope.

HIV replication has marked influences on the metabolism of the cell membrane and of second messengers [10–14] and melittin in turn was shown to interfere with second messenger systems, possibly due to its high affinity for calmodulin and several calmodulin-binding proteins [7,15,16]. So there were three points — similarity to gp41, membrane interaction and interference with second messenger metabolism — which made it relevant to test the influence of melittin on HIV production.

In this paper, we show that the amphipathic  $\alpha$ -helix of melittin is able to reduce HIV-1 production in persistently infected KE37/1-III<sub>0</sub> cells and present data suggesting that this is achieved by interference of the melittin with the processing of the gag/pol precursor protein.

## 2. MATERIALS AND METHODS

### 2.1. Cells and virus

Human T lymphoma cells, uninfected (KE37/1) and permanently infected with the HIV-1 isolate HIV-III<sub>0</sub> (KE37/1-III<sub>0</sub>) [17] were obtained from M. Popovic (The Laboratory of Tumor Cell Biology, NCI, NIH, Bethesda, MD, USA). Human fibroblastoid cells (LC5) susceptible to HIV infection were derived from human embryonic lung [18]. C8166 T lymphoma cells [19,20] were used for HIV-1 titration. The cells were cultivated in RPMI 1640 Medium (Gibco) supplemented with NaHCO<sub>3</sub> (2.2 g/l), FCS (10%) and, in the case of microtitre cultures, with antibiotics (100 U penicillin, 100  $\mu$ g streptomycin, and 0.25  $\mu$ g Fungizone (Amphotericin B, Gibco) per ml). Cells were grown at 37°C in a humid atmosphere of 5% CO<sub>2</sub>.

### 2.2. Synthesis and purification of peptides

The peptides were synthesized on the resin Ultrosyn C using the

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Fmoc strategy and a linker releasing the peptides as amides. Synthesis was carried out on an automatic peptide synthesizer (model Biolyne, Novabiochrome, UK) according to the instructions of the manufacturer. The peptides were purified on a TSK 120T reverse-phase column  $7.5 \times 300$  mm (Pharmacia Biosystem *s/s*, Denmark), and the amino acid content was verified by mass spectrometry. All peptides used were more than 95% pure.

### 2.3. Treatment of T lymphoma cell cultures with peptides

Cells were cultivated in microtitre plates. On day 0 washed cells were seeded by adding 10  $\mu$ l of a cell suspension ( $10^5$  cells/ml) to cell-free medium (90  $\mu$ l) containing the drug in the appropriate concentrations. The final culture volume was designed to be 100  $\mu$ l. If the cultures were to be treated repeatedly, the final culture volume of 100  $\mu$ l was achieved with the last treatment on day 4 or 7 after start of cultivation. Repeated treatment was performed by feeding the cultures every 24 h with 10  $\mu$ l fresh medium, where the added medium contained the substance in a concentration which compensated for the growing culture volume. The substances were applied in concentrations between 0.5 and 30  $\mu$ g/ml depending on their toxicity. The corresponding molarities, assuming that the substances are completely in free solution, are given in parentheses at the proper places.

For the determination of cell growth and viability (on day 4 or 7 after start of cultivation), the MTT test was carried out in the microtitre cultures according to Mosmann [21]. The test was slightly modified in the following way: 10  $\mu$ l of an MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolimbromide) solution (5 mg/ml MTT dissolved in PBS, and sterilized by filtration) were added to every well. The cells were incubated with the MTT for 4 h at 37°C in a 5% CO<sub>2</sub> atmosphere to allow MTT metabolism. The reaction was stopped by adding 180  $\mu$ l of 0.04 N HCl in isopropanol to every well and the dye was extracted. The resulting solutions were vigorously mixed in order to dissolve all formazan crystals. The optical density (OD) of the solution was determined at  $\lambda = 600$  nm and corrected for blank OD values (cell-free culture medium plus MTT and isopropanol).

### 2.4. Determination of HIV replication

The production of infectious HIV in treated infected cells was analysed in parallel to the cell growth determination. Microtitre cultures of HIV-infected cells were grown as described above. On days 4 or 7, the microtitre cultures were pooled in duplicates (200  $\mu$ l, 4-fold) and the plates were centrifuged (10 min, 3,000 rpm). The cell-free supernatants were added to uninfected LC5 cells (100  $\mu$ l to 3,000 cells/well in 96 well plates, 4 wells per drug concentration). The supernatants were removed from the LC5 cells after 1 h incubation at 37°C and replaced by fresh culture medium. After four days the infected cells in the LC5 cultures were determined by the indirect immunoperoxidase staining method (IPS) [22]. The colonies of HIV positive cells were counted as infectious centres. The 4 different counts per drug dose were averaged and related to the OD value of the corresponding lymphoma cell culture as obtained by the MTT test. The result obtained is the ratio of infectious HIV particles per viable, treated T lymphoma cell.

For the determination of reverse transcriptase activity in the supernatants of T lymphoma cultures, six 1 ml cultures were pooled and centrifuged (10 min, 3,000 rpm). Half the volume of a 30% PEG 6000 solution was added and protein was precipitated overnight at 4°C. The precipitated protein was collected by centrifugation (20 min, 3,000 rpm, 4°C) and tested for reverse transcriptase activity as described elsewhere [23]. The reverse transcriptase activity (RT) value was calculated in cpm/ml supernatant and related to the OD value as obtained by the MTT test of the corresponding T lymphoma cell culture.

The quantitative HIV p24 antigen capture ELISA was obtained from DuPont and performed according to the manufacturer's instructions. The p24 amounts in tested supernatants were normalized to the cell number in the culture.

### 2.5. ELISA for quantitative determination of melittin

Melittin breakdown in cell cultures (LC5 cells) was measured by an ELISA. The medium and the cells were assayed separately. Briefly, the

medium was removed from the cells for estimation of free melittin and an equal volume of medium was added to the remaining cells including 2% Triton X-100. The cells were scraped off in this buffer, left for 1 h at 0°C, and frozen. Triton X-100 was also added to the culture medium before this was frozen. All subsequent incubations with samples were carried out at 0°C.

The ELISA was a competition assay using a solid phase of melittin coupled N-terminally to albumin. Melittin was coupled to albumin (RIA grade, Sigma) using 2.5% glutaraldehyde in a borate buffer (50 mM, pH 8.5) for 24 h followed by dialysis against the ELISA coupling buffer. This antigen preparation was used as the first layer in the ELISA (10  $\mu$ g/well).

The ELISA plates were coated overnight at 4°C with the first layer, followed by blocking and washing with 0.1% albumin, 1% Tween 20, 50 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.5 (washing buffer).

The second layer consisted of a polyclonal antibody (dilution 1:1,000) to the amide end of melittin (ALK, Copenhagen), and the sample or standards of melittin (samples and blanks contained 2% Triton X-100). The buffer was 1% Tween 20, 50 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.5. The incubation was carried out overnight at 4°C. Then the wells were washed 3 times with the washing buffer.

The third layer was an anti-rabbit antibody coupled to peroxidase (Dako, Copenhagen) and incubation was 1 h. The peroxidase reaction was carried out for 10 min by addition of substrate (0.2 mg/ml *ortho*-phenylene diamine, 0.1% hydrogen peroxide). The reaction was terminated by addition of 100  $\mu$ l 100 mM H<sub>2</sub>SO<sub>4</sub> and resulted in an absorption of 0.1–1.2 units.

### 2.6. Analysis of the virolytic effect of melittin-containing culture medium

Aliquots of RPMI 1640 medium containing melittin in concentrations of 0, 4 or 10  $\mu$ g/ml were prepared and kept at 37°C for various times. In a parallel experiment, uninfected KE37/1 T lymphoma cells (about  $10^5$ /ml) were added to the melittin-containing- and the melittin-free medium.

At time 0, 3, 16, 24 and 40 h, all incubated medium/melittin samples were made cell free and added to fresh HIV preparations (supernatant of KE37/1-III<sub>2</sub> cells, concentrated by ultracentrifugation at 120,000  $\times$  g, 90 min over a 20% w/w sucrose cushion). The virus was resuspended and incubated for 1 h at 37°C. Then the virus was titrated on C8166 cells in 96-well microtitre plates ( $4 \times 10^5$  cells/100  $\mu$ l, duplicate per dilution), either immediately or following another ultracentrifugation which was designed to remove non-virus-bound melittin from the samples. Syncytia formation was estimated 4–6 days after titration; syncytia were checked for HIV proteins by indirect immunoperoxidase staining [22], and only wells with HIV-positive syncytia were considered positive. The titre is given as the negative logarithm of the highest dilution rendering HIV-positive syncytia, titre reduction is given as the difference between titres of melittin-free and melittin-containing medium samples.

### 2.7. Western blotting

SDS-PAGE and Western blotting was carried out according to standard protocols, using a low-salt extraction buffer and ammonium sulfate precipitation for the preparation of cell extracts. Equal amounts of protein were loaded onto lanes of 12% polyacrylamide gels. The blots were stained with a human serum positive for HIV and detected with a second antibody coupled to alkaline phosphatase (reaction substrate: NBT/BCIP).

## 3. RESULTS AND DISCUSSION

The effect of melittin treatment of human KE37/1 T lymphoma cells persistently infected with HIV-1 was tested in doses of 0.5–5  $\mu$ g/ml. Melittin had no effect on the growth of uninfected and HIV-1 infected T lymphoma cells in concentrations from 0.5 to 2.5  $\mu$ g/ml

Table I

HIV infectivity (% HIV production) of supernatants following melittin treatment of the cultures (KE37/1-III<sub>8</sub> cells; daily melittin treatment); comparison of several experiments

	Melittin concentration ( $\mu\text{g/ml}$ )						
	0	0.5	1.5	2.5	3	4.5	5
Exp. 1	100		50		20	tox.	
Exp. 2	100	80		10			tox.
Exp. 3	100			10			tox.
Exp. 4	100	60		5			tox.

(0.2–0.9  $\mu\text{M}$ ; repeated application), but was growth inhibiting at 5  $\mu\text{g/ml}$  (1.8  $\mu\text{M}$ ) after 4 doses within 4 days. There was no difference in the growth inhibition of infected or uninfected cell lines (data not shown). Analysis of the supernatants of the treated lymphoma cultures for infectious HIV showed already an almost total absence of infectious virus particles in the cultures treated with 2.5  $\mu\text{g/ml}$  (0.9  $\mu\text{M}$ ) and a 20–40% reduction at 0.5  $\mu\text{g/ml}$  (0.2  $\mu\text{M}$ ), as seen in several independent experiments (Table I).

In order to test how long the described effect of the melittin can be measured after melittin application, we grew lymphoma cultures which were only treated once with melittin and analyzed on day 7. In these cultures, melittin had no cytotoxic effect on both uninfected and infected cells up to 8  $\mu\text{g/ml}$  (2.8  $\mu\text{M}$ ). Virus production (related to the corresponding cell number) at this concentration was 10% of the untreated infected cells (Fig.

% virus production/cell number

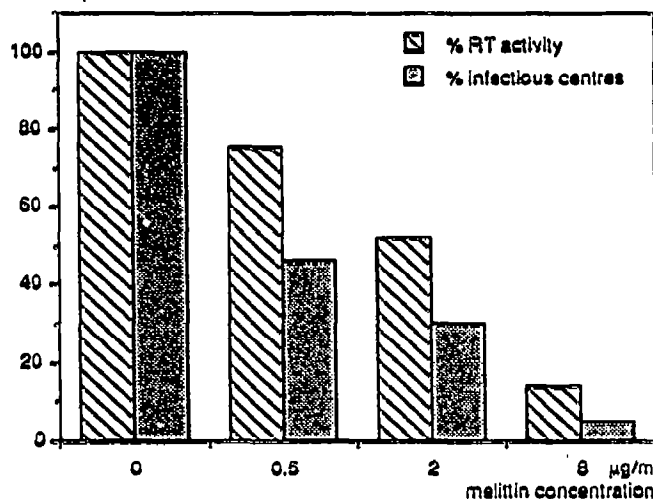


Fig. 1. Infectious HIV production in permanently HIV-1 infected lymphoma cultures which had undergone *single* melittin treatment (7 days before analysis). The culture supernatants were checked for infectious centres and for reverse transcriptase activity (see Materials and Methods). Infectious virus (stippled bars) and reverse transcriptase activity (hatched bars) are given in % of the values obtained with the supernatants from untreated, infected lymphoma control cultures.

% (p24/OD)

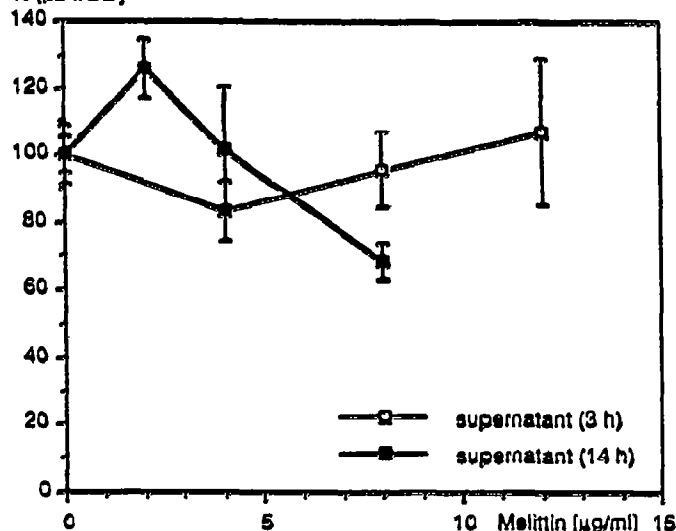


Fig. 2. Quantitative determination of p24 in culture supernatants of KE37/1-III<sub>8</sub> following melittin treatment in several concentrations and at different times after application of melittin. The values are normalized to the cell number and given in % ( $\pm$  S.D.) of the value of the untreated control. Melittin does not disturb the quantification of p24 by ELISA (data not shown).

1). Reverse transcriptase activity in the supernatants of melittin-treated cells declined in parallel with the decrease of production of infectious virus (Fig. 1).

The reduction of HIV production by melittin was further confirmed by quantitative measurement of HIV p24 in the supernatants of melittin-treated cultures. 3 h after melittin application there was no reduction of p24 in the culture supernatant even at 12  $\mu\text{g/ml}$  (4.2  $\mu\text{M}$ ), a concentration which is lytic to the cells in prolonged incubation. After 14 h, there is a marked reduction (70%

Table II

Log titre reduction of HIV at various melittin concentrations

Pre-incubation time (h)		Melittin concentration	
		4 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$
0	-	1	2
	+	n.d.	n.d.
3	-	0.5	1.5
	+	0	1
16	-	0	0
	+	0	1
24	-	0	0.5
	+	0	0
40	-	n.d.	n.d.
	+	0	0

Titre reduction represents titre differences of treated and mock-treated virus preparations.

-, pre-incubation in the absence of metabolizing KE37/1 cells

+, pre-incubation in the presence of metabolizing KE37/1 cells

n.d., not determined

of the value in untreated cultures) following single treatment with 8  $\mu\text{g/ml}$  (2.8  $\mu\text{M}$ ) (Fig. 2).

Since melittin has been reported to be lytic to retroviruses [24] and in higher concentrations is used to extract reverse transcriptase from virus preparations [25], we were interested whether the described reduction of HIV production in melittin-treated cultures could be accounted for by a direct effect of the melittin on the virus. This question was addressed by a set of experiments determining the direct effect of melittin on HIV infectivity. As shown in Table II, there was no titre reduction when melittin (4  $\mu\text{g/ml}$ ) was kept at 37°C in the presence of metabolizing KE37/1 cells for 3 h or more, but a slight titre reduction at 3 h when the melittin was kept in the absence of metabolizing cells. The same experiment done with a cytotoxic melittin concentration of 10  $\mu\text{g/ml}$  showed an enhanced titre reduction when compared to the non-cytotoxic concentration (4  $\mu\text{g/ml}$ ). There was some difference between melittin kept in the presence or in the absence of metabolizing T lymphoma cells. After 40 h of pre-incubation no virolytic activity was left at all.

To find out whether melittin is actually removed from the culture medium by the cells, we analyzed the presence of melittin in LC5 fibroblast cultures by quantitative ELISA. Fig. 3 shows that almost all melittin is removed from the medium within 2 h and found in the cell extract, whereas there is a quite long melittin half-life within the cells (about 24 h). This circumstance reflects our finding that the melittin concentration tolerated by cells is lower under daily treatment than single treatment (see Table I and Fig. 2 for a comparison) because a half-life of 24 h combined with total melittin

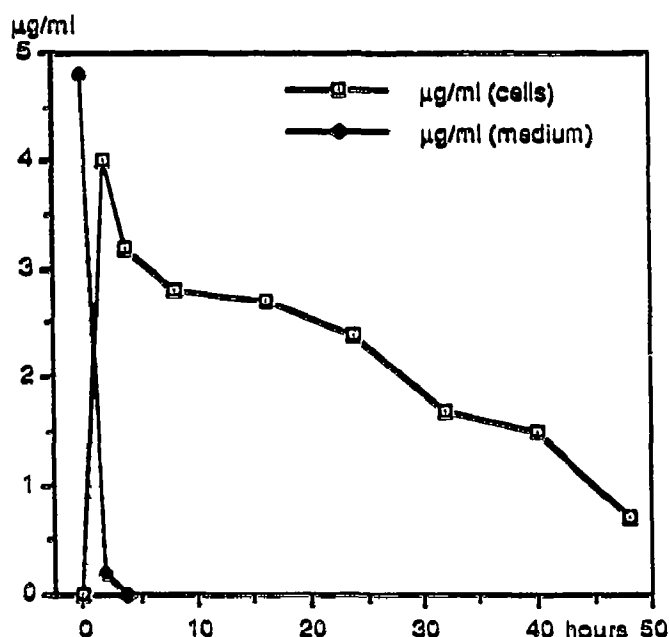
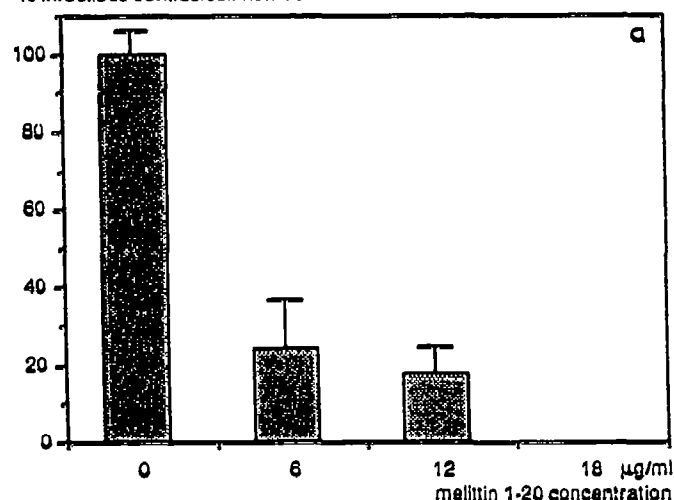


Fig. 3. Time dependency of the disappearance of melittin from the medium (filled symbols) and the uptake into and the disappearance from the cells (open symbols) after addition of 5  $\mu\text{g/ml}$  of melittin at  $t = 0$  (measured by competition ELISA).

uptake allows an accumulation of melittin under daily treatment. The long half-life of melittin inside the cells could also account for the long-lasting effect on HIV production.

Since the melittin is so rapidly removed from the culture medium (Fig. 3) and since there is no HIV titre reduction by melittin even at 10  $\mu\text{g/ml}$  when kept in the

% infectious centres/cell number



% infectious centres/cell number

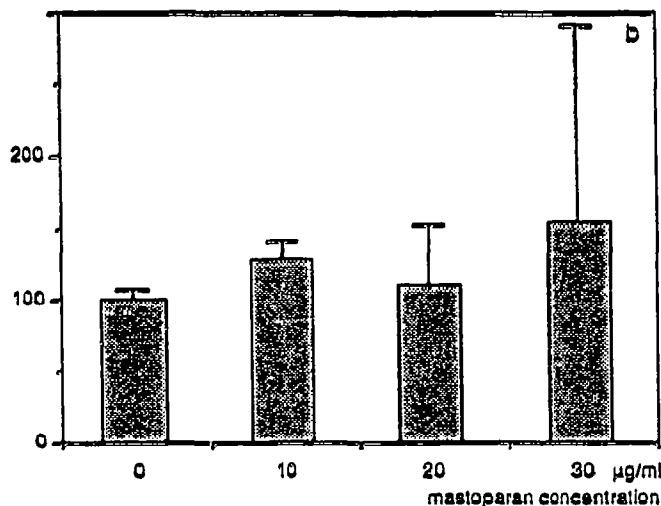


Fig. 4. Infectious HIV production in HIV-1 infected lymphoma cultures treated (a) with melittin 1-20 (amphipathic helix of the melittin without the carboxy-terminal basic hexapeptide) and (b) mastoparan. Dosage was repeated every 24 h for 5 days. Determination of the infectious centres was done as described in Materials and Methods. Infectious centres (about 100 were obtained with supernatant from untreated, infected lymphoma cultures) were counted, averaged and related to the cell densities obtained from the MTT test. This ratio of infectious centres to virus-producing cells is given in % ( $\pm$  S.D.) of the value obtained with supernatants from untreated infected lymphoma control cultures.

presence of cells for 24 h or more (Table II), the virolytic ability of melittin cannot account for the reduction of infectious virus particles found in cultures of chronically HIV infected KE37/1 cells when treated with melittin (Table I and Fig. 1), where analysis for infectious virus was performed between 24 h and 7 days after the last treatment with melittin.

We therefore supposed an intracellular rather than a membranolytic effect to be responsible for the described observations. Since the membranolytic effect is mediated by the basic carboxy-terminal hexapeptide at the tail of the melittin [26,27], we tested a melittin derivative consisting only of the amino-terminal helix-forming 20 amino acids (designated 'melittin 1-20'). This substance was less toxic than melittin as it is tolerated by the lymphoma cells up to 12  $\mu\text{g/ml}$  (5.9  $\mu\text{M}$ ) after repeated treatment. 18  $\mu\text{g/ml}$  of melittin 1-20 (8.9  $\mu\text{M}$ ) are 90% growth inhibiting. At concentrations of 6 and 12  $\mu\text{g/ml}$  (3 and 5.9  $\mu\text{M}$ ) of melittin 1-20 the HIV production was reduced to about 20% of the level in untreated cultures (Fig. 4a).

The comparison of melittin and melittin 1-20 (Table I and Fig. 4a) showed that the carboxy-terminal tail (Lys-Arg-Lys-Arg-Gln-Gln) seems to modulate the effective concentration of the peptide, however, it does not affect the principal action of the substance. In order

to further analyze this phenomenon, and possibly find substances even more active than melittin, we synthesized a set of melittin derivatives with different carboxy-terminal tails attached to the  $\alpha$ -helix. Our strategy was to design tails with different characteristics in their charge distributions, because the high positive charge in the tail of native melittin is responsible for the interaction with membrane phospholipids and consequently the membrane affinity [27], so derivatives with altered tail structures might exhibit altered membrane affinities and altered effects on HIV production. We synthesized melittin derivatives with the following modified carboxy-terminal tails: (i) six glycines, 'melittin E', (ii) ornithin-ornithin, 'melittin F', (iii) native sequence in D-conformation, 'melittin 3', (iv) Lys-Arg-Lys-Arg-Gly-Gly, 'melittin 4', (v) four lysines, 'melittin 6'. The effects of these derivatives are summarized in Table IIIa. Melittin E (six glycines) and melittin 6 (four lysines) had an effect comparable to melittin 1-20, the no-tail  $\alpha$ -helix, indicating that the charge distribution within the tail alone does not explain the effect of melittin on HIV production. The substance with D-amino acids (melittin 3) had a slightly reduced effect when compared to melittin, whereas melittin 4 (only the last two glutamines were substituted by glycines) had a stronger effect than native melittin on HIV production. Taken together, the

Table IIIa

Effect of melittin tail derivatives on the supernatant infectivity of cultures treated with subtoxic substance concentrations

Peptide	Melix	Tail	Conc. ( $\mu\text{M}$ )	Infectivity (%)
Melittin	GIGAVLKVLTTGLPALISWI	KRKRQQ	0.9	10
Melittin 1-20	"	none	5.9	20
Melittin E	"	GGGGGG	5.1	30
Melittin F	"	Orn-Orn	1.3	40
Melittin 3	"	D-(KRKRQQ)	2.1	30
Melittin 4	"	KRKRGG	0.6	5
Melittin 6	"	KKKK	4.7	50

Table IIIb

Effect of other amphipathic peptides on the supernatant infectivity of cultures treated with subtoxic substance concentrations

Peptide	Sequence	Conc. ( $\mu\text{M}$ )	Infectivity (%)
gp41 transmembrane	FIMIVGGLYGLRIVFAVLSIVNRVRRQ	10.3	100
MHC class I transmembrane	VAAKANRYADEIRHKREKLE	19.3	100
Mastoparan	INLKALAALAKKIL	13.5	100

Table IIIc

Effect of a non-amphipathic peptide (negative control) on the supernatant infectivity of cultures treated with subtoxic substance concentrations

Peptide	Sequence	Conc. ( $\mu\text{M}$ )	Infectivity (%)
Gastrin releasing factor peptide	FAESGVDTPVFENSY	29.4	100

data suggest that the positive charges in the tail influence the inhibitory effect on HIV-1 production but also that other tail constructions can give a similar result.

Since the amphipathic helix of the melittin seems to be sufficient for the inhibition of HIV production, we synthesized other  $\alpha$ -helices that could exert similar effects. We tested mastoparan, the wasp venom peptide [7], which is an amphipathic  $\alpha$ -helix of 14 amino acids. Mastoparan was not toxic to the lymphoma cells up to 20  $\mu\text{g/ml}$  (13.5  $\mu\text{M}$ ) and had no influence on the HIV production in this concentration. 30  $\mu\text{g/ml}$  (20.3  $\mu\text{M}$ ) were 80% growth inhibiting and did not have a significant influence on the HIV production in the surviving cells (Fig. 4b and Table IIb). An amphipathic peptide from the transmembrane portion of the MHC class I protein did not influence HIV production either (Table IIb), nor did a non-amphipathic negative control peptide from the gastrin releasing factor (Table IIc).

As indicated above, one of the reasons to test melittin was its similarity to transmembrane regions of gp41. We synthesized a peptide from the gp41 transmembrane region and tested it in our system. However, there was no effect on HIV production in the concentrations tested (up to 10  $\mu\text{M}$ ; Table IIb). A summary of the tested amphipathic peptides other than melittin-derivatives, not inhibitory to HIV production, is given in Table IIb.

To further investigate a possible intracellular effect of melittin on HIV production, we analyzed the spectrum of HIV proteins in the extract of melittin-treated cells by Western blotting using a human serum which reacts with all known HIV-1 structural proteins. Fig. 5 shows a Western blot where a 31 kDa protein is reduced following a single melittin treatment. This protein might correspond to the p31 integrase, or to some intermediate fragment of the processed gag/pol precursor polypeptide. We are in favour of the latter, since the high molecular weight bands are more prevalent in the extract of the treated cells, suggesting that the processing of the gag/pol precursor is impaired and immature cleavage products are enriched under melittin treatment.

We conclude that the inhibition of HIV-1 production by melittin, as described in this letter, is mediated by the amphipathic  $\alpha$ -helical part (amino acids 1–20) of melittin as a result of intracellular impairment of HIV protein production rather than a membrane effect. This is suggested by the observations that (i) melittin is readily taken up into cells and (ii) a melittin derivative lacking the basic carboxy-terminal hexapeptide, which is required for lytic activity [26,27], has a similar inhibitory effect on HIV-1 as the melittin, albeit at somewhat higher concentrations. The experiments with mastoparan and other  $\alpha$ -helical amphipathic peptides show that the inhibition of HIV-1 production cannot be achieved just by any amphipathic  $\alpha$ -helix. This fact suggests that there are some structural requirements for

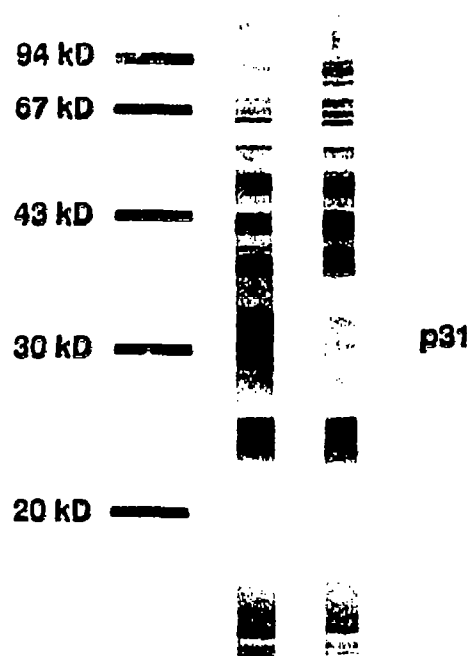


Fig. 5. Western blot of the extracts of untreated cells (left) and cells that had undergone single melittin treatment (3  $\mu\text{g/ml}$ ) 3 days before extract preparation. Staining was performed with a human HIV-positive serum.

HIV inhibition which are fulfilled by melittin but not other amphipathic helices.

At the moment, we speculate that melittin interferes with the processing of the gag/pol precursor protein. This concept is strongly supported by reports that calmodulin is a cellular substrate of the HIV protease [28,29]. Since melittin is also known to have high affinity for calmodulin [7,8], a considerable affinity of melittin for the viral substrate of the HIV protease, the gag/pol precursor, is likely. So melittin might bind to the gag/pol precursor thus preventing its being processed by the HIV protease.

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