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The nonapeptide leucinostatin A acts as a weak ionophore and as an immunosuppressant on T lymphocytes

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Abstract

Earlier studies have disclosed that leucinostatin A, a hydrophobic nonapeptide antibiotic, assumes an α-helical secondary structure in nonpolar environments. The present report demonstrates that the peptide acts as a weak ionophore facilitating the transport of mono-and divalent cations through the plasma membrane of T lymphocytes and through artificial membranes. Leucinostatin A does not change the thymidine uptake of both resting mouse thymocytes and peripheral blood lymphocytes but dose-dependently prevents the activation of T lymphocytes by tetradecanoyl-phorbol-acetate and by anti-T cell receptor antibody.

Key words: Leucinostatin A; Ionophore; Antibiotic; T lymphocyte; Immunosuppression; Cyclosporin A

1. Introduction

Leucinostatin A has been isolated simultaneously and independently from culture filtrates of *Paecilomyces lilacinus* A-257 [1] and from *Paecilomyces marquandii* (Massee) Hughes [2,3]. As it is attested by its primary structure portrayed in Fig. 1, leucinostatin A exhibits a highly lipophilic character. Some major cometabolites, e.g., leucinostatins B and C, differ from leucinostatin A in the number of methyl groups at their C terminal blocking moiety [2–7] while another one, leucinostatin D, features a Leu residue in lieu of the 2-amino-6-hydroxy-4-methyl-8-oxo-decanoic acid [8]. More recently further minor cometabolites, leucinostatins F, H and K, were isolated and identified [9–11].

Leucinostatin A was shown to act as an inhibitor of the mitochondrial ATP synthesis [12–18] and photophosphorylation [19,20]. It also exhibits remarkable biological activity against several Gram-positive bacteria and fungi as well as cytotoxic and phytotoxic effects [1–4,7,9,10,21,22]. CD, IR [23] and high field NMR (Radics, L. and Rossi, C., unpublished data) studies have shown that, in its positively charged ($-N^+(CH_3)_2$) form, leucinostatin A assumes a highly preferred helical conformation in nonpolar media, a secondary structure that resembles the α-helix conformation found recently in an X-ray study of crystalline leucinostatin A/HCl salt [24]. These findings raised the possibility that leucinostatin A may prove to be a novel peptide ionophore. The results reported in the sequel show that leucinostatin A does, in fact, facilitate the transport of mono- and divalent cations through artificial membranes and acts as a weak ionophore on T lymphocytes. Leucinostatin A also acts as an immunosuppressant attennuating the tetradecanoyl-phorbol-acetate (TPA) and anti-T cell receptor antibody-induced activation of T cells.

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Abbreviations: BCECF, (bis(carboxyethyl))-carboxyfluorescein; Hepes, *N*-2-hidroxyethylpiperazine-N^1-ethanesulfonic acid; Me, methyl group; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate

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2. Materials and methods

Materials

Mouse thymocytes and human peripheral blood lymphocytes were prepared as described earlier [25]; the viability of the cells was regularly checked and was never below than 95% during the experiments. RPMI-1640 medium and fetal calf serum were from Gibco. Eagle's Minimum Essential Medium was from the National Institute of Hygiene (Budapest, Hungary). Leucinostatin A was prepared as described earlier [2,3] and its purity was tested by NMR spectroscopy. BMA 031 anti-T cell receptor (anti-CD3) antibody was kindly provided by Drs. F. Seiler and R. Kurrle (Behringwerke, Marburg, Germany). Ionomycin and the acetoxymethyl esters of fura-2 and (bis(carboxyethyl))-carboxy-fluorescein (BCECF) were from Calbiochem. Bovine serum albumin, Hepes, Nonidet P-40, penicillin, streptomycin and 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) were Sigma products. $^{45}CaCl_2$ (4.3 GBq/mmol) and $^{86}RbCl$ (94 GBq/mmol) were from the National Institute of Isotopes (Budapest, Hungary). [^{3}H]Thymidine (185 GBq/mmol) and $^{65}ZnCl_2$ (4.0 GBq/mmol) were purchased from Amersham (UK). All the other reagents used were of best analytical purity.

Measurement of $^{45}Ca^{2+}$ and $^{65}Zn^{2+}$ uptake

Uptake of $^{45}Ca^{2+}$ and $^{65}Zn^{2+}$ was measured as described earlier [26]. Briefly, mouse thymocytes were incubated in Eagle's Minimum Essential Medium supplemented with 20 mM Hepes (pH 7.4), the appropriate amount of leucinostatin A and 40 kBq/ml $^{45}CaCl_2$ or $^{65}ZnCl_2$. Incubation was done in Eppendorf tubes at a cell density of 10^7 cells/ml at 37°C for times indicated. The final volume of the sample was 1 ml, the final total concentrations of Ca^{2+} and Zn^{2+} were 1.8 mM and 10 μM, respectively. After incubation cells were centrifuged in a microfuge, the supernatant was quantitatively removed, the tips of Eppendorf tubes were cut and placed into scintillation vials. Radioactivity was determined by liquid scintillation. The effect of ethanol or dimethylsulfoxide, the solvents of leucinostatin A, was negligible for the transport processes even at the highest concentration (0.5% v/v).

Measurement of intracellular calcium concentration

Measurement of intracellular calcium concentration was performed using the fluorescent indicator fura-2 as described earlier [26,27]. Briefly, mouse thymocytes ($5 \cdot 10^7$ cells/ml) were incubated with fura-2-acetoxymethyl ester at a final concentration of 2 μM for 20 min at 37°C in a modified Hank's medium [26,27]. Cells were centrifuged and resuspended at a final cell density of 10^7 cells/ml. Fluorescence measurements were performed using a Jobin Yvon JY3 spectrofluorimeter at an excitation/emission wavelength pair of 340/510 nm. The individual samples were calibrated and the intracellular calcium concentration was calculated using the 'digitonin-EGTA' method described earlier [26,27]. Ethanol or dimethylsulfoxide, the solvents of leucinostatin A, caused a maximum 5% increase in intracellular calcium concentration even at their highest concentration (0.5% v/v). Our results have been corrected for this unspecific effect.

Measurement of intracellular pH

The intracellular pH was measured using the acetoxymethyl ester of the fluorescent pH indicator bis(carboxyethyl)carboxifluorescein (BCECF). Thymocyte suspensions ($5 \cdot 10^7$ cells/ml) in modified Hank's medium were loaded at 37°C for 20 min with 2 μM bis(carboxyethyl)carboxyfluorescein acetoxymethyl ester. Cells were then sedimented and resuspended in the same medium at a cell density of 10^7 cells/ml. The fluorescence of the indicator was monitored at an excitation of 495 nm and emission of 525 nm with 4-nm slits in a Jobin Yvon JY3 spectrofluorimeter. Calibration of the fluorescence vs. pH was obtained releasing the indicator from the cells by digitonin and setting the pH to known values in the range of 6.5–8.0.

Measurement of $^{86}Rb^+$ release

$2 \cdot 10^8$ mouse thymocytes were incubated at a cell density of $5 \cdot 10^6$ cells/ml with 40 kBq $^{86}RbCl$ in Eagle's Minimum Essential Medium supplemented with 20 mM Hepes (pH 7.4), bovine serum albumin (1 mg/ml), streptomycin and penicillin (100 U/ml each) at 37°C. After 4 h cells were centrifuged and resuspended in 20 ml of Eagles's Minimum Essential Medium supplemented with 20 mM Hepes (pH 7.4). 1-ml samples were immediately incubated with the amount of leucinostatin indicated for 6 min at 37°C. After incubation cells were centrifuged in a microfuge and a 0.5-ml aliquot of the supernatant was removed for the determination of its radioactivity. The maximum amount (100%) of released $^{86}RbCl$ was determined by a treatment with the detergent Nonidet P-40 at a final concentration of 0.1% (v/v). The radioactivity of the Nonidet P-40-treated cellular pellet was negligible. The radioactivity in the supernatant of the samples was expressed as the percentage of the Nonidet P-40-released maximum amount of $^{86}RbCl$.

Measurement of [^{3}H]thymidine uptake

Mouse thymocytes or human peripheral blood lymphocytes ($5 \cdot 10^6$ cells/ml) were incubated for 48 h in RPMI-1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, penicillin and streptomycin (100 U/ml each), $5 \cdot 10^{-5}$ M 2-mercaptoethanol the in case of mouse thymocytes and with the appropriate amount of various stimulants specified in Tables 1 and

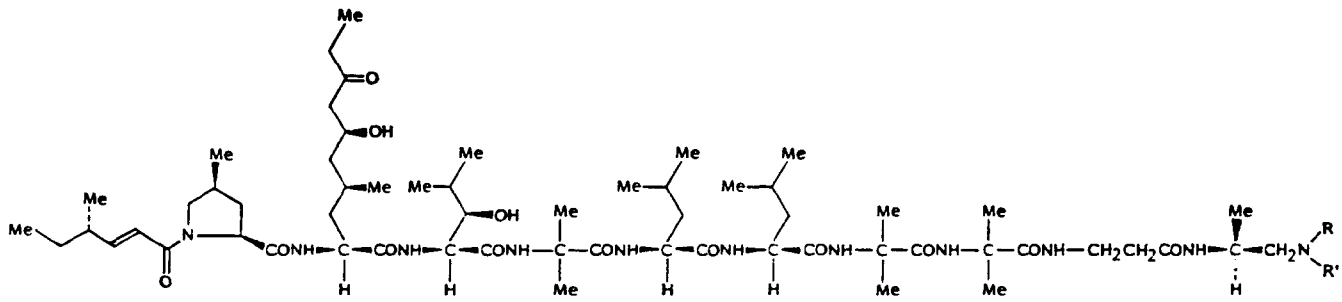


Fig. 1. Chemical structure of leucinostatin. Leucinostatin A: R=R'=CH_3; leucinostatin B: R=H, R'=CH_3; leucinostatin C: R=R'=H (Me, methyl group).

2. Cells were pulsed with [^{3}H]thymidine (18.5 kBq/ml) and were incubated further for 4 h. After incubation cells were harvested and their [^{3}H]thymidine uptake was determined by liquid scintillation. Viability of the cells was routinely checked by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay [28] showing no significant drop of cell viability in the absence or presence of leucinostatin.

Measurement of mono- and divalent ion transport through artificial membranes

Soybean lecithin liposomes (final concentration: 1 mg/ml lipid) were produced with sonication in the presence of either ^{86}RbCl or EGTA at final concentrations of 40 kBq/ml and 1 mM, respectively, in a buffer of 50 mM Hepes (pH 7.4). Trapped ^{86}RbCl or EGTA of 1 ml samples were separated from the excess using Sephadex G-50 columns (1 × 8 cm) equilibrated with 50 mM Hepes (pH 7.4). ^{86}RbCl release was measured by the addition of various amounts of leucinostatin A and valinomycin as indicated in the experiments. Released ^{86}RbCl was detected by separating it from trapped ^{86}RbCl by passing through a small Sephadex G-50 column (0.5 × 3 cm), collecting 0.1-ml fractions and analyzing their radioactivity by liquid scintillation. The first peak corresponded to the bound, and the second to the released ^{86}RbCl. Detection of $^{45}CaCl_2$ uptake was performed similarly. After the addition of 40 kBq/ml $^{45}CaCl_2$ and various amounts of leucinostatin A and A23187 as indicated above, separation and detection of bound and excess $^{45}CaCl_2$ was done as described with ^{86}RbCl.

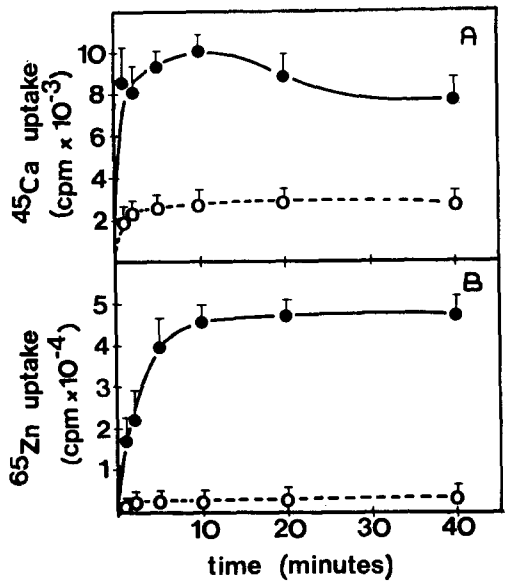


Fig. 2. Time dependence of $^{45}Ca^{2+}$ (panel A) and $^{65}Zn^{2+}$ (panel B) uptake of mouse thymocytes in the absence (○) and in the presence (●) of leucinostatin A at a final concentration of 1.0 μg/ml. Mouse thymocytes were isolated and uptake measurements were performed as described in Materials and methods. Cells were incubated with radioactive cations for times indicated. Data are means ± S.D. of three independent experiments.

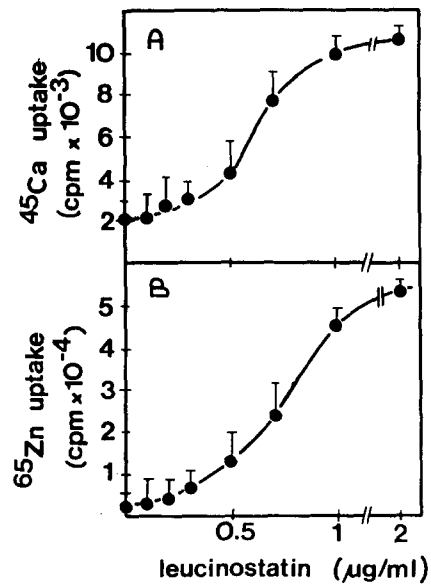


Fig. 3. The effect of various concentrations of leucinostatin A on the uptake of $^{45}Ca^{2+}$ (panel A) and $^{65}Zn^{2+}$ (panel B) by mouse thymocytes. Mouse thymocytes were isolated and uptake measurements were performed as described in Materials and methods. The incubation time was 3 and 6 min in the case of $^{45}CaCl_2$ and $^{65}ZnCl_2$, respectively. Data are means ± S.D. of three independent experiments.

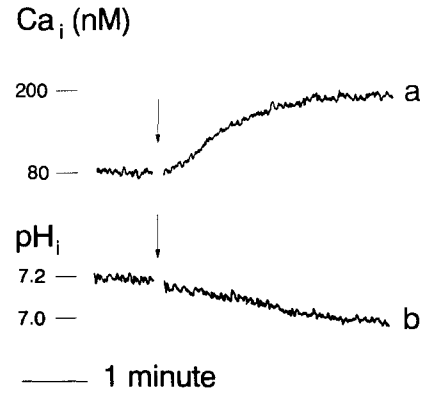


Fig. 4. The effect of leucinostatin A on intracellular calcium (panel A) and proton (panel B) concentration of mouse thymocytes. Mouse thymocytes were isolated and measurements of intracellular calcium and proton concentrations were performed as described in Materials and methods. The bar indicates a time period of 1 min. At the arrow leucinostatin A was added at a final concentration of 1.0 μg/ml. Traces are representatives of five separate experiments.

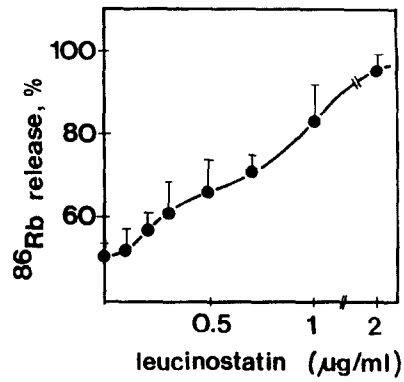


Fig. 6. The effect of various concentrations of leucinostatin A on the release of $^{86}Rb^+$ by mouse thymocytes. Mouse thymocytes were isolated and the release of $^{86}Rb^+$ was measured as described in Materials and methods. Data are means ± S.D. of three separate experiments.

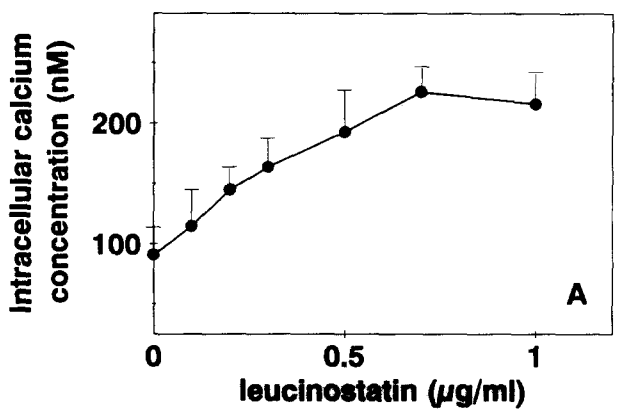
3. Results

Fig. 2 shows the time dependence of $^{45}Ca^{2+}$ (panel A) and $^{65}Zn^{2+}$ (panel B) uptake of mouse thymocytes in the absence and presence of 1.0 μg/ml leucinostatin A. Leucinostatin A increases the uptake of both cations several-fold compared to the control cells. In case of $^{45}Ca^{2+}$ the uptake is relatively fast (it is essentially completed after 1 min) while in the case of $^{65}Zn^{2+}$ the uptake is much slower reaching a plateau only after 5 min. In case we repeatedly washed the cells after the addition of leucinostatin A but *before* the addition of the radioactive isotopes, no significant changes in ion fluxes could be observed (data not shown).

The concentration dependence of leucinostatin A-induced uptake of $^{45}Ca^{2+}$ and $^{65}Zn^{2+}$ indicates a half-maximal effect at leucinostatin A concentrations of 0.6 and 0.8 μg/ml, respectively (Fig. 3).

As expected from the results of Figs. 2 and 3, leucinostatin A increases the intracellular calcium concentration of mouse thymocytes (Fig. 4, curve a). The half-maximal effect of the peptide is in the same concentration range as it was in the case of $^{45}Ca^{2+}$ uptake measurements (cf. panels A of Figs. 3 and 5). Interestingly, the effect of leucinostatin A is maximal at around a twofold increase of the intracellular calcium concentration and the peptide does not equilibrate the intra- and extracellular calcium concentrations, in contrast to well-known calcium ionophores such as A23187 or ionomycin (Fig. 5A and data not shown).

Leucinostatin A causes an intracellular acidification as demonstrated in Fig. 4, curve b. The intracellular pH decreases from 7.20 ± 0.03 to 6.96 ± 0.06 after the addition of 1 μg/ml leucinostatin A ($P < 0.001$). The



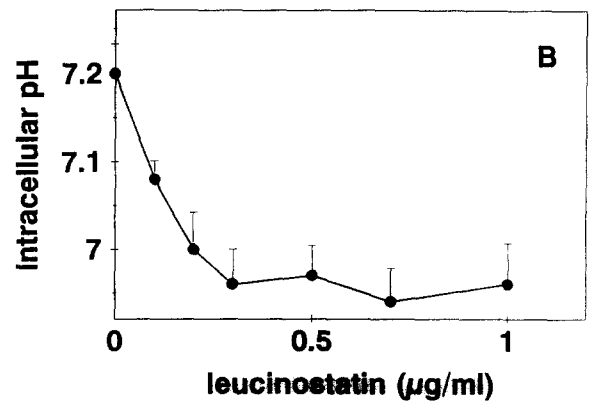


Fig. 5. The effect of various concentrations of leucinostatin A on the intracellular calcium (panel A) and proton (panel B) concentrations of mouse thymocytes. Mouse thymocytes were isolated and measurements of intracellular calcium and proton concentrations were performed as described in Materials and methods. Increasing concentrations of leucinostatin A were added sequentially. Re-measurement of some data points after the addition of the whole amount of leucinostatin at once did not gave different results. Traces were followed until the equilibrium had been reached, usually not longer than for 3 min. Data are means ± S.D. of five separate experiments.

Table 1
Effect of leucinostatin A on the [^{3}H]thymidine uptake of mouse thymocytes

Stimulus	[^{3}H]Thymidine uptake (cpm/10^6 cells)
Control	563 ± 48
Ionomycin (0.5 μg/ml)	633 ± 96
TPA (10 ng/ml)	4312 ± 159
TPA (10 ng/ml) + ionomycin (0.5 μg/ml)	6412 ± 645
Leucinostatin A	
0.1 μg/ml	579 ± 83
0.25 μg/ml	512 ± 65
0.5 μg/ml	623 ± 53
1.0 μg/ml	455 ± 92
TPA (10 ng/ml) + leucinostatin A	
0.1 μg/ml	3449 ± 275
0.25 μg/ml	3234 ± 106
0.5 μg/ml	1724 ± 89
1.0 μg/ml	492 ± 66

[^{3}H]Thymidine uptake was measured as described in Materials and methods. Results are means ± S.D. of triplicates from two independent experiments.

time course of the increase in intracellular calcium and proton concentration is very similar (cf. curves a and b in Fig. 4). The half-maximal effect of the peptide is also in the range of 0.2 μg/ml, like in the case of the leucinostatin A-induced increase in intracellular calcium concentration (cf. panels A and B of Fig. 5).

The peptide increases the release of ^{86}Rb from mouse thymocytes as shown in Fig. 6. The half-maximal effect can be observed at a leucinostatin A concentration of 0.8 μg/ml.

Table 2
Effect of leucinostatin A on the [^{3}H]thymidine uptake of human peripheral blood lymphocytes

Stimulus	[^{3}H]Thymidine uptake (cpm/4·10^5 cells)
Control	1233 ± 125
Ionomycin (0.5 μg/ml)	1386 ± 305
TPA (10 ng/ml)	8443 ± 51
TPA (10 ng/ml) + ionomycin (0.5 μg/ml)	15042 ± 620
Leucinostatin A	
0.1 μg/ml	1268 ± 101
0.25 μg/ml	1121 ± 92
0.5 μg/ml	1364 ± 40
1.0 μg/ml	1006 ± 47
TPA (10 ng/ml) + leucinostatin A	
0.1 μg/ml	7553 ± 377
0.25 μg/ml	7082 ± 283
0.5 μg/ml	3995 ± 226
1.0 μg/ml	1051 ± 86
BMA 031 (10 μg/ml)	14562 ± 702
BMA 031 (10 μg/ml) + leucinostatin A	
0.1 μg/ml	6208 ± 314
0.25 μg/ml	5821 ± 349
0.5 μg/ml	3103 ± 124
1.0 μg/ml	885 ± 106

[^{3}H]Thymidine uptake was measured as described in Materials and methods. Results are means ± S.D. of triplicates from two independent experiments.

Table 3
Effect of leucinostatin A on the transport of mono- and divalent cations through artificial membranes

Treatment	$^{86}Rb^+$ Release (cpm·10^{-3})	$^{45}Ca^{2+}$ Uptake (cpm·10^{-3})
Control	1.2 ± 0.2	7.0 ± 1.3
A23187 (0.5 μM)	N.M.	150 ± 32
Valinomycin (0.2 μM)	44 ± 5	N.M.
Leucinostatin A		
0.25 μg/ml	6.4 ± 0.9	10 ± 2
0.5 μg/ml	14 ± 2	36 ± 9
1.0 μg/ml	33 ± 5	55 ± 11

$^{86}Rb^+$ release and $^{45}Ca^{2+}$ uptake from and to soybean lecithin liposomes were measured as described in Materials and methods. Results are means ± S.D. of triplicates of released $^{86}Rb^+$ and trapped $^{45}Ca^{2+}$, respectively, from three independent experiments. N.M., not measured.

Leucinostatin A alone does not induce any significant change in the [^{3}H]thymidine uptake of both mouse thymocytes and human peripheral blood lymphocytes. However, the peptide dose-dependently inhibits the stimulating effects of both 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) and BMA 031 anti-T cell receptor (anti-CD3) antibody on the proliferation of these cells. The half-maximal inhibiton occurs in a similar concentration range of leucinostatin A, like its other effects on the uptake and release of various mono- and divalent cations (Tables 1 and 2).

Leucinostatin A induces significant changes in the release of $^{86}Rb^+$ and in the uptake of $^{45}Ca^{2+}$ from and to soybean lecithin liposomes (Table 3). The leucinostatin A-induced release of $^{86}Rb^+$ almost reaches the effect of valinomycin, a well-known potassium-specific ionophore, while the uptake of $^{45}Ca^{2+}$ after leucinostatin A treatment is about a third as much than after the addition of A23187, a calcium ionophore. The dose-dependence of leucinostatin A-induced effects in this artificial membrane system is similar to the concentration dependence of the effects of the nonapeptide on T lymphocytes (Table 3).

4. Discussion

According to the data reported above, addition of leucinostatin A results in an increased transport of both monovalent and divalent cations such as $^{86}Rb^+$, H^+, $^{45}Ca^{2+}$ and $^{65}Zn^{2+}$ through the plasma membrane of mouse thymocytes, and leucinostatin A promotes the transport of both $^{45}Ca^{2+}$ and $^{86}Rb^+$ through artificial membranes. This general effect reflects that the peptide behaves as an ionophore.

While the inward transport of cations such as Ca^{2+} or Zn^{2+} causing the decrease of the pertinent concen-

tration gradients across the plasma membrane is typical for most ionophores, H^+ ions usually tend to move in the opposite, outward direction. This intracellular alkalinization results from the tightly coupled exchange of Ca^{2+} or Zn^{2+} and permits the ionophore to decrease the gradient of H^+. Contrary to this expectation, leucinostatin A induces the acidification of the intracellular milieu.

Electrical potential in most cells is determined by the potassium or chloride gradients. Lymphocytes have a rather high intracellular Cl^- concentration of approx. 100 mM [29] which suggests that the contribution of this mechanism to the regulation of charge balance at the plasma membrane of these cells may be rather small. The influx of Cl^- ions should cause the swelling of the cells in order to offset the increased osmotic pressure. Since leucinostatin A neither induces any significant difference in light scattering of mouse thymocytes nor results in a measurable increase in intracellular Cl^- concentration as measured by the fluorescent indicator 6-methoxy-*N*-(sulfopropyl)quinolinium (SPQ) [30] (data not shown), one has to assume that the charge balance is maintained by the other mechanism, namely by the outward movement of K^+. Convincing evidence has been provided by the data in Fig. 6 which demonstrate that leucinostatin A increases the release of $^{86}Rb^+$, cations which, by their ionic properties, may effectively replace potassium ions. Leucinostatin A induces a significant efflux of $^{86}Rb^+$, presumably causing a large potassium efflux from the cells. Since the nonapeptide does not change the volume of the cells (data not shown), this may reflect a leucinostatin A-induced Rb^+/K^+ exchange.

Leucinostatin A facilitates the transport of both mono- and divalent cations. This rather unspecific action is a little 'biased' towards monovalent cations, as is clear from the direct comparison in liposomes and from the fact that leucinostatin does not equilibrate the extra- and intracellular calcium levels, whilst inducing a massive release of Rb^+ (K^+) ions. The action of the nonapeptide is not that pronounced as that of the 'traditional' mono- or divalent cation-specific ionophores, like valinomycin or A23187. This may partially result from its weak selectivity.

Leucinostatin A changes the fluxes of both monovalent and divalent ions through the plasma membrane of T lymphocytes. This may happen via the activation/inhibition of ion transport systems and via a direct ionophoric effect. The effects of leucinostatin A were reversible, which makes a tight interaction of the nonapeptide with the ion-transporters of the T lymphocyte plasma membrane rather unlikely. The leucinostatin A-induced changes of ion fluxes through artificial membranes show a great similarity both in concentration dependence and in selectivity with those induced on T lymphocytes. These similarities strongly suggest that leucinostatin A – at least partially – acts as a weak ionophore at the cellular level as well. However, the nonapeptide-induced activation/inhibition of ion transport systems of T lymphocytes may also contribute to the ion fluxes observed.

Leucinostatin A acts similarly to well-known activators of T lymphocytes, increasing the influx of Ca^{2+} [31], the efflux of K^+ [32,33] and causing an intracellular acidification [34]. These properties led us to investigate the effects of leucinostatin A on the activation of T lymphocytes. Our results demonstrate that leucinostatin A does not induce the activation of these cells, but acts rather as an inhibitor of cell proliferation when added together with 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) or an anti-T cell receptor (anti-CD3) antibody. These findings, together with the observation that leucinostatin A induces fluxes of mono- and divalent cations through artificial membranes, further support our conclusion that the peptide acts as a rather non-specific ionophore and not as an activator of T lymphocytes. The inhibitory effect of leucinostatin A on the activation of T lymphocytes is similar to leucinostatin A-induced inhibiton of the growth of other cell types [1–4].

The ionophoric properties of leucinostatin A are in accordance with earlier findings. Reed and Lardy as well as Shima et al. [12,13,18] have demonstrated that the peptide is a specific inhibitor of the mitochondrial ATPase, but at higher concentrations it acts as an uncoupler. Leucinostatin A had similar effect on rat liver mitochondria like valinomycin and X-537A (Lasalocid), well-known ionophores [17]. Leucinostatin A-induced increase in proton translocation was also observed in chloroplasts [19] and chromatophores of *Rhodospirillum rubrum* [20]. The peptide dissipated the H^+ and K^+ gradient in maize root segments [22] and liberated the trapped glucose from liposomes [21]. However, our study is the first report demonstrating directly the ionophoretic properties of this compound.

The half-maximal effect of leucinostatin A is in the range of 0.2–1.0 μg/ml in all effects tested. This corresponds well to its ID_{50} values on various cell lines [1–4], which raises the possibility that there is a correlation between the leucinostatin A-induced inhibition of cell growth and the ionophoretic property of the peptide.

Leucinostatin A transports both mono- and divalent cations through artificial membranes and activates ion fluxes through the plasma membrane of T lymphocytes. This relative unspecificity might be explained if we assume that the peptide forms rings or helical oligomers and acts like a carrier, or an ion channel, respectively. The membrane-insertion of the peptide might be enhanced by its non-polar N- and C-termini. Since both termini of leucinostatin A are 'masked', changes in membrane potential do not cause differences in leuci-

nostatin A-induced membrane damage [20] as was predicted by DeGrado et al. [35]. On the other hand increased fluidity of the membrane enhances the insertion of leucinostatin A to the lipid bilayer [20]. The importance of these hydrophobic 'caps' in both the N- and C-termini is emphasized by the fact that leucinostatin C, which has a free amino group at one end, acts much less effectively as an antibacterial and antimycotic agent [7].

Leucinostatin acts as an inhibitor of the activation of T lymphocytes. However, the ionophoretic property of leucinostatin A is different from the behavior of cyclosporine, the hydrophobic endecapeptide, a well-known inhibitor of the activation of T lymphocytes. Cyclosporine is believed to act via binding to a class of intracellular proteins, cyclophyllins [36]. The basic structural difference between leucinostatin A and cyclosporine that the latter is a cyclic compound, hence it does not need H-bonds to stabilize its structure. Therefore the N-methylation can be present in cyclosporine at a much higher degree than in leucinostatin A [36]. This structural difference might contribute to the inability of cyclosporine to form helices, and to behave like an ionophore. Despite the clear differences in the mechanism of cyclosporine- and leucinostatin A-induced inhibition of T lymphocyte activation, the high hydrophobicity of both peptides may contribute to their inhibitory effect.

Programmed cell death, apoptosis, can be both induced and inhibited by ionophores. Thus apoptotic effects of calcium, proton and potassium ionophores were demonstrated [37–39], while in certain cell types calcium ionophores behaved as inhibitors of apoptosis of lymphokine-deprived cells [40,41] and zinc ionophores proved to be general inhibitors of apoptosis [42]. Our control experiments, which measured no significant change in cell viability after leucinostatin addition, together with the morphological stability of the cells, do not indicate that leucinostatin A induces an extensive apoptosis in T lymphocytes. A detailed analysis of the effect of leucinostatins on the apoptosis of thymus cells will be an interesting task of further investigations.

The present report demonstrates that leucinostatin A is a novel peptide ionophore and acts as an immunosuppressant of T lymphocytes. These findings may provide a new clue for the explanation of the pleiotropic effects of this antibiotic.

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6. References

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