ARTICLE
Journal of Cellular Biochemistry 113:2077–2085 (2012)

Journal of Cellular Biochemistry

Sphingosine-1-Phosphate is a Mediator of TNF-α Action on the Na^+/K^+ ATPase in HepG2 Cells

Zeina Dakroub and Sawsan Ibrahim Kreydiyyeh*

Department of Biology, Faculty of Arts and Sciences, American University of Beirut, Beirut, Lebanon

ABSTRACT

We showed previously that TNF-α down-regulates the Na^+/K^+ ATPase in HepG2 cells. This work was undertaken to study the role of ceramide and its metabolites in TNF-α action. Treating HepG2 cells with the cytokine in presence of an inhibitor of sphingomyelinase, abrogated the effect of TNF-α on the ATPase. To confirm the involvement of ceramide or its metabolites, cells were incubated with exogenous ceramide. Ceramide reduced time-dependently the activity of the ATPase and its effect disappeared in presence of CAY 10466 or SHKI, respective inhibitors of ceramidase and spingosine kinase, suggesting that ceramide acts via sphingosine or sphingosine-1-phosphate (S1P). However, HepG2 cells treated with exogenous sphingosine showed a higher Na^+/K^+ ATPase activity inferring that S1P is the one responsible for the down-regulatory effect of TNF-α and ceramide. This hypothesis was confirmed by the observed inhibitory effect of exogenous S1P on the pump, which was maintained when JNK and NF-κB were inhibited separately or simultaneously. The concurrent, but not individual inhibition of the kinase and transcription factor in the absence of S1P imitated the effect of S1P. It was concluded that S1P down-regulates the ATPase by inhibiting both JNK and NF-κB. This conclusion was supported by the observed decrease in the phosphorylation of c-jun and the enhanced protein expression of IκB and lower NK-KB activity. J. Cell. Biochem. 113: 2077–2085, 2012.

KEY WORDS: Na^+/K^+ ATPase; CERAMDIE; SPHINGOSINE; SPHINGOSINE-1-PHOSPHATE; TNF-α; JNK; NF-κB

We demonstrated previously a down-regulatory effect of TNF-α on hepatic Na^+/K^+ ATPase [Kassardjian and Kreydiyyeh, 2008], a ubiquitous transporter that plays an important role in liver physiology and that is responsible for the trans-membrane Na^+ and K^+ gradients. By coupling the energy from ATP to membrane transport, the Na^+/K^+ ATPase provides the driving force for the active uptake of bile acids, resulting in the so-called bile acid-dependent bile flow [Erlinger, 1982]. Changes in the ATPase activity are correlated with changes in cell volume, bile flow, cytoplasmic pH, and to changes in the metabolism of carbohydrates, lipids, and proteins [Graf and Haussinger, 1996]. An inhibition or a deficiency of the ATPase was associated with cell death [Yu, 2003], while an increase in its activity was observed during liver regeneration [Martinez-Mas et al., 1995] Although we demonstrated modulation of the ATPase by TNF-α, yet the mechanism of action of the cytokine has not been fully elucidated.

TNF-α is known to induce the generation of sphingolipids by activating sphingomyelinases which hydrolyse sphingomyelin, a sphingolipid abundantly present at the cell membrane, generating ceramide [Marchesini and Hannun, 2004; Taha et al., 2006; Wartewig and Neubert, 2007]. Ceramide is the precursor of many sphingolipids [Gómez-Muñoz et al., 2005] and can be transformed to sphingosine, the basic building block of sphingolipids [Morales et al., 2007] in a reversible reaction involving ceramidase and ceramide synthase [Gómez-Muñoz et al., 2005]. Similar to ceramide, sphingosine exerts pro-apoptotic effects but unlike ceramide, the effects are cell type dependent and occur via different mechanisms [Spiegel and Milstien, 2003].

Although ceramide and sphingosine may lead to apoptosis in response to stress, they can be antagonized by metabolites of the sphingomyelin pathway possessing pro-growth activities, defining the concept of the "sphingolipid rheostat" [Spiegel and Milstien, 2003]. Ceramide kinase (CERK) and sphingosine kinase (SPHK 1and 2) phosphorylate ceramide and sphingosine respectively [Pyne and Pyne, 2000; Levade et al., 2002], leading to the formation of ceramide 1-phosphate (C1P) and sphingosine 1-phosphate (S1P). Ceramide phosphatase converts C1P back to ceramide [Gómez-Muñoz et al., 2005], and S1P phosphatase (SPPs) converts S1P back to sphingosine [Spiegel and Milstien, 2003]. However, only the cleavage of S1P by S1P lyases constitutes the main route of the irreversible S1P degradation and the only exit from the pathway of sphingolipids metabolism because it yields, unlike the lysosomal

Grant sponsor: University Research Board.

*Correspondence to: Sawsan Ibrahim Kreydiyyeh, Department of Biology, Faculty of Arts and Sciences, American University of Beirut, Beirut, Lebanon. E-mail: sawkreyd@aub.edu.lb

Manuscript Received: 25 September 2011; Manuscript Accepted: 17 January 2012

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 23 January 2012

DOI 10.1002/jcb.24079 • © 2012 Wiley Periodicals, Inc.

degradation pathway, two non-sphingolipids that are not recycled back into the sphingolipids pathway [Spiegel and Milstien, 2003].

The biological effects of ceramide depend on its concentration, the differentiation status of the cell, and the time frame [Lin et al., 2006], which defines which metabolite of the sphingomyelin pathway is exerting its effect. These sphingolipids target c-Jun-N-terminal kinase (JNK), NF-κB and caspases, the same mediators that we showed to be involved in the effect of TNF-α on the Na^+/K^+ pump. Whether these sphingolipids are modulators of the Na^+/K^+ ATPase and mediators of TNF-α action is a question that we attempt to address in this work.

MATERIALS AND METHODS

MATERIALS

Recombinant Human TNF alpha was purchased from Thermo Fisher Scientific, Rockford, IL, GW4869, the N-SMase inhibitor, and SP600125 the inhibitor of JNK were obtained from Calbiochem, San Diego, CA. The human liver carcinoma cell line (HepG2) was purchased from American Type Culture Collection (ATCC), VA. Dulbecco modified Eagle's medium (DMEM), fetal bovine serum (FBS) and anti – IκBα antibody were purchased from Invitrogen (CA). D-Erythro-Sphingosine, which will be referred to as sphingosine was purchased from MP biomedicals (Illkirch, France). Ceramidase inhibitor CAY10466, sphingosine-1-phosphate and sphingosine kinase inhibitor 2 (SPHKI) were purchased from Cayman (MI). The protein assay reagent was purchased from Bio-Rad (München, Germany). C-Jun, P-c-Jun antibodies, the secondary antibodies and the luminol reagent were obtained from Santa Cruz (CA).

N-acetyl-D-sphingosine, which will be referred to as ceramide, Pyrrolidinedithiocarbamate (PDTC), Adenosine 5′-triphosphate disodium salt (ATP), and all other materials were purchased from Sigma (MO).

METHODS

Culture and treatment of HepG2 cells. HepG2 cells were grown in DMEM supplemented with 10% FBS and 1% streptomycin in a humidified incubator (5%CO_2). At 60–70% confluence, the cells were treated with TNF-α (100 ng/ml) or different sphingolipids after an overnight starvation. Ceramide was dissolved in DMSO and used at a final concentration of 3 μM, sphingosine was dissolved in pure ethanol and used at 10 μM, while sphingosine-1-phosphate was dissolved in a mixture of 72.57% methanol, 6.25% water, 2.88% dodecane and 18.3% ethanol and used at 5 μM.

When the involvement of neutral sphingoyelinase (N-SMase) was studied, its inhibitor GW4869 (20 μM) was added to the cells 15 min before TNF-α.

The ceramidase inhibitor CAY10466 and the sphigosine kinase inhibitor 2 (SPHKI) were dissolved in DMSO and used at a respective final concentration of 100 μM and 10 μM.

The involvement of NF-κB and JNK was studied by pre-treating the cells, for 30 min, with their respective specific inhibitors, PDTC (100 mM) and SP600125 (50 μM) before the addition of exogenous sphingolipids. Changes in the activity of NF-κB were determined by a kit and by assaying for changes in the expression of the NF-κB inhibitor protein IκB, by Western blot analysis.

At the end of the incubation period, HepG2 cells were washed, lysed, and homogenized on ice. Proteins were quantified using the BioRad Reagent and following the Bradford method.

ASSAY FOR Na^+/K^+ ATPase ACTIVITY

The activity of the Na^+/K^+ ATPase was assayed by measuring the amount of inorganic phosphate liberated in the presence and absence of Na^+/K^+ ATPase inhibitors as described by Esmann [1988]. The cell homogoneate was diluted in histidine (150 mM) pH = 7.4 to a protein concentration of 2 μg/μl. Samples were then withdrawn and incubated with saponin (0.2%) for 30 min at room temperature in the presence of 0.03 M glycerophosphate and 0.03 M pyrophosphate. The reaction was then initiated by the addition of ATP (30 mM) in the presence and absence of KCl (200 mM), NaCl (1,240 mM), and $MgCl_2$ (40 mM) and continued for 30 min at 37°C. Choline chloride was added in replacement of the omitted salts to maintain the same chloride concentration. The reaction was stopped by the addition of trichloroacetic acid and samples were spun at 3,000*g* for 5 min. The amount of inorganic phosphate liberated in the supernatant was measured colorimetrically according to the method of Taussky and Shorr [1953]. Enzymatic activity was determined by calculating the difference in inorganic phosphate (Pi) liberated in the presence and absence of the substrates and cofactors (Na^+/K^+, and Mg^{2+}) and reported as a percentage of the value observed in the control untreated group.

WESTERN BLOT ANALYSIS

Equal amounts of protein were resolved on 10% acrylamide gel then transferred to PVDF membranes which were incubated with a primary anti Na^+/K^+ ATPase α1 subunit, anti-IκB, anti-c-Jun, or anti P-c-Jun, followed by incubation with a secondary antibody. Signals were detected by enhanced chemiluminescence using luminol reagent.

In all electrophoresis work, equal loading was checked by determination of GAPDH protein expression.

CELL VIABILITY

The effect of S1P on cell viability was studied using the trypan blue exclusive assay.

NF-κB ACTIVITY

The activity of NF-κB was assayed using the TransAM® NFκB p65 kit (Active Motif, Carlsbad, CA) as recommended by the manufacturer. The assay relies on the binding of activated NF-κB in nuclear extracts to oligonucleotides containing NF-κB consensus binding sites immobilized on 96-well plates. The bound NF-κB p65 is then recognized by a primary antibody, which is detected by a secondary antibody conjugated to horseradish peroxidase, providing thus a signal that can be quantified with a microplate spectrophotometer at 450 nm. Nuclear extracts were prepared using the TransAM® nuclear extract kit. Nuclear extracts (10 μg) were added to each well and the assay was run in triplicates.

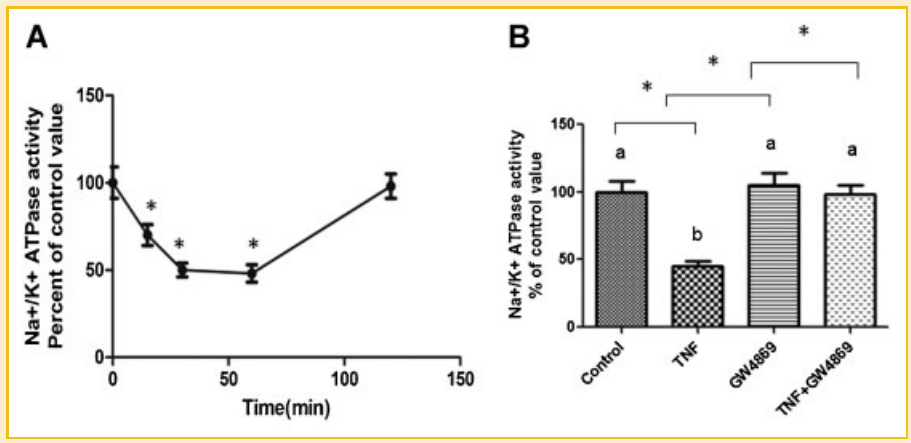

Fig. 1. Effect of TNF-α (100 ng/ml) on the Na^+/K^+ ATPase (A) Time response study. Values are means ± SE. N = 4. *: significantly different from the control $P < 0.01$. B: Effect of GW4869 (20 μM), an inhibitor of N-SMase, on TNF action (100 ng/ml, 30 min). The inhibitor was added 15 min bofore TNF-α Values are means ± SE. N = 5. *: significantly different at $P < 0.01$.

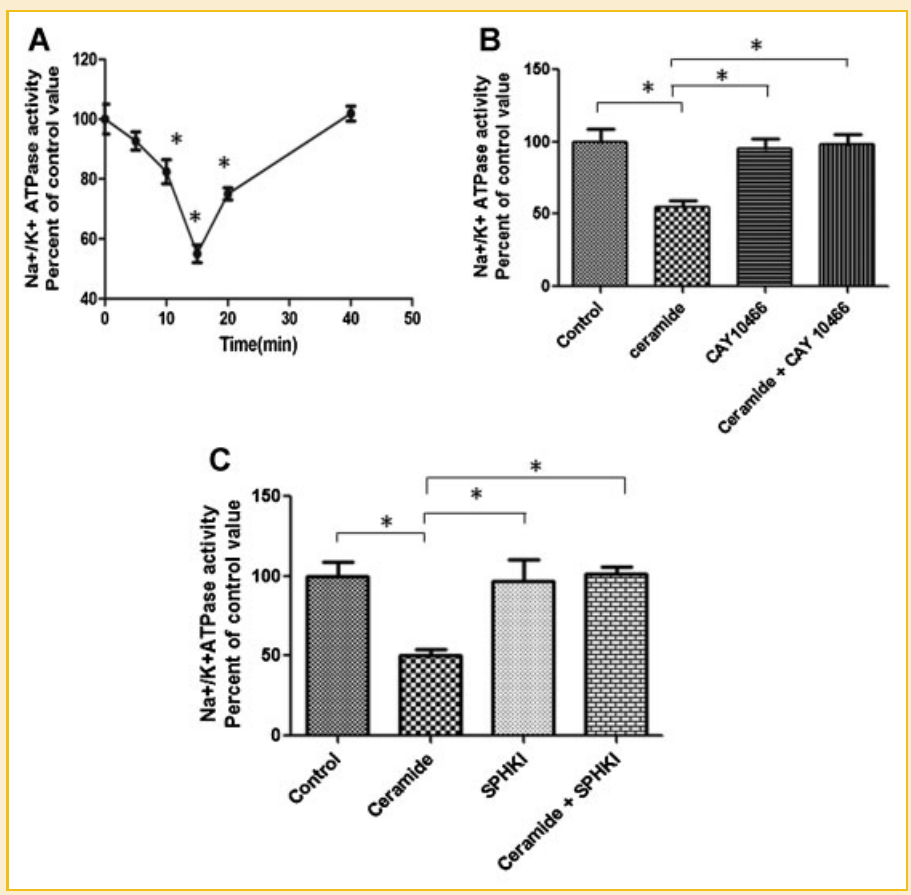

Fig. 2. Effect of exogenous ceramide (3 μM) on the Na^+/K^+ ATPase (A) Time response study. Values are means ± SE. N = 5 *: significantly different from the control at $P < 0.01$. B: Ceramide effect (3 μM, 15 min) on the ATPase in presence of CAY10466 (100 μM), an inhibitor of ceramidase. The inhibitor was added 15 min before ceramide. Values are means ± SE. N = 5. *: significantly different at $P < 0.01$. C: Ceramide effect (3 μM, 15 min) on the ATPase in presence of sphingosine kinase inhibitor 2 (10 μM). The inhibitor was added 15 min before ceramide. Values are means ± SE. N = 6. *: significantly different at $P < 0.01$.

STATISTICAL ANALYSIS

The data were analyzed for significant differences by ANOVA followed by Tukey–Kramer test using GraphPad InStat software.

RESULTS

TNF-α AND N-SMASE

TNF-α reduced time-dependently the activity of the pump (Fig. 1A). The most prominent effect was observed at 30 and 60 min and disappeared at 2 h. This down-regulatory effect was abolished in presence of GW4869, an inhibitor of N-SMase (Fig. 1B).

CERAMIDE

A time-response study revealed a significant inhibitory effect of ceramide on the Na^+/K^+ ATPase activity, which reached a maximum at 15 min (Fig. 2A). Consequently 15 min was considered as the optimal time for the ceramide effect and was adopted in all subsequent work.

The effect of ceramide was not manifested in presence of an inhibitor of ceramidase (CAY104660; Fig. 2B) or sphingosine kinase (SPHKI; Fig. 2C). The inhibitors alone had no effect on the pump.

Ceramide increased the protein level of IκB and decreased the activity of NF-κB (Fig. 3A,B), revealing thus an inhibitory effect on the transcription factor NF-κB. This inhibitory effect disappeared however, when sphingosine kinase was inhibited (Fig. 3B).

SPHINGOSINE

HepG2 cells treated with exogenous sphingosine for different time intervals had a significantly higher Na^+/K^+ ATPase activity (Fig. 4A). A maximal effect was observed at 30 min. Since sphingosine was considered as a mediator of ceramide action, its effect on the pump was studied at 15 min, the time at which ceramide exerted its maximal effect.

The sphingosine-induced increase in the pump activity disappeared in presence of SP600125 (Fig. 4B), but was maintained in presence of SPHKI (Fig. 4C). The inhibitors alone had no effect on the ATPase.

Sphingosine, unlike ceramide, did not have any significant effect on the protein expression of IκB or activity of NF-κB (Fig. 5A,B), but increased the phosphorylation of c-Jun (Fig. 5C).

SPHINGOSINE-1-PHOSPHATE (S1P)

Exogenous S1P reduced the activity and protein expression of the Na^+/K^+ pump (Fig. 6A,B). This down-regulatory effect was maximal at 15 min, and overlapped in time with the maximal effect of ceramide.

At 15 min, S1P up-regulated IκB expression and reduced NF-κB activity (Fig. 7A,B), but its inhibitory effect on the Na^+/K^+ ATPase appeared unchanged in presence of PDTC (Fig. 6C), an inhibitor of NF-κB, or in presence of SP600125 (Fig. 6C), an inhibitor of JNK. The inhibitors added individually had no effect on the pump, but caused a significant decrease in the activity of the ATPase when added simultaneously. S1P decreased the phosphorylation of c-Jun but did not affect the level of total c-Jun (Fig. 7C).

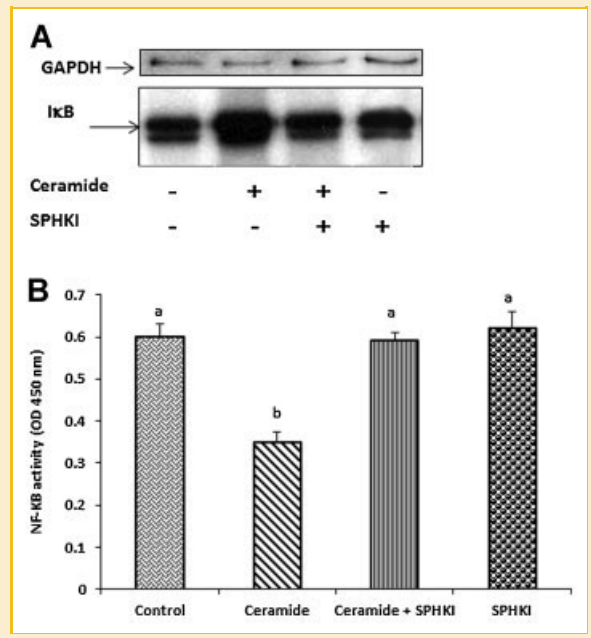

Fig. 3. Effect of Ceramide on NF-κB (A) Effect of ceramide (3 μM, 15 min) on the protein expression of IκB in presence and absence of SPHKI (10 μM). The blot is representative of an experiment repeated three times. B: Effect of ceramide (3 μM, 15 min) on NF-KB activation. To each well 10 μg of nuclear extracts were added and optical density was read at 450 nm. Values are means ± SE. N = 3. Bars sharing a common superscript are not significantly different from each other at $P < 0.01$.

The trypan blue exclusion assay showed a decrease in cell survival of HepG2 cells treated with S1P for 15 min by 32% as compared to non-treated cells.

DISCUSSION

TNF-α is known to induce via the enzyme sphingomyelinase, the production of ceramide, [Wullaert et al., 2009], which may be transformed in turn into different sphingolipid intermediates including sphingosine, sphingosine-1-phosphate (S1P) and ceramide-1-phospahate (C1P) [Levade et al., 2002; Lin et al., 2006]. We speculate that any of these sphingolipid intermediates may be, in addition to ceramide, a potential mediator of TNF-α action on the Na^+/K^+ ATPase. This hypothesis was confirmed by the disappearance of TNF-α effect in presence of the inhibitor of sphingomyelinase GW4869, inferring an involvement of ceramide in the effect of the cytokine. Exogenous ceramide imitated the effect of TNF-α and inhibited the Na^+/K^+ ATPase activity.

Quick changes in ATPase activity have been observed before and were ascribed to phosphorylation [Therien and Blostein, 2000] or translocation. Chibalin et al. [1999] reported in renal epithelial cells, a dopamine induced translocation of around 40% of the α1 subunits of the Na^+/K^+ATPase from the plasma membrane to the endosomes, within 15 min. Shuttling of ATPase molecules between plasma membrane and intracellular stores is triggered by phosphorylation [Efendiev et al., 2000]. Similarly, insulin was shown to induce in

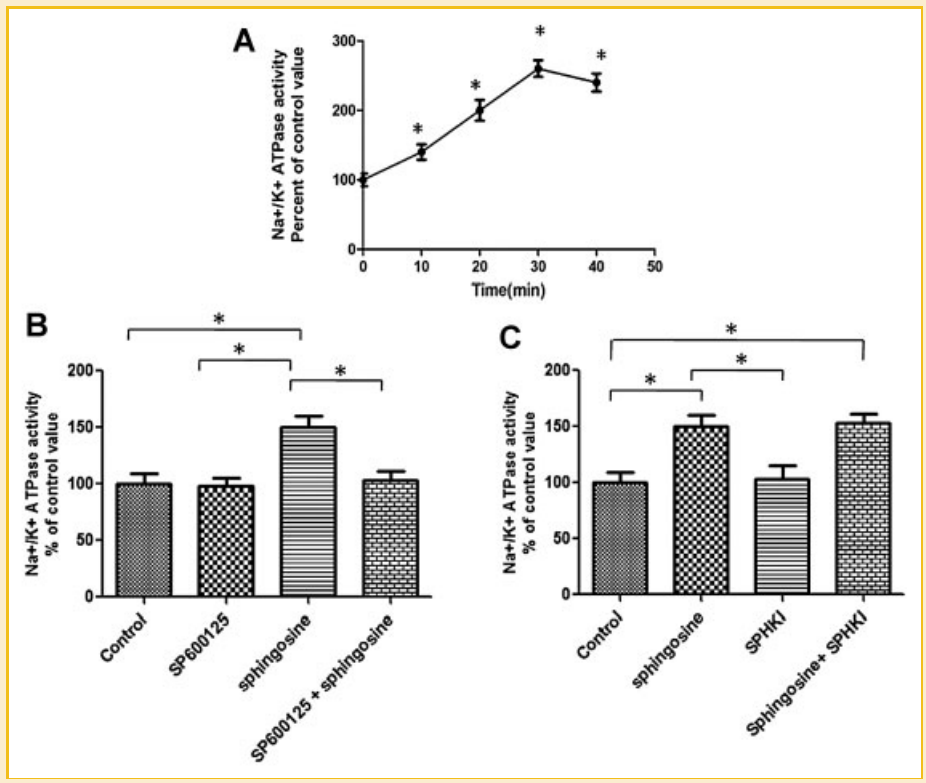

Fig. 4. Effect of exogenous sphingosine (10 μM) on the activity of the Na^+/K^+ ATPase (A) Time response study. Values are means ± SE. N = 5. *: significantly different from the control at $P < 0.01$. B: Effect of sphingosine (10 μM, 15 min) on the ATPase in presence of SP600125 (50 μM), an inhibitor of JNK. The inhibitor was added 30 min before sphingosine. N = 5. *: significantly different at $P < 0.01$. C: Effect of sphingosine (10 μM, 15 min) on the ATPase in presence of SPHKI (10 μM). Cells were pre-treated with the inhibitor 15 min before sphingosine. N = 6. *: significantly different at $P < 0.01$.

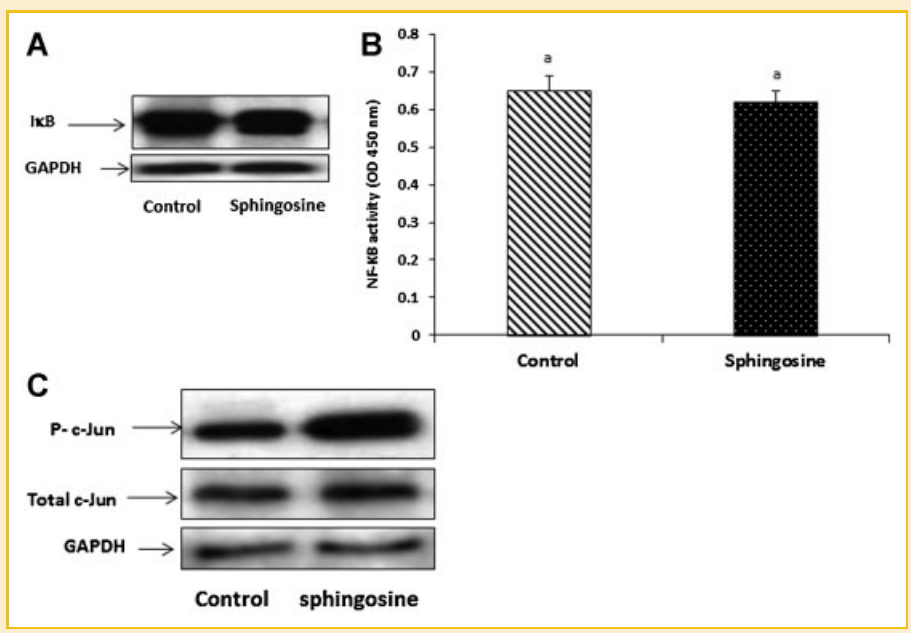

Fig. 5. Effect of sphingosine on NF-KB and c-jun (A) Effect of sphingosine (10 μM, 15 min) on the protein expression of IκB. The blot is representative of an experiment repeated three times. B: Effect sphingosine (10 μM, 15 min) on the activity of NF-κB. To each well 10 μg of nuclear extracts were added and optical density was read at 450 nm. Values are means ± SE. N = 4. Bars sharing a common superscript are not significantly different from each other at $P < 0.01$ (C) Effect of sphingosine (10 μM, 15 min) on c-jun phosphorylation. The blot is representative of an experiment repeated three times.

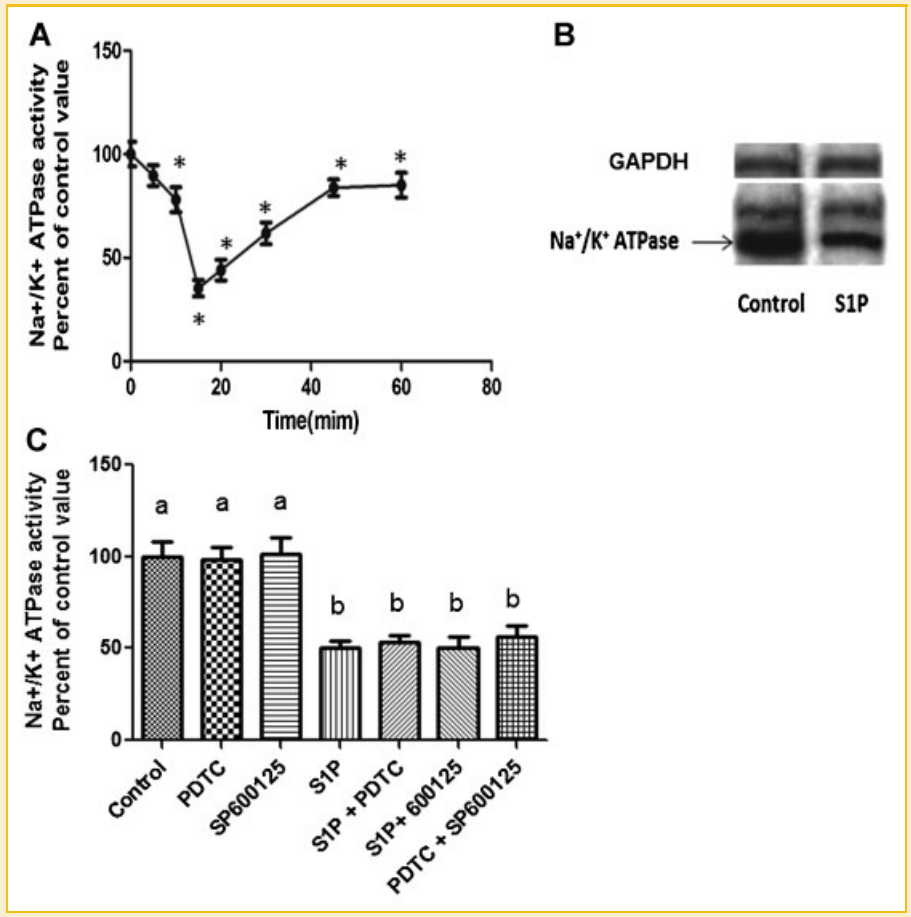

Fig. 6. Effect of exogenous sphingosine 1 phosphate (5 μM) on the Na^+/K^+ ATPase (A) Time response study on the effect of S1P (5 μM, 15 min) on the activity of the Na^+/K^+ ATPase. Values are means ± SE.N = 5. *: significantly different from the control at $P < 0.01$. B: Effect of S1P on the protein expression of the Na + /K+ ATPase. The results are representative of an experiment repeated three times. C: Effect of S1P (5 μM, 15 min) on the ATPase in presence of PDTC (100 mM) or SP600125. The inhibitors were applied 30 min before S1P. Values are means ± SE. N = 5. Bars sharing a common superscript are not significantly different from each other at $P < 0.01$.

skeletal muscle cells, the translocation of intracellular Na^+/K^+ ATPase molecules stored in sealed vesicles to the plasma membrane within 20 min [Omatsu-Kanbe and Kitasato, 1990].

The inhibitory effect of ceramide did not appear when ceramidase was inhibited with CAY 10466, i.e., when the production of sphingosine or sphingosine-1-phosphate was blocked. The data imply that the inhibitory effect observed with ceramide is due to sphingosine or its phosphorylated form and not to ceramide.

Since SPHK transforms sphingosine into S1P, inhibiting the kinase would determine which of the two sphingolipids exerts the real effect.

The down-regulatory effect of ceramide disappeared when SPHK was inhibited, indicating that S1P and not sphingosine is responsible for the inhibitory effect of ceramide on the pump. On the other hand, sphingosine increased the Na^+/K^+ ATPase activity and expression exerting thus an opposite effect to that of ceramide. These results confirm our previous assumption that S1P is the one responsible for the inhibitory effect of ceramide and not sphingosine.

These findings are in line with similar reported antagonistic effects of ceramide and sphingosine on another ATPase, the $Ca2^+$ ATPase [Colina et al., 2002].

The inhibitory effect of S1P on the Na^+/K^+ ATPase was confirmed also by the results of the treatment of HepG2 cells with exogenous S1P. Since inhibition of the ATPase correlates with cell death, [Nobel et al., 2000], our findings provide an explanation to the reported S1P role in promoting cell death in PC3 cell [Liao et al., 2005], mediating apoptotic signals in human hepatic myofibroblats [Davaille et al., 2002] and inducing growth arrest, differentiation and apoptosis in other specific cell types [Mao and Obeid, 2008; Schuppel et al., 2008].

As we previously demonstrated an involvement of JNK and NF-κB in TNF-α action on the pump [Kassardjian and Kreydiyyeh, 2008], we investigated if they are also involved in sphingolipid signaling.

Ceramide up-regulated IκB and reduced NF-κB activity, indicating an inhibitory effect of ceramide on NF-κB. Other studies have shown a similar NF-κB mediated ceramide-induced cell death in MCF7 and Jurkat cells [Signorelli et al., 2001].

Addition of SPHKI in the presence of ceramide abrogated the inhibitory effect of ceramide on NF-κB, which implies that neither ceramide nor sphingosine has a direct action on the transcription factor, but S1P does.

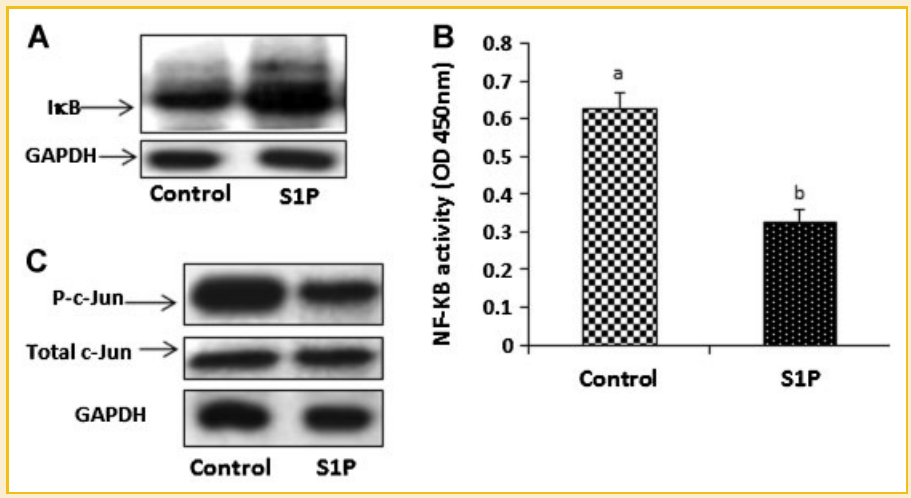

Fig. 7. Effect of exogenous sphingosine 1 phosphate (5 μM) on NF-κB and c-jun (A) Effect of S1P (5 μM, 15 min) on the protein expression of IκB. The blot is representative of an experiment repeated three times. B: Effect of S1P (5 μM, 15 min) on the activity of NF-κB. To each well 10 μg of nuclear extracts were added and optical density was read at 450 nm. Values are means ± SE. N = 4. Bars sharing a common superscript are not significantly different from each other at $P < 0.01$ (C) Effect of S1P (5 μM, 15 min) on c-jun phosphorylation. The blot is representative of an experiment repeated three times.

Sphingosine in fact did not have any effect on IκB expression and NF-κB activity, but lost its stimulatory effect on the pump when JNK was inhibited, indicating that sphingosine acts through an active JNK.

Treating the cells with exogenous S1P confirmed its inhibitory effect on NF-κB. However, PDTC and SP600125, respective specific inhibitors of NF-κB and JNK did not alter the inhibitory effect of S1P on the Na^+/K^+ ATPase implying that either they are not involved in the S1P-induced inhibitory effect on the ATPase, or if they do, they are inhibited by S1P.

Importantly, the individual and separate additions of PDTC or SP600125 to HepG2 cells had no effect on the Na^+/K^+ pump but their simultaneous addition resulted in a down regulation similar to the one observed with S1P. These results imply that inhibition of the Na^+/K^+ ATPase necessitates the concurrent inhibition of both NF-κB and JNK.

The data suggest that S1P inhibits the pump at 15 min via an inhibition of both JNK and NF-κB. The suspected inhibitory effect of S1P on JNK was confirmed by a lower phosphorylation of its substrate c-jun. Furthermore, the fact that sphingosine requires an active JNK to activate the pump confirms once again that it is not mediating ceramide effects on the pump.

Whether NF-κB and JNK are targeted directly or indirectly by TNF-α induced S1P cannot be determined from the current work. S1P produced in response to TNF-α may function intracellularly as a signaling molecule, or may act after being secreted, in an autocrine or paracrine way through a family of 5 G protein coupled receptors called S1P1–S1P5. S1P2 and SIP3 couple to Gi/o, Gq, and G12/13, SIP4 and SIP 5 couple to Gi/o and G12/13, and SIP1 couples to Gi/o only. [Rosen et al., 2009].

The export of intracellularly produced S1P is mediated by the ATP-binding cassette (ABC) family of transporters [Mitra et al., 2006; Sato et al., 2007; Takabe et al., 2010], and the putative spinster homologue 2 (Spns2) transporter [Kawahara et al., 2009].

A receptor independent direct inhibitory effect of S1P on NF-κB was shown previously in human testis germ cells [Suomalainen et al., 2005]. On the other hand, S1P may modulate the activity of the Na^+/K^+ ATPase via activation of its G protein-coupled receptors, which are known to target JNK and NF-κB [Ye, 2001].

As to exogenously added S1P, its effects on the Na^+/K^+, ATPase, NF-κB, and c-jun are mediated via its G protein coupled proteins. More work will be conducted to determine the type of receptor (s) involved.

The S1P-induced decrease in the activity of the pump is expected to reduce the Na^+ and K^+ gradient across the membrane and alter cell volume. A decrease in cell volume [Kerr et al., 1972], a loss of K^+ and a gain of Na^+ [Arrebola et al., 2005] are all recognized hallmarks of apoptosis. By inhibiting the ATPase through an inhibition of NF-κB and JNK, S1P may be contributing to cell death. This is in line with the known pro-survival role of NF-κB exerted via an activation of genes encoding anti-apopatotic proteins [Fan et al., 2008; Wullaert et al., 2009], and in line also with the pro-survival role of

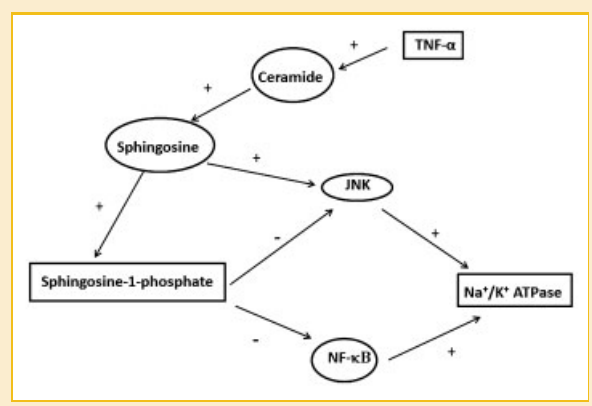

Fig. 8. Suggested signaling pathway activated by TNF-α.

JNK mediated via c-Myc and Egr-1, [Svensson et al., 2011] or via phosphorylation and inactivation of the Bcl-2 family protein BAD [Yu et al., 2004]. The apoptotic role of S1P was confirmed by a 68% residual cell viability observed in HepG2 cells incubated with S1P for 15 min. Figure 8 represents a potential mechanism of action of TNF-α on the Na^+/K^+ ATPase.

ACKNOWLEDGMENTS

This work was supported by a grant from the University Research Board.

REFERENCES

Arrebola F, Zabiti S, Canizares FJ, Cubero MA, Crespo PV, Fernandez-Segura E. 2005. Changes in intracellular sodium, chlorine, and potassium concentrations in staurosporine-induced apoptosis. J Cell Physiol 204:500–507.

Chibalin A, Ogimoto G, Pedemonte G, Presley T, Katz A, Féraille E, Berggren P, Berterello M. 1999. Dopamine-induced endocytosis of Na+,K+-ATPase is initiated by phosphorylation of Ser-18 in the Rat α subunit and is responsible for the decreased activity in epithelial cells. J Biol Chem 274:1920–1927.

Colina C, Cervino V, Benaim G. 2002. Ceramide and sphingosine have an antagonistic effect on the plasma-membrane Ca2+ ATPase from human erythrocytes. Biochem J 362:247–251.

Davaille J, Li L, Mallat A, Lotersztajn S. 2002. Sphingosine triggers both apoptotic and survival signals for human hepatic myofibroblasts. J Biol Chem 277:37323–37330.

Efendiev R, Bertorello AM, Pressley TA, Rousselot M, Féraille E, Pedemonte CH. 2000. Simultaneous phosphorylation of Ser11 and Ser18 in the alpha-subunit promotes the recruitment of Na(+),K(+)-ATPase molecules to the plasma membrane. Biochemistry 39:9884–9892.

Erlinger S. 1982. Does Na^+/K^+ ATPase have any role in bile secretion? Am J Physiol Gastrointest Liver Physiol 243:G243–G247.

Esmann E. 1988. ATPase and phosphatase activity of Na+, K+ -ATPase: Molar and specific activity, protein determination. In: Fleischer S, Fleischer B, editors. Methods in enzymology. Vol: 156, Biomembranes part P—ATP-driven pumps and related transport: The Na, K-pump. London: Academic Press, pp. 105–115.

Fan Y, Dutta J, Gupta N, Fan G, Gélinas C. 2008. Regulation of programmed cell death by NF-kappaB and its role in tumorigenesis and therapy. Adv Exp Med Biol 615:223–250.

Gómez-Muñoz A, Kong JY, Parhar K, Wang SW, Gangoiti P, González M, Eivemark S, Salh B, Duronio V, Steinbrecher UP. 2005. Ceramide-1-phosphate promotes cell survival through activation of the phosphatidylinositol 3-kinase/protein kinase B pathway. FEBS Lett 579:3744–3750.

Graf J, Haussinger D. 1996. Ion transport in hepatocytes: Mechanisms and correlations to cell volume, hormone actions and metabolism. J Hepatol 24(Suppl 1):53–77.

Kassardjian A, Kreydiyyeh SI. 2008. JNK modulates the effect of caspases and NF-kappaB in the TNF-alpha-induced down-regulation of Na+/K+ATPase in HepG2 cells. J Cell Physiol 216:615–620.

Kawahara A, Nishi T, Hisano Y, Fukui H, Yamaguchi A, Mochizuki N. 2009. The sphingolipid transporter spns2 functions in migration of zebrafish myocardial precursors. Science 323:524–527.

Kerr JF, Wyllie AH, Currie AR. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–357.

Gómez-Muñoz A, Kong JY, Parhar K, Wang SW, Gangoiti P, González M, Levade T, Cazenave SM, Gouazé V, Ségui B, Tardy C, Betito S, Abadie NA, Cuvillier O. 2002. Ceramide in apoptosis: A revisited role. Neurochem Res 27:601–607.

Liao JJ, Huang YT, Lee H. 2005. Inhibitory effects of sphingosine 1-phosphate on proliferation of PC-3human prostate cancer cells. Zool Stud 44:219–227.

Lin CF, Chen CL, Lin YS. 2006. Ceramide in apoptotic signaling and anticancer therapy. Curr Med Chem 13:1609–1616.

Mao C, Obeid LM. 2008. Ceramidases: Regulators of cellular responses mediated by ceramide, sphingosine, and sphingosine-1-phosphate. Biochim Biophys Acta 1781:424–434.

Marchesini N, Hannun YA. 2004. Acid and neutral sphingomyelinases: Roles and mechanisms of regulation. Biochem Cell Biol 82:27–44.

Martinez-Mas JV, Peinado-Onsurbe J, Ruiz-Montasell B, Felipe A, Casado FJ, Pastor-Anglada M. 1995. Na+, K(+)-ATPase expression during the early phase of liver growth after partial hepatectomy. FEBS Lett 362:85–88.

Mitra P, Oskeritzian CA, Payne SG, Beaven MA, Milstien S, Spiegel S. 2006. Role of ABCC1 in export of sphingosine-1-phosphate from mast cells. Proc Natl Acad Sci USA 103:16394–16399.

Morales A, Lee H, Goni FM, Kolesnick R, Checa JCF. 2007. Sphingolipids and cell death. Apoptosis 12:923–939.

Nobel CS, Aronson JK, van den Dobbelsteen DJ, Slater AF. 2000. Inhibition of Na+/K(+)-ATPase may be one mechanism contributing to potassium efflux and cell shrinkage in CD95-induced apoptosis. Apoptosis 5:153–163.

Omatsu-Kanbe M, Kitasato H. 1990. Insulin stimulates the translocation of Na+/K(+)-dependent ATPase molecules from intracellular stores to the plasma membrane in frog skeletal muscle. Biochem J 272:727–733.

Pyne S, Pyne NJ. 2000. Sphingosine 1-phosphate signalling in mammalian cells. Biochem J 349:385–402.

Rosen H, Gonzalez-Cabrera PJ, Sanna MG, Brown S. 2009. Sphingosine 1-phosphate receptor signaling. Annu Rev Biochem 78:743–768.

Sato K, Malchinkhuu E, Horiuchi Y, Mogi C, Tomura H, Tosaka M, Yoshimoto Y, Kuwabara A, Okajima F. 2007. Critical role of ABCA1 transporter in sphingosine 1-phosphate release from astrocytes. J Neurochem 103:2610–2619.

Schuppel M, Kürschner U, Kleuser U, Schäfer-Korting M, Kleus B. 2008. Sphingosine 1-phosphate restrains insulin-mediated keratinocyte proliferation via inhibition of Akt through the S1P2 receptor subtype. JID 128:1747–1756.

Signorelli P, Luberto C, Hannun YA. 2001. Ceramide inhibition of NF-kappaB activation involves reverse translocation of classical protein kinase C (PKC) isoenzymes: Requirement for kinase activity and carboxyl-terminal phosphorylation of PKC for the ceramide response FASEB 15:2401–2414.

Spiegel S, Milstien S. 2003. Sphingosine-1-phosphate and enigmatic signaling lipid. Nat Rev 4:307–407.

Suomalainen L, Pentikäinen V, Dunkel L. 2005. Sphingosine-1-phosphate inhibits nuclear factor kappaB activation and germ cell apoptosis in the human testis independently of its receptors. Am J Pathol 166:773–781.

Svensson C, Part K, Künnis-Beres K, Kaldmäe M, Fernaeus SZ, Land T. 2011. Pro-survival effects of JNK and p38 MAPK pathways in LPS-induced activation of BV-2 cells. Biochem Biophys Res Commun 406:488–492.

Taha TA, Mullen TD, Obeid LM. 2006. A house divided: Ceramide, sphingosine, and sphingosine-1-phosphate in programmed cell death. Biochim Biophys Acta 1758:2027–2036.

Takabe K, Kim RH, Allegood JC, Mitra P, Ramachandran S, Nagahashi M, Harikumar KB, Hait NC, Milstien S, Spiegel S. 2010. Estradiol induces export of sphingosine 1-phosphate from breast cancer cells via ABCC1 and ABCG2. J Biol Chem 285:10477–10486.

Taussky HH, Shorr E. 1953. Microcalorimetric method for determination of inorganic phosphorous. J Biol Chem 202:675–685.

Therien AG, Blostein R. 2000. Mechanisms of sodium pump regulation. Am J Physiol Cell Physiol 279:C541–C566.

Wartewig S, Neubert RHH. 2007. Properties of ceramides and their impact on the stratum corneum structure: A review. Skin Pharmacol Physiol 20:220–229.

Wullaert A, van Loo G, Heyninck K, Beyaert R. 2009. Hepatic tumor necrosis factor signaling and nuclear factor-kB: Effects on liver homeostasis and beyond. Endocr Rev 28:365–386.

Ye RD. 2001. Regulation of nuclear factor kappaB activation by G-protein-coupled receptors. J Leukoc Biol 70:839–848.

Yu SP. 2003. Na^+, K^+-ATPase: The new face of an old player in pathogenesis and apoptotic/hybrid cell death. Biochem Pharmacol 66:1601–1609.

Yu C, Minemoto Y, Zhang J, Liu J, Tang F, Bui TN, Xiang J, Lin A. 2004. JNK Suppresses apoptosis via phosphorylation of the proapoptotic Bcl-2 family protein BAD. Mol Cell 13:329–340.