MECHANISMS OF ACTION OF SPHINGOMYELINASE INDUCED ENHANCED VASORELAXATION IN DB/DB MICE

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Introduction

Sphingolipids, derived from sphingomyelin metabolism, have been implicated as important mediators in the cardiovascular system (1-2). Sphingomyelinase (SMase) catalyzes the conversion of sphingomyelin to ceramide (3), which is the precursor of other sphingolipid mediators, e.g. shingosine-1-phosphate (S1P) (Fig. 1). Both the endothelium and the vascular smooth muscle express receptors of S1P which mediate diverse vascular effects. On the other hand, sphingolipid mediators may have biological effects independently of the activation of S1P receptors. The first step of sphingolipid biosynthesis from sphingomyelin is catalyzed by sphingomyelinase (SMase) enzymes which are reportedly upregulated in certain cardiovascular and metabolic disorders such as type 2 diabetes mellitus (4).

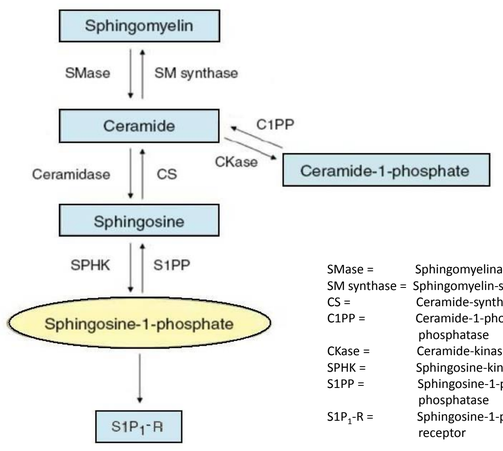

Fig. 1. Pathways of sphingomyelin metabolism.

It was shown earlier by our group and others that sphingomyelinase (SMase) induces changes of vascular tone with an initial contraction and subsequent relaxation in aortic rings of wild type mice (5). However, the exact mechanisms of the vascular effects of sphingomyelinase on vessels from wild type and diabetic mice are unknown. Our earlier studies revealed that the effect is not related to S1P2 or S1P3 receptors and subsequent experiments indicated that the contraction is related to thromboxane and the relaxation is NO-dependent.

Recently, hydrogen sulfide (H_2S) has been implicated as an important gasotransmitter in physiological and pathophysiological states (6) and our and others' data indicate that it has profound effect on the on vascular tone (7-9). A possible mechanism could be its inhibitory effect on the phospodiesterase 5A (PDE5A) leading to increased levels of cGMP, thus relaxation (Fig 2.). Furthermore, H_2S levels were shown to be altered in db/db mice (10) suggesting a possible role for H_2S in the endothelial dysfunction that is associated with this condition.

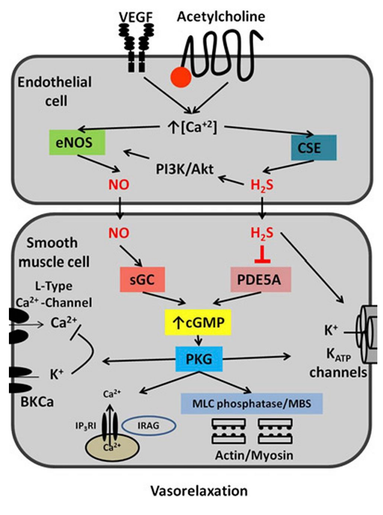

Fig. 2. Possible involvement of H_2S in vasorelaxation. (Modified after reference 9.)

Purpose

We aimed to analyze the effects of SMase on vascular tone in diabetic conditions and to elucidate the signal transduction mechanisms involved with special emphasis on hydrogen sulfide.

Materials and Methods

Thoracic aorta segments were isolated from adult db/db and non-diabetic littermate male mice following measurement of blood glucose levels and body weights. After establishing a dose-response curve for acetyl-choline (Ach from 10^{-9} to 10^{-5} M) the effect of 0.2 U/ml SMase was investigated after a precontraction with 0.1 µM phenylephrine under isometric conditions in myographs. Vascular segments were first tested the presence or absence of eNOS inhibitor L-NAME (100 µM), the selective thromboxane receptor (TP-R) antagonist SQ 29,548 (1 µM) or both. Results of these experiments are expressed in two ways, one showing the maximal changes in vascular tone in percentages and the other indicating the relative level of vascular tone over time after the application of SMase. In the following experiements that were designed to better understand the signal transduction mechanisms we used sphingosine-1-phosphate receptor 1 inhibitor W146 (1 µM), ceramidase inhibitor D-erythro-MAPP (50 µM), PI3-kinase inhibitor Wortmannin (0.1 µM), Akt-inhibitor MK2206 (1 µM) and cystathionine-γ-liase (CSE) inhibitor propargylglycin (PAG, 10 mM). In case of PLC inhibitor U73122 the inactive analog U73343 was used as a control and these drugs were given to the animals in perfusion. We also tested the dose-response relationships of phenylephrine (PE, 10^{-8}-10^{-5} M) and sodium hydrosulfide (NaHS, 10^{-5}-10^{-3} M) and sodium nitroprusside (SNP, 10^{-10}-10^{-5} M) after precontraction induced by 1 µM phenylephrine. Results are expressed as percentage changes comparing the maximal contraction and maximal relaxations to the tone of precontraction.

Results

Blood glucose levels and body weights of the investigated animals were significantly higher in the db/db group (Fig 3.A and B) and vessels from these animals developed marked endothelial dysfunction as shown by the right-shift of their acetyl-choline dose-response curve (Fig 3.C).

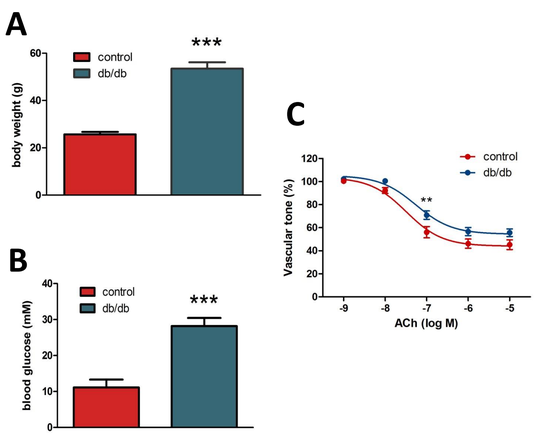

Fig. 3. The investigated db/db mice developed manifest diabetic state as indicated by their blood glucose level (A), body weight (B) and endothelial dysfunction (C).(n=12-20; **:p<0.01; ***: p<0.001)

SMase evoked an initial contraction (13.8±6.3 %) in control vessels followed by relaxation to the original tone, while it caused a marked relaxation (-21.9±7.1%) in the diabetic vessels (Fig. 4A). L-NAME administration further increased the contraction (25.3±7.6 %) in the control and strong contraction (36.8±3.1%) occured in the diabetic vessels as well (Fig. 4B). TP-R inhibitor SQ 29,548 led to relaxation in both groups, but these were more pronounced in the db/db group (-25.1±6.3% vs. -63.1±14.9%; Fig. 4C). Co-administration of the inhibitors resulted in no change in vascular tone after SMase treatment (Fig 4D).

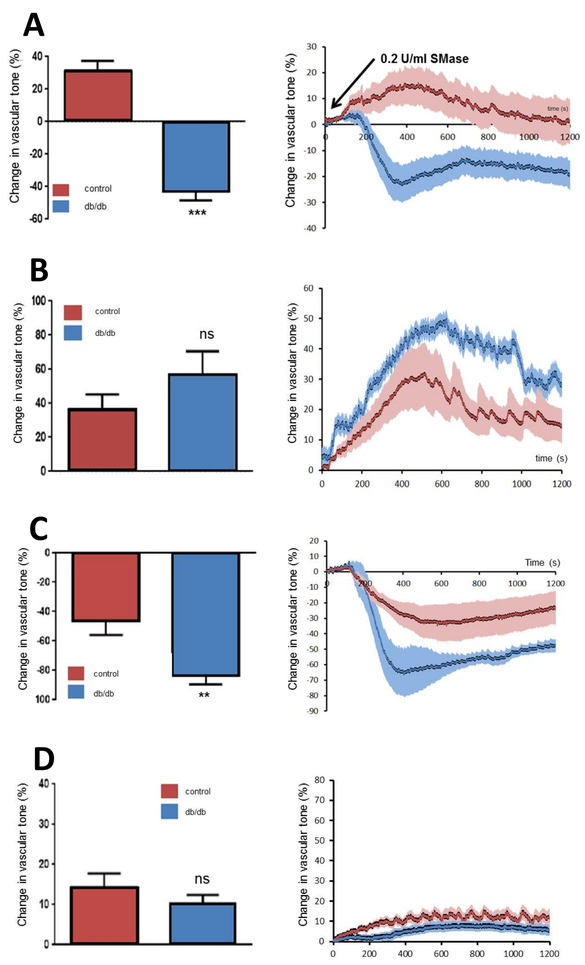

Fig. 4. Changes in vascular tones of thoracic aorta segments derived from control and db/db mice after the application of 0.2 U/ml SMase. Maximal relaxations or contractions are shown on the left and changes over time are presented on the right. (A) no inhibition; (B) NOS-inhibition by L-NAME; (C) TP-R inhibition by SQ 29,548; (D) combined inhibition of NOS and TP-R by L-NAME and SQ 29,548 (mean±SEM, n=7-26).

The use of W146, D-erythro-MAPP, Wortmannin and MK2206 did not alter the vasoactive effects of SMase in the wild type or db/db mice (Fig. 5). However, the PLC inhibitor U73122 and propargylglycin decreased (-2.21±1.06% and -22.02±4.95% respectively, p<0.05) the SMase induced vasorelaxation in diabetic vessels (Fig. 6). Dose-response curves for PE and NaHS were shifted to the left while dose-response curves for SNP were shifted to the right in the db/db group (Fig. 7. and Fig. 8).

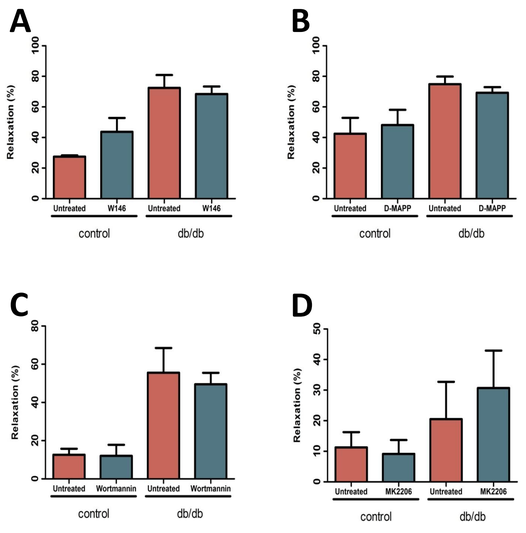

Fig. 5. Changes in vascular tones of thoracic aorta segments derived from control and db/db mice after the application of 0.2 U/ml SMase. Maximal relaxations are shown with or without the inhibition of S1P1R inhibition (W146, A), ceramidase blocking (D-MAPP, B), PI3K inhibition (wortmannin, C) and Akt inhibition (MK2206, D) (mean±SEM, n=2-6).

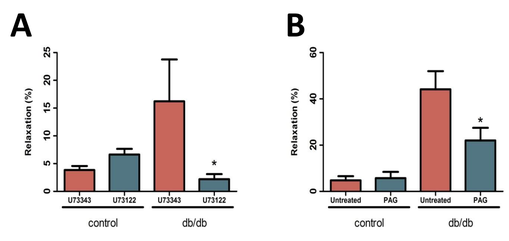

Fig. 6. Relaxations in db/db derived vessels are dependent on PLC (A) and on CSE (B) (mean±SEM, n=5-9).

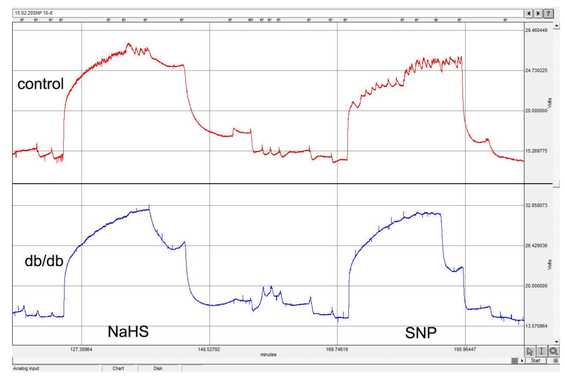

Fig. 7. Representative recordings of NahS and SNP dose-repsonse experimenst from a wild type (upper, red curve) and a db/db mice derived vessel (lower, blue curve).

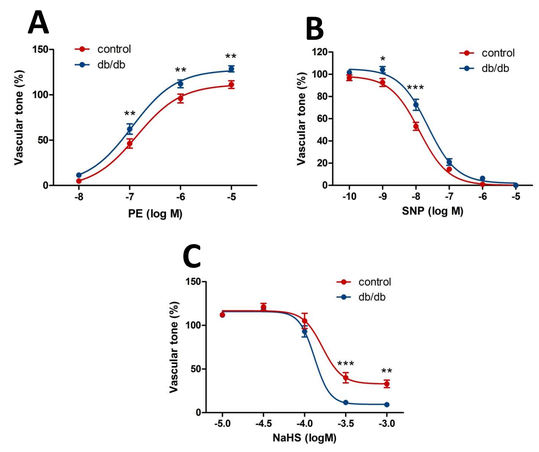

Fig. 8. Dose-response curves for (A) PE; (B) SNP; (C) NaHS (mean±SEM, n= 5; *: p<0.05, **: p<0.01, ***: p<0.001).

Discussion and Conclusions

SMase induces enhanced biphasic changes in the tone of db/db mice derived vessels and these effects are related to thromboxane A_2 and endothelial nitric oxide. The increased relaxations require the activation of PLC and involve cystathionine-γ-liase (CSE). The altered dose-response curves for NaHS, SNP and PE suggest that phosphodiesterase (PDE) activity might be enhanced in the aortas of db/db mice. Taken together our results indicate that an altered balance of gasotransmitters might contribute to the SMase induced and surprisingly enhanced vasorelaxations in db/db animal derived aortas. According to literature there is a 10-fold increase in the SMase-sensitive lipid raft area in db/db mice that inhibits eNOS activation by CAV-1 (10). Parallelly, H_2S levels in cardiovascular tissues from db/db mice are reported to be lower (11). These suggest decreased availability of the endogenous inhibitor of PDE and indicate that sphingomyelinase might lead to enhanced vasorelaxations by massively increased NO production. An interesting aspect of our results is that type 2 diabetic vascular tissues have bigger sensitivity to either endogenously or exogenously altered H_2S levels. Taken together our observations strengthen the importance to achieve proper H_2S levels in type 2 diabetic vascular tissues.

Acknowledgements

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