Time-dependent model to mimic acetylcholine induced vasodilatation in arterial smooth muscle cells

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ABSTRACT Computational approaches for spatial modeling of dynamics of the intercellular distribution of molecules can parse, simplify, classify and organize the spatiotemporal richness of any biochemical pathway and demonstrate its impact on the cells function by simply coupling it with the downstream effecters. One such online system biology modeling package is Virtual cell that provides a unique open source software and it's used for making mathematical models to simulate the cytoplasmic control of molecule that interact to produce certain cellular behavior. In our present study, a spatial model for time dependent acetylcholine induced relaxation of vascular endothelial cells lining the lumen of blood vessel that regulate the contractility of the arteries was generated. The time-dependent action of neurotransmitter acetylcholine for total time period for 1 second was studied on the endothelial cell at an interval of every 0.05 seconds. Such time simulated spatial models may be useful for testing and developing new hypotheses, interpretation of results and understand the dynamic behavior of cells.

INTRODUCTION

The development of computational approaches for modeling and understanding the time dependent dynamics of any intracellular physiologies are of profound interest for researchers [1]. In this field a widely used systems biology and computational simulation, software package is Virtual Cell (VCell: http://www.nrcam.uchc.edu/). It is used to model the intracellular biological pathways and understanding the downstream effects of biochemical reactions that impact the overall cellular function in a time-course manner [2].

Virtual Cell has a unique computational environment for modeling and simulation of a cell and has applications in a wide range of scientific disciplines ranging from experimental cell biology to theoretical biophysics. The models can be based on experimental data and/or purely theoretical assumptions The biological or mathematical models created can be either simple or complex multi-layered and may be used in interpreting experimental data or probing the predicted behavior of complex.

The human physiology has a well developed and coordinated system of doing the same process within a time scale of few milliseconds with the help of the release of neurotransmitter Ach. The endothelial cells contain a Go protein–coupled receptor (GPCR) that binds this Ach and activates its associated effectors Phospholipase C which has one of its associated secondary messenger as inositol trisphosphate (IP3). IP3 mediates the opening of the IP3 gated $Ca2+$ channels in the endoplasmic reticulum with subsequent rise of the cytosolic $Ca2+$ concentration which forms a complex with Calmodulin. This $Ca2+$/calmodulin further activate the isoenzyme eNOS (endothelial NO Synthase) which then converts L-Arginine (Arg) and Oxygen (O2) to citruline and nitric oxide. The diffusion of NO through the non-striated smooth muscle cells found within the walls of blood vessels causes contraction of muscle fibers resulting in a more relaxed state of conformation leading to relaxation of the muscle cells i.e. vasodilatation [6-10] [Fig. 1].
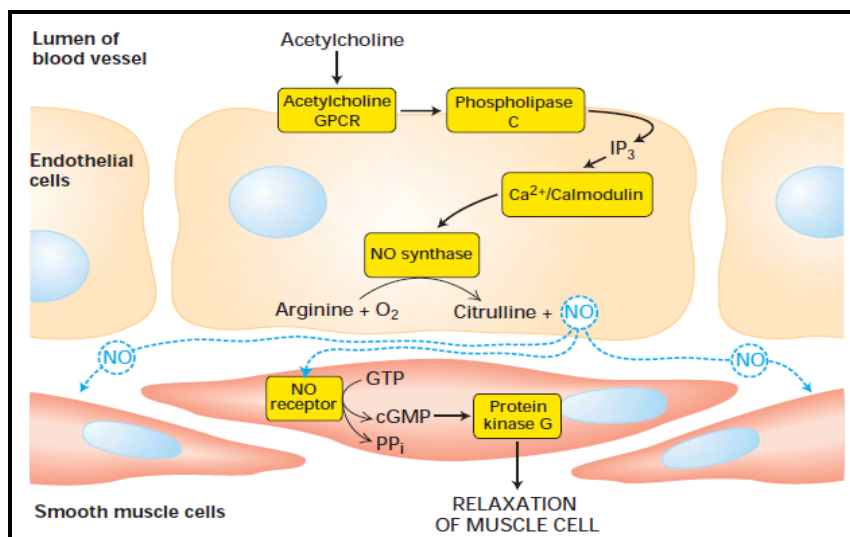
Fig. 1: Mechanism of Ach induced regulation of arterial smooth muscle relaxation [12]

Therapeutically nitroglycerin has been widely used for treating intense chest pain of angina as it is known to slowly decomposes in the body to Nitric Oxide (NO), thereby being able to mimic the natural pathway of vasodilatation [6, 7].

In the present work, acetylcholine induced vasodilatation of the smooth muscle cell that surrounds the blood vessels which feed the heart muscle itself [3-5, 8] has been studied using an *in-silico* approach.

MATERIAL AND METHODS

Signaling events are initiated on the plane of the membrane which is further propagated through the volumes of the cytosol and multiple intracellular compartments dictated in terms of the cellular pathway [11]. Spatial, mathematical modeling and simulation in these situations is an important component to understand the behavior of signaling of the cell and further decode the information of the physiological working of the cell. Web-based VCell modeling environment was used in the present study to build a spatial model for modeling Ach induced arterial smooth muscle relaxation.

Virtual Cell is a web based application in which compartmental topology, geometry, molecular characteristics, and relevant interaction parameters can be used defined. The VCell then automatically forms a corresponding mathematical system of ordinary and/or partial differential equations from the given biological description.

The following workflow was used to address the various parameters that are involved in the process of model building:

(i) Physiology Building and Defining the chemical and physiological input parameter of the model:

In the navigation tree, physiology icon was selected and it was used to specify structure diagram in the main workspace i.e. to add compartments and membranes in the model being generated. The compartments and membranes were then populated with molecular species and thereafter reactions or fluxes were then created in the reaction diagram tab [Fig. 2] or the table view [Fig. 3]. The reaction kinetic tab for the initial reacting species was selected to mass action and the expression was given as (Kf.Ach–Kr.Ach_GPCR) with the units (molecules/µm2s). The rate constant for forward reaction (Kf) was chosen as 10.0 µm2s and reverse rate constant (Kr) as 0.1 µm2s [13]. Other reacting species were also studied under the condition of mass action.
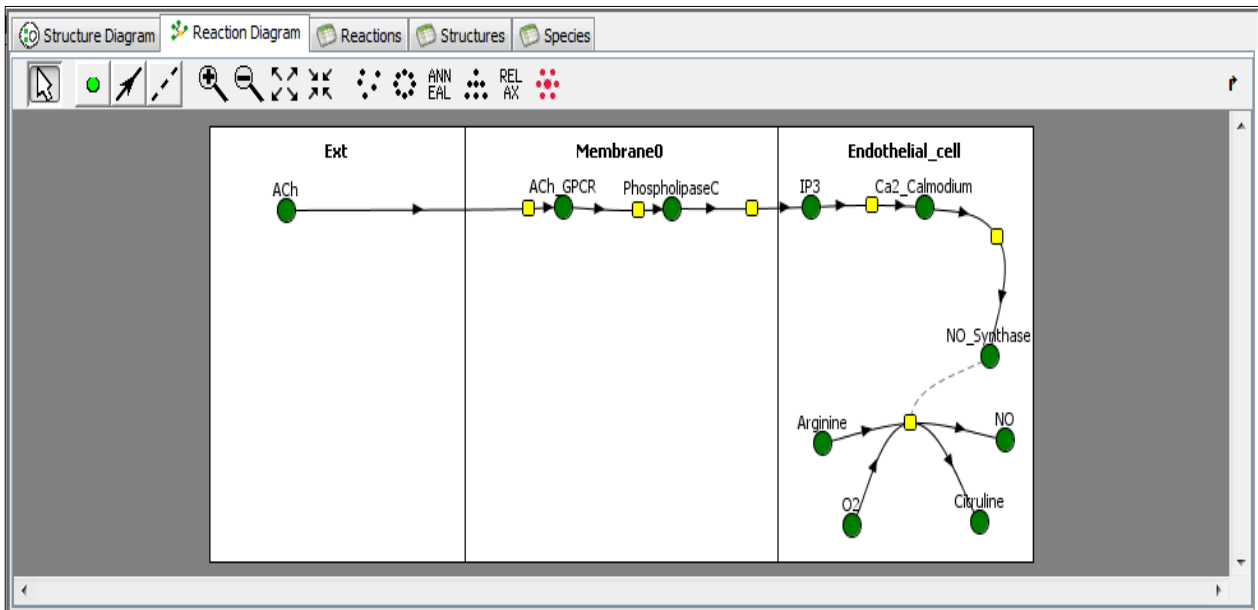
Fig. 2: The cell model created in VCell with reaction diagram tool depicting the presence of different molecular species in various intracellular compartments.

Reaction	Link	Name	Structure	Kinetics
ACh -> ACh_GPCR		Binds	Membrane0	MassAction
ACh_GPCR -> PhospholipaseC		Acticates	Membrane0	MassAction
PhospholipaseC -> IP3		Activates	Membrane0	MassAction
IP3 -> Ca2_Calmodium		Increasing level	Endothelial_cell	MassAction
Ca2_Calmodium -> NO_Synthase		activates	Endothelial_cell	MassAction
Arginine + O2 -> Citruline + NO		catalyses	Endothelial_cell	HenriMichaelisMenten (Irreversible)
(add new here, e.g. a+b->c)				

Fig. 3: Reaction tab of VCell showing the transport kinetics of the various molecular species of the model generated.

(ii) Simulation studies and geometry definition:

Virtual Cell was then used to create the simulation models using its graphical user interface. After building the physiological model its various components were mapped to the geometric domains of cell structures given in VCell [Fig. 4]. The input parameters of the model were specified and the mathematical description of the model was automatically generated by the software in the form of differential equations, which were accessed using the menu tab of MathModels. The geometry was defined with the line tool and was followed by specifying the initial concentration of Ach as 10 μm as reported by Taylor *et al.* [13] [Fig. 5], thus completing the steps of structure mapping.
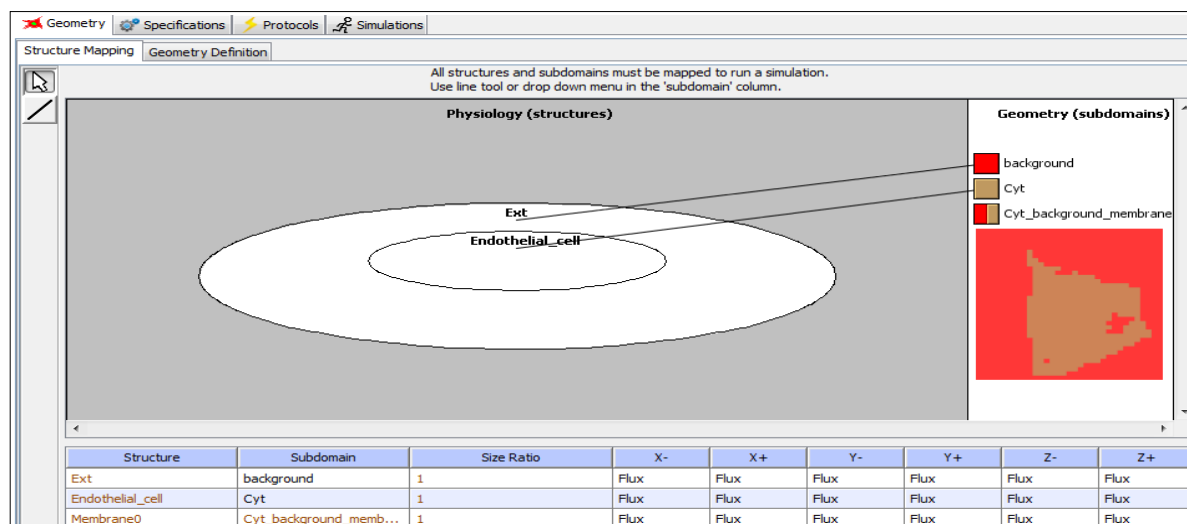
Structure	Subdomain	Size Ratio	X-	X+	Y-	Y+	Z-	Z+
Ext	background	1	Flux	Flux	Flux	Flux	Flux	Flux
Endothelial_cell	Cyt	1	Flux	Flux	Flux	Flux	Flux	Flux
Membrane0	Cyt_background_memb...	1	Flux	Flux	Flux	Flux	Flux	Flux

Fig. 4: Defining the geometry of the Ach model generated by mapping the cellular components.

Once structure mapping was completed, a model to study the time-dependent action of neurotransmitter Ach for 1 second on the endothelial cell at time intervals of every 0.05 seconds was generated.

Species	Structure	Clamped	Initial Condition	Well Mixed	Diffusion Constant
ACh	Ext	☐	10.0	☐	10.0
IP3	Endothelial_cell	☐	30.0	☐	10.0
Ca2_Calmodium	Endothelial_cell	☐	45.0	☐	10.0
NO_Synthase	Endothelial_cell	☐	50.0	☐	10.0
Arginine	Endothelial_cell	☐	5.0	☐	10.0
O2	Endothelial_cell	☐	5.0	☐	10.0
Citruline	Endothelial_cell	☐	5.0	☐	10.0
NO	Endothelial_cell	☐	5.0	☐	10.0
ACh_GPCR	Membrane0	☐	15.0	☐	0.1
PhospholipaseC	Membrane0	☐	15.0	☐	0.1

Fig. 5: The initial concentration of the reacting species specified for the model.

RESULTS AND DISCUSSION

A time simulated spatial model can be used as an exceptional tool for testing and developing new hypotheses and interpret their results. Another benefit of the time simulated spatial modeling is that it can help to extract quantitative information from the dynamic behavior of the cell [1, 14].

Virtual cell is a novel platform to emulates real life process and perform virtual experiments that. In the present work, VCell was used to construct and study the time dependent variation in the concentration of various species of molecules (namely Ach, GPCR, phospholipase C and NO) that are involved in Ach induced vasodilatation of endothelial muscle cells [8-9, 12].

The initial concentration of Ach was taken as 10.0 µM [13] and the reaction model used was mass action kinetic type reaction, to study time dependent changes in the concentration of Ach on a single endothelial muscle cells, as VCell studies a single cell at a time. In the entire muscle segment having large number of cells, the secreted 10.0 µM concentration of Ach would have an exponential effect.

Acetylcholine that was secreted in the cells exterior binds to the GPCR present on the cell membrane resulting in the decrease in concentration on Ach [Fig. 6A] and increase in Ach bound to GPCR [Fig. 6B] and Table 1.
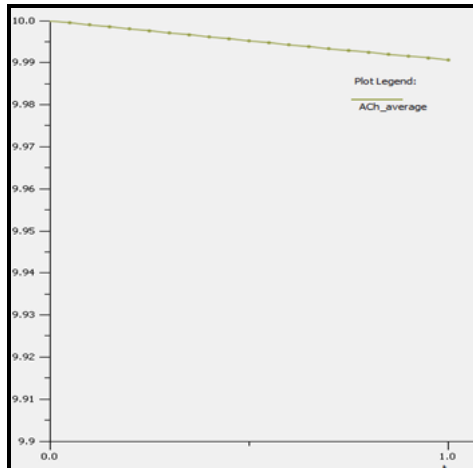
Fig. 6A: Time plot showing the secretion of Ach on the exterior of the cell.
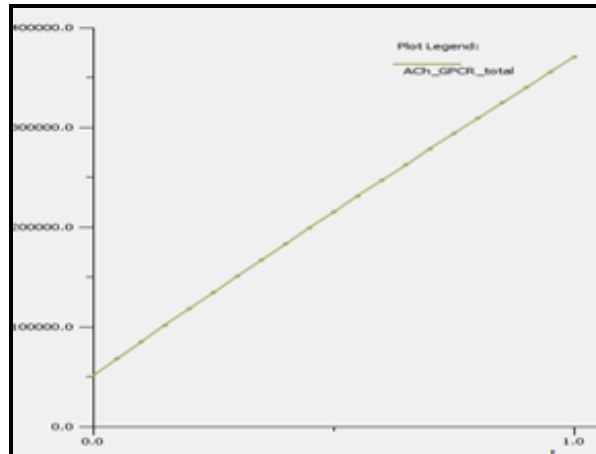

Fig. 6B: Time plot showing the showing the binding of the secreted Ach to GPCR's of a single endothelial cell.

The difference of slopes of the time plot was due to the fact that the concentration of ACh in the cellular exterior changes slowly [Fig.6A] as compared to the binding of the ACh to the GPCR receptor [Fig.6B] which was given for only one cell in the VCell model.

Table 1: The change in concentration of ACh with time

Time^	ACh_average *	ACh_GPCR_total #		Time^	ACh_average *	ACh_GPCR_total #
0.00	10	51233.815		0.55	9.9947397	231132.45
0.05	9.9995087	68182.837		0.60	9.9942763	246947.36
0.10	9.9990203	84939.98		0.65	9.9938154	262680.45
0.15	9.9985345	101560.96		0.70	9.9933568	278332.23
0.20	9.9980514	118071.84		0.75	9.9928007	293903.24
0.25	9.9975708	134485.26		0.80	9.9924469	309393.95
0.30	9.9970927	150807.56		0.85	9.9919954	324804.86
0.35	9.9966172	167042.08		0.90	9.9915463	340136.44
0.40	9.9961441	183190.76		0.95	9.9910995	355389.15
0.45	9.9956735	199254.81		1.00	9.9906549	370563.45
0.50	9.9952054	215235.14		Units:	^ seconds	* & # μM
*Ach secreted in the exterior of the cell decreases continuously with time. # ACh bound to GPCR on one cell studied in the model generated using VCell						

Binding of Ach to GPCR initiates the signal transduction and its downstream effect is the activation of the membrane component Phospholipase C, which starts a chain of reaction in the cytoplasm of the endothelial cell ultimately leading to the production NO. Nitric oxide has a very short half life of around 2 to 20 seconds [12,15-16] and it quickly diffuses through the endothelial cells adhering to Fick's law of diffusion, into the neighboring smooth muscle cells where it triggers the actual muscle relaxation [7-9]. The change in concentration of NO in the extracellular medium was seen in Fig. 7A while in Fig.7B the increase in concentration of NO in a single cell as it enters into it, was observed.
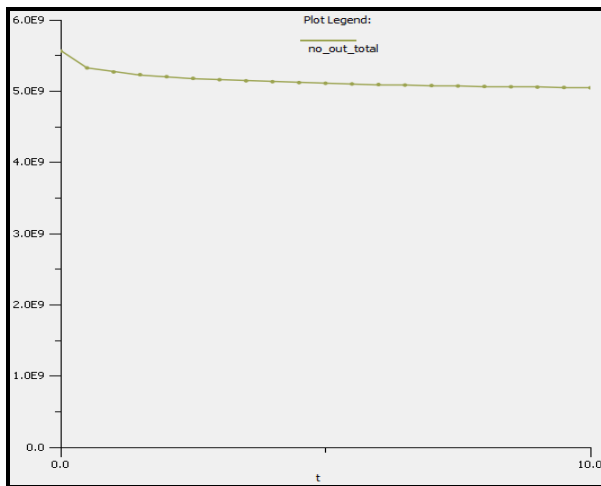
Fig. 7A: Time plot showing the change in concentration of Nitric oxide present in the cells exterior.
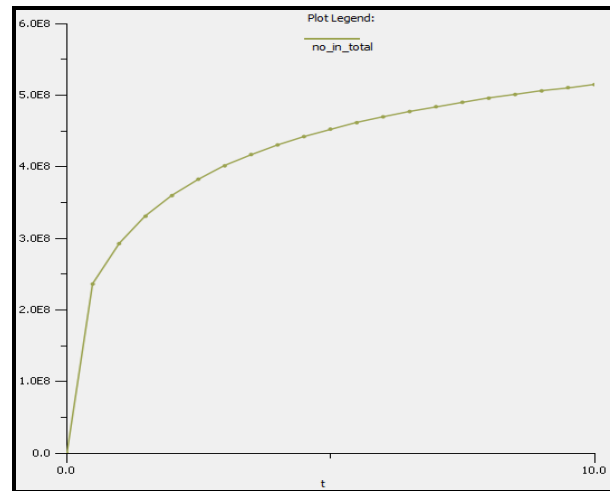

Fig. 7B: Time plot showing the entry of Nitric oxide molecule into a single cell.

In the present work, a biological model was generated to study the spatial distribution and time-dependant change in concentration of the various species reported to be involved in vasodilatation [8-9, 12] using VCell. The model generated for Ach induced vasodilatation helps us to understand the time-dependence of the secretion of Ach, the process of vasodilatation and regulation of contractibility of the blood vessel supplying the cardiac muscles. Such models can greatly help us in understanding biological systems using a systems biology approach [17].

CONCLUSION

Therefore from the results we have been able to ellucidate the time-dependance of various biochemical parameters involved in Ach induced vasoilation and their spacial distribution in different compartments of arterial smooth muscle cells.

REFERENCES:

[1]   N.A.W van Riel, Dynamic modeling and analysis of biochemical networks: mechanism-based models and model-based experiments, Briefings in bioinformatics 7(4) (2006) 364-374.

[2]   L.M. Loew, J.C. Schaff, The Virtual Cell: a software environment for computational cell biology, Trends in Biotechnology 19(10) (2001) 401–406.

[3]   D. Dröge, Free radicals in the physiological control of cell function, Physiological reviews, 82(1) (2002), 47-95.

[4]   G.P. Robb and I. Steinberg, Visualization of the chambers of the heart pulmonary circulation and the great blood vessels in man: summary of method and results, JAMA (1940) 474-480.

[5]   J.D. Coffin, T.J. Poole, Endothelial cell origin and migration in embryonic heart and cranial blood vessel development, Anat. Rec. 231(3) (1991) 383-95.

[6]   D.J. Kurz, B. Naegeli, O. Bertel, A double-blind, randomized study of the effect of immediate intravenous nitroglycerin on the incidence of postprocedural chest pain and minor myocardial necrosis after elective coronary stenting, Am. Heart J. 139(1) (2000) 35-43.

[7]   T. Münzel, H. Li, H. Mollnau, U. Hink, E. Matheis, M. Hartmann, M. Oelze, M. Skatchkov, A. Warnholtz, L. Duncker, T. Meinertz, U. Förstermann, Effects of long-term nitroglycerin treatment on endothelial nitric oxide synthase (NOS III) gene expression, NOS III–mediated superoxide production, and vascular NO bioavailability, Circ. Res.86 (1) (2000) E7-E12.

[8]   R.F. Furchgott , J.V. Zawadzki, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature 288 (1980) 373-6.

[9]   R.F. Furchgott, P.D. Cherry, J.V. Zawadzki, D. Jothianandan, Endothelial cells as mediators of vasodilation of arteries, J. Cardiovasc. Pharmacol. 6(2) (1984) S336-343.

[10]   Y. Hirooka, T. Imaizumi, T. Tagawa, M. Shiramoto, T. Endo, S. Ando, A. Takeshita, Effects of L-arginine on impaired acetylcholine-induced and ischemic vasodilation of the forearm in patients with heart failure, Circulation 90(2) (1984) 658-668.

[11]   J.M. Berg, J.L. Tymoczko, L. Stryer, Biochemistry, fifth edition, New York: W. H. Freeman, 2002.

[12]   H. Lodish, A. Berk, S.L. Zipursky, P. Matsudaira, D. Baltimore, J. Darnell, Molecular Cell Biology, fourth edition, New York: W.H. Freeman, 2000.

[13]   P. Taylor, J.H. Brown, Synthesis Storage and Release of Acetylcholine, in: G.J. Siegel, B.W. Agranoff, R.W. Alberts (Eds.), Basic Neurochemistry: Molecular, Cellular and Medical Aspects, sixth edition, Philadelphia: Lippincott-Raven, 1999.

[14]   Information   on   http://med.stanford.edu/news/all-news/2010/02/virtual-cell-could-bring-benefits-of-simulation-to-biology.html

[15]   M. Klem, Nitric oxide metabolism and breakdown, Biochimica et Biophysica Acta (BBA) Bioenergetics, 1411 (2)(1999) 273-289.

[16]   J.T.S. Hakim, K. Sugimori, E.M. Camporesi, G. Anderson, Half-life of nitric oxide in aqueous solutions with and without haemoglobin, Physiol Meas.17(4) (1996) 267-277.

[17]   T.R. Sahrawat, S. Bhalla, Identification of Critical Target Protein for Cystic Fibrosis using Systems Biology Network Approach, Int. J. Bioautomation 17(2013) 227-240.

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DOI References

[2] L.M. Loew, J.C. Schaff, The Virtual Cell: a software environment for computational cell biology, Trends in Biotechnology 19(10) (2001) 401-406.

10.1016/s0167-7799(01)01740-1

[3] D. Dröge, Free radicals in the physiological control of cell function, Physiological reviews, 82(1) (2002), 47-95.

10.1152/physrev.00018.2001

[4] G.P. Robb and I. Steinberg, Visualization of the chambers of the heart pulmonary circulation and the great blood vessels in man: summary of method and results, JAMA (1940) 474-480.

10.1001/jama.1940.02810060020005

[5] J.D. Coffin, T.J. Poole, Endothelial cell origin and migration in embryonic heart and cranial blood vessel development, Anat. Rec. 231(3) (1991) 383-95.

10.1002/ar.1092310312

[6] D.J. Kurz, B. Naegeli, O. Bertel, A double-blind, randomized study of the effect of immediate intravenous nitroglycerin on the incidence of postprocedural chest pain and minor myocardial necrosis after elective coronary stenting, Am. Heart J. 139(1) (2000).

10.1016/s0002-8703(00)90306-5

[7] T. Münzel, H. Li, H. Mollnau, U. Hink, E. Matheis, M. Hartmann, M. Oelze, M. Skatchkov, A. Warnholtz, L. Duncker, T. Meinertz, U. Förstermann, Effects of long-term nitroglycerin treatment on endothelial nitric oxide synthase (NOS III) gene expression, NOS III-mediated superoxide production, and vascular NO bioavailability, Circ. Res. 86 (1) (2000).

10.1161/01.res.86.1.e7

[8] R.F. Furchgott , J.V. Zawadzki, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature 288 (1980) 373-6.

10.1038/288373a0

[9] R.F. Furchgott, P.D. Cherry, J.V. Zawadzki, D. Jothianandan, Endothelial cells as mediators of vasodilation of arteries, J. Cardiovasc. Pharmacol. 6(2) (1984) S336-343.

10.1097/00005344-198406002-00008

[10] Y. Hirooka, T. Imaizumi, T. Tagawa, M. Shiramoto, T. Endo, S. Ando, A. Takeshita, Effects of L-arginine on impaired acetylcholine-induced and ischemic vasodilation of the forearm in patients with heart failure, Circulation 90(2) (1984) 658-668.

10.1161/01.cir.90.2.658

[12] H. Lodish, A. Berk, S.L. Zipursky, P. Matsudaira, D. Baltimore, J. Darnell, Molecular Cell Biology, fourth edition, New York: W.H. Freeman, (2000).

10.1016/s1470-8175(01)00023-6

[15] M. Klem, Nitric oxide metabolism and breakdown, Biochimica et Biophysica Acta (BBA) Bioenergetics, 1411 (2)(1999) 273-289.

10.1016/s0005-2728(99)00020-1

[16] J.T.S. Hakim, K. Sugimori, E.M. Camporesi, G. Anderson, Half-life of nitric oxide in aqueous solutions with and without haemoglobin, Physiol Meas. 17(4) (1996) 267-277. 10.1088/0967-3334/17/4/004