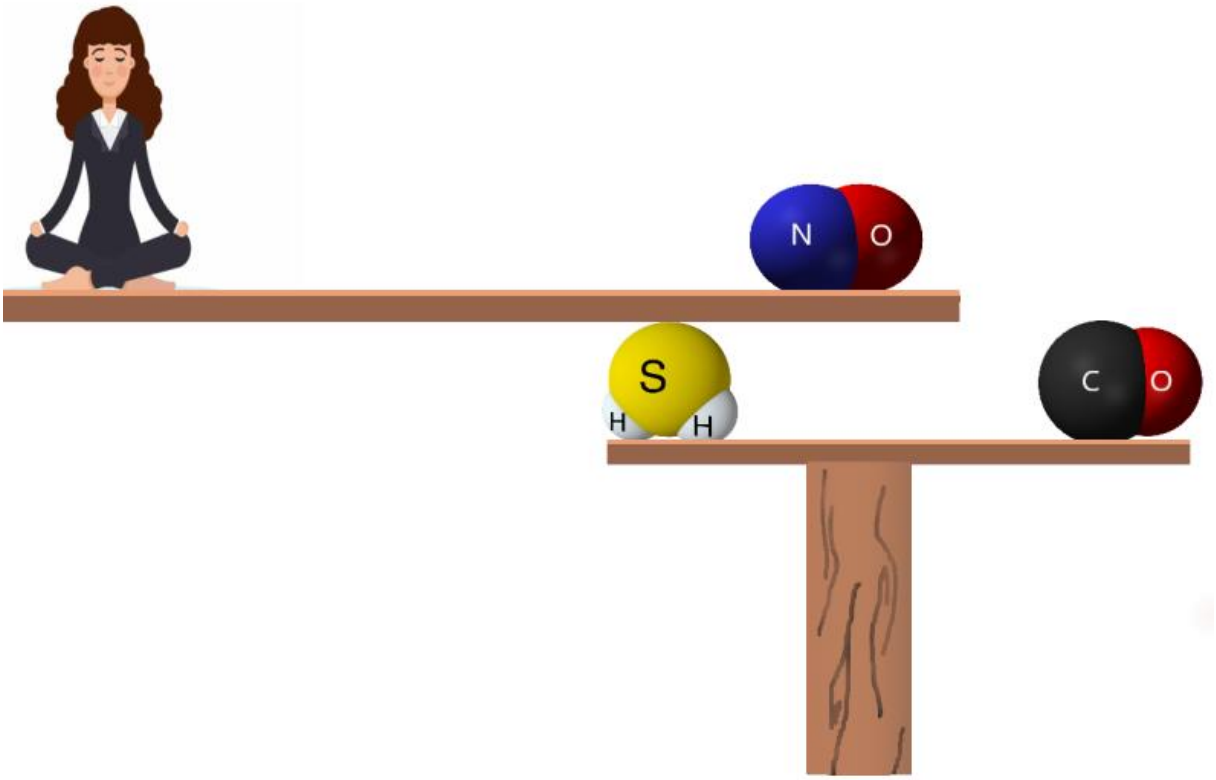
The signaling pathways and cross communication between the three gasotransmitters Nitric Oxide, Carbon Monoxide and Hydrogen Sulfide in our vascular system.



Bachelor thesis
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Content

Abstract

The endothelial cells are of high importance to the regulation of our vascular resistance. This cell type is able to produce various signaling molecules to induce either vasorelaxation, or by inhibition of the relaxation, vasoconstriction. Among these signaling molecules are the gasotransmitters nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S). All of these gasses have shown to induce vasorelaxation, but next to relaxation also posttranslational modification of proteins is mediated by these gasotransmitters.

In this literature research I would like to identify the signaling mechanisms of the gasotransmitters to induce vasodilatation, including the possibility of cross communication between the gasotransmitters. Additionally, since the three gasotransmitters share the ability to induce vasorelaxation, I would like to debate if one gasotransmitter is able to compensate for the loss of another.

The gasotransmitters have shown to induce vasorelaxation through overlapping pathways. All via cyclic guanosine monophosphate (cGMP) mediated pathways and via reducing intracellular calcium availability. Additionally, NO and H_2S mediate posttranslational protein modification, which regulates the activity of their target proteins.

The three gasotransmitters have shown to influence production of each other by altering enzyme activity. Next to affecting enzyme activity, H_2S influences gene transcription of the CO producing enzyme heme oxygenase 1. Cross communication independent of the producing enzymes is mostly via competition. NO and CO share cofactors and signaling targets for the induction of vasorelaxation, cross communication between these gasses is mediated through competition for those cofactors and targets. Cross communication between NO and H_2S is mediated via competition for the same cysteine residues to mediate posttranslational protein modification.

When the NO system is dysregulated CO reveals to compensate some vasorelaxation, a protective mechanism to keep the resistance under control. NO signaling is dependent on the endothelial cells, whereas H_2S mediated relaxation is able to continue if the endothelial cells are damaged. However, the compensated vasorelaxation remains to be partial, in the absence of one gasotransmitter the others are unable to regenerate full activity which would be mediated by the missing gas.

1. Introduction

The first time that a gas, nitric oxide, was considered as a potential signaling molecule was during the 1980's[1]. In the following years the hypothesis was supported by accumulating evidence, proving that not only nitric oxide is produced; but that it is also able to selectively activate soluble gyanylate cyclase, a key enzyme in the regulation of the vascular resistance. In 1998 Robert F. Furchgott, Louis J. Ignarro and Ferid Murad were awarded with the Nobel Prize of Medicine for their findings that nitric oxide is a signaling molecule in our cardiovascular system[1].Now, over twenty years later we are aware of three gaseous signaling molecules. Next to nitric oxide (NO), carbon oxide (CO) and hydrogen sulfide (H_2S) are identified as such 'gasotransmitters' as well.

The three gasotransmitters share some characteristics. All are small lipid permeable gaseous molecules, allowing free paracrine distribution through cells; all possess over short half-life times, varying from seconds to minutes[2]; and all are able to bind to heme. The mentioned lipophilic permeability properties complicates storage of these compounds[3]. The gasses will not stay in vesicles for later release, since they simply are capable of diffusing through the membrane. Thus for cell signaling purposes, the gasotransmitter availability comes from enzymatic production, making tight regulation of the enzymes important. Since gasotransmitter availability is directly connected to enzyme activity, the production of NO, CO or H_2S is the first step in the signaling cascade mediating vasorelaxation. For this reason, the producing enzymes and the regulation of their activity will be discussed in the next chapter of this literature research.

Despite the mentioned similarities, the molecules also differ from each other. Structurally NO and CO are planar molecules, whereas H_2S possesses over a 3D tetrahedral structure. Furthermore, whereas CO is a stable molecule; is NO is highly reactive, existing as a radical, a nitroxyl anion and a nitrosonium cation. H_2S switches at a physiological pH between the acid and hydrogen-sulfide anion, and it is (yet) unknown if the acid, the anion or both are responsible for its physiological effects. The stability of these gasses determines the range of activity, NO and H_2S are able to induce physiological effects close to their production site, while CO is able to induce effect further from where it is produced[4].

Various studies have proven that all three gasotransmitters are able to induce vasorelaxation[5,6,7]. Whereas other studies have proven that the gasotransmitters cross communicate and regulate each other as well[1,4,8,9]. When two gasotransmitters are present at the same time, the physiological effect observed varied. Through cross communication one gas is able to influence the availability or signaling cascade of the other, e.g. leading to stimulation or inhibition of the vasorelaxation. Because of the ability of the gasotransmitters to influence each other explains why we are far from understanding the (patho)physiological effect mediated by the gasotransmitters if two or more are produced simultaneously. Additionally, the cross communication among the three gasotransmitters explains why, if one gasotransmitter is out balanced, the pathophysiological effects can vary to great contents[1,5].

In this literature research I would like to explain how the gasotransmitters NO, CO and H_2S are produced and via which signaling mechanisms each gas is able to induce vasorelaxation. Furthermore I would like to investigate how the gasotransmitters are communicating among each other, including the effects resulting from this cross communication. And lastly, with all this information I would like to debate whether these gasotransmitters are substitutable for each other; is one gas able to make up for the loss of another?

2. Gasotransmitter production and signaling

2.1 Nitric oxide

Enzymatic production
Being the first identified gaseous signaling molecule the production route of nitric oxide is carefully mapped out over the years. The enzyme recognized as the source of NO production is nitric oxide synthase (NOS). Of this enzyme three isoforms are identified, neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS)[10]. Researchers M.D Seddon and colleges have proven that in humans, the main arterial pressure is regulated by nNOS, expressed in the vascular wall; while the NO produced by eNOS facilitates dynamic alterations in blood pressure[11]. eNOS is mainly expressed in the endothelium, but also slightly expressed in vascular smooth muscle cells and in perivascular adipose tissue[12].
All isoforms of NOS require the same substrates, co-substrates and cofactors to allow NO production. L-arginine (L-Arg), oxygen and NADPH are the (co)substrates necessary for the reaction, together with the cofactors flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), (6R-)5,6,7,8-

tetrahydro-L-biopterin (BH4), calmodulin (CaM) and iron protoporphyrin IX(heme) NOS is able to produce NO. The actual NO production is mediated through NOS by catalyzation of the following 2-step reaction: firstly hydroxylation of L-arginine to hydroxyl-L-arginine takes place, followed by the oxidation of hydroxyl-L-arginine producing NO and L-citrulline (L-Cit)[10]. Presented in figure 1 is a schematic overview of the mechanism by which a NOS dimer is able to catalyze this NO producing reaction.

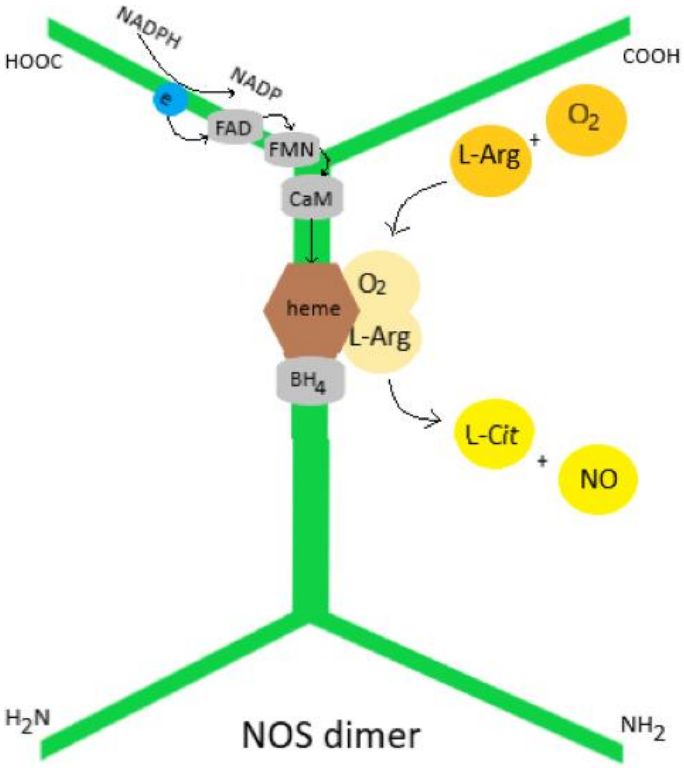

Figure 1: a schematic overview of how NOS catalyzes NO production. The NOS dimer is able to reduce NADPH to NADP, obtaining an electron. This electron is passed via the cofactors FAD, FMN and CaM to the heme group. The heme group together with BH_4 is able to use this electron for oxidation of L-arginine to L-citrulline, by which NO is produced as well.

Enzyme regulation

Under resting conditions NOS is bound to caveolin-1 (CAV-1), an inhibitory mediator of NOS. Increasing calcium concentrations results in binding of calcium to calmodulin, after which this calcium-calmodulin complex is able to displace CAV-1 bringing NOS to a higher active state[10,13]. The NOS activity can also be influenced through post-translational phosphorylation, avoiding the calcium mediated pathway. The kinases protein kinase A (PKA), protein kinase B (Akt1) and phosphatases are able to phosphorylate NOS and can be induced by shear stress. Phosphorylation of the NOS enzyme can either induce or inhibit the enzyme in activity[13]. The activity of NOS can also be influenced through substrate competition, e.g. arginase is able to compete with NOS for the substrate L-arginine. An endogenous inhibitor of eNOS is asymmetric dimethyl-L-arginine (ADMA), which is next to competing for L-arginine, also able to inhibit phosphorylation of NOS[10,14]. Uncoupled eNOS monomers, in the absence of L-arginine, are biologically active as well, producing O_2^- radicals instead of NO[13]. These radicals can act as NO scavengers, forming NO-derived peroxynitrite ($ONOO^-$). Peroxynitrite is able to oxidize BH_4, inhibiting NOS activity by reducing availability of this cofactor[12].

Signaling mechanisms

NO production in the vascular endothelial cells leads to diffusion of NO from the endothelial cells to the vascular smooth muscle cells. Once arrived in the smooth muscle cells, NO is able to bind soluble guanylyl cyclase (sGC). Resulting in an increased production of the second messenger cyclic guanosine monophosphate (cGMP). Increasing cGMP levels in the vascular smooth muscle cells activates the cGMP-dependent protein kinase (PKG) signaling cascade, this PKG mediated signaling is able to reduce intracellular calcium availability through three mechanisms. In figure 2 these three signaling mechanisms are presented in a schematic overview. The first mechanism is by which

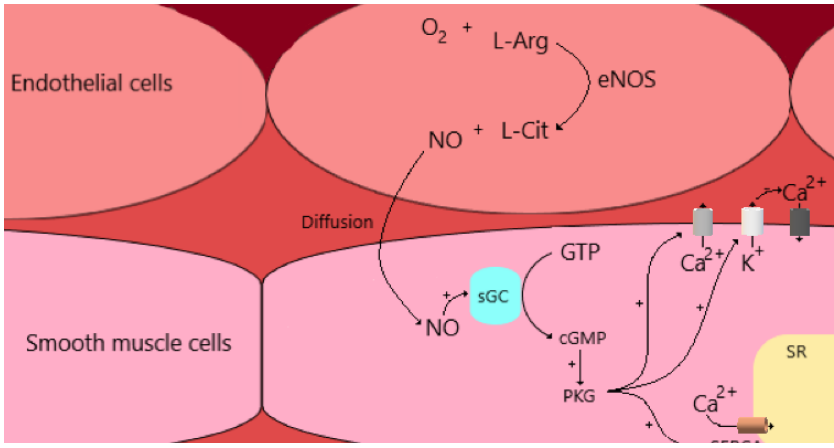

Figure 2: Signaling mechanism mediated by NO. Firstly NO is produced by eNOS in the endothelial cells. The produced NO diffuses to the smooth muscle cells to mediate vasodilatation. NO activates sGC which produces cGMP, then cGMP is able to activate PKG. By PKG 3 ion channels are activated to reduce the intracellular calcium availability. Firstly the calcium channels which allow calcium to leave the cell. Secondly, potassium channels which mediate a potassium efflux are activated, this potassium efflux inhibit voltage gated calcium channels and thus prevent calcium influx. Lastly the SERCA channels are activated, which mediates a calcium flow from the cytoplasm into the sarcoplasmic reticulum (SR).

PKG reduces calcium levels is through encouraging calcium reuptake into the sarcoplasmic reticulum; secondly via mediating calcium efflux transport; and lastly by opening calcium-activated potassium channels, or BK channels, which leads to closing of voltage gated calcium channels. Without calcium, myosin light chain kinase is unable to mediate myosin phosphorylation needed for contraction. Thus vasorelaxation is induced[1,13]. NO is also able to induce vasorelaxation independent of cGMP. By stimulation of the sarco/endoplasmic reticulum calcium ATPase (SERCA), intracellular calcium levels are reduced and vasorelaxation is induced. Additionally, the earlier discussed peroxynitrite ($ONOO^-$), formed by NO and CO_2^-, is also able to stimulate the SERCA activity through s-glutathiolation[13].
Next to vasorelaxation NO is also able to mediate post translational protein modification called s-nitrocylation. Covalent binding between NO and cysteine thiols form s-nitrosothiols, a process catalyzed by the enzyme nitrosylase. This posttranslational protein modification is involved in downstream signaling processes mediated by NO. Not only is this protein modification associated with mostly decreased activity, expression or function of those proteins, it also mediates a short-time storage possibility. The s-nitrosoproteins, existing mostly in the mitochondria, possess over a half-life time of about one hour, release of the NO is in this case mediated by dithiothreitol and vitamin C[4,13].

2.2 Carbon monoxide

Enzymatic production
Carbon monoxide was the second gaseous molecule discovered, and is produced by heme oxygenase (HO). Heme oxygenase is the enzyme responsible for degradation of heme producing bilirubin, iron and CO in the process. Three isoforms of HO are present in the human body, HO-1, HO-2 and HO-3. Of these HO-1 and HO-2 are the main producers of CO, HO-3 is highly homologous to HO-2 but showed little catalytic activity. HO-1 and HO-2 differ in where they are expressed, HO-1 is mainly expressed in the liver, spleen and kidneys whereas HO-2 is expressed in the brain, smooth muscle and endothelial cells[3]. HO-1 and HO-2 also differ in the regulation mechanisms, HO-1 expression needs to be induced for the production of CO, whereas HO-2 is constitutively expressed[15]. And because of the differences in expressions HO-1 is considered responsible for changes in endogenous CO production over the longer term, since it may take some time before the active enzyme is translated and active. On the contrary, HO-2 is considered to be a continuous producer of CO.

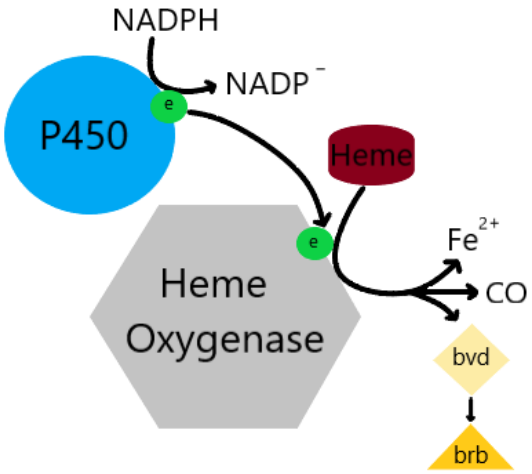

Figure 3: Schematic overview of heme oxygenase mediated CO production. Cytochrome P450 (P450) is able to reduce NADPH to $NADP^-$, obtaining an electron. This electron is given to heme oxygenase which is then able to degrade protoheme IX (heme) into iron (Fe^{2+}), CO and biliverdin (bvd). Biliverdin is rapidly converted to bilirubin (brb).

Like NOS heme oxygenase requires NADPH, oxygen and protoheme IX to be able to catalyze the CO producing reaction. Cytochrome P450 obtains an electron by reducing cofactor NADPH to $NADP^-$, this electron is transferred to HO. With the electron HO is able to catalyze the cleavage of heme to biliverdin, iron and CO[2,3,8].

Enzyme regulation
An overflow of CO is highly toxic, CO is able to bind hemoglobin with an affinity of around 200 times better than of oxygen[4]. Tight regulation of the CO availability is necessary to prevent the toxic heme-CO bonding. The heme availability is the rate limiting factor in CO production, regulation of heme production is indirectly controlling CO production. The production of heme is a negative feedback mechanism, the produced heme is able to inhibit the heme production. Additionally, oxidized heme (hemin) is also able to inhibit heme production[8]. Direct regulation of CO production is via the producing enzymes.
As mentioned above, HO-1 activity is mainly regulated through gene expression, the transcription factor Nrf2 is able to induce expression of HO-1[15]. CAV-1, an inhibitor of NOS activity, has proven to

also be able to inhibit HO-1 activity[3]. Furthermore, a second inhibitory mechanism of HO-1 activity is potentially mediated via negative feedback of CO[16]. The cellular environment is more important in HO-2 activity regulation. Since HO-2 activity is more potent to directly respond to the cellular environment, altered enzyme activity presents itself far more acute than with HO-1 activity. These environmental stimulators of HO-2 include elevating heme or calcium concentrations, or post translational protein modification mediated by PKC[3,8].

Signaling mechanisms

HO-2 is expressed in both vascular endothelial and smooth muscle cells. Suggesting that the CO mediated vasorelaxation can be independent of the vascular endothelial cells, since the production of CO can take place in the smooth muscle cells themselves. CO is able to mediate vasorelaxation via activation of sGC, similar to how NO mediates vasorelaxation. However, CO is far less effective in activating sGC than NO[4]. For this reason, it is most likely that another signaling mechanism of CO is mediating vasorelaxation. Next to activating sGC, CO is able to influence large-conductance calcium-activated potassium channels (BK_{Ca} channels). CO influences both the alfa and the beta subunit of the BK_{Ca} channels, resulting in BK_{Ca} channel coupling and an increased sensitivity of the channels to calcium. How CO is mediating the changes in the BK_{Ca} channels is not completely determined. Of the several hypotheses is the one describing that CO binds the heme group of the BK_{Ca} channels the most supported. Additionally, CO is able to able to induce so-called intracellular calcium sparks, in combination with the increased calcium sensitivity this activates the BK_{Ca} channels. Opening of the BK_{Ca} channels results in an efflux of potassium, which induces hyperpolarization of the cellular membrane. Voltage gated calcium channels (VGCC) will respond to this hyperpolarization and close, leading to a decreased calcium availability in the smooth muscle cell resulting in relaxation. For better understanding of this signaling mechanism a schematic overview is provided in figure 4.

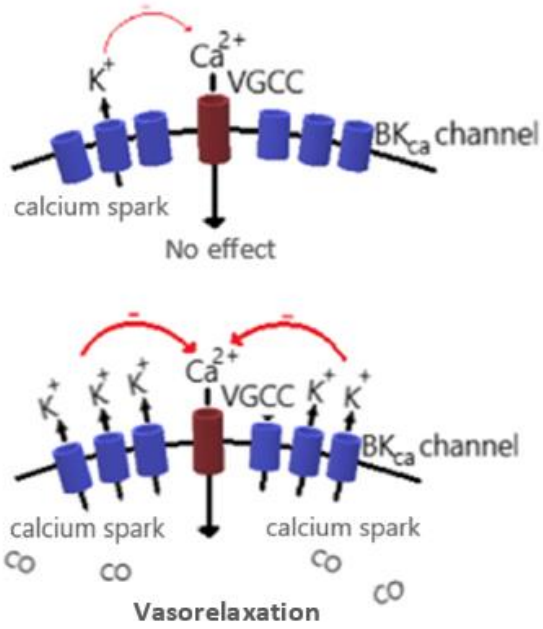

Figure 4: the activation of the BK_{Ca} channels mediated by CO. In the upper scheme is visible how the BK_{Ca} channels mediate little potassium efflux under influence of a calcium spark, providing minimal inhibition of the voltage gated calcium channels (VGCC). These minimal effects do not result in an effect. In the lower scheme the effect CO on these BK_{Ca} channels is shown. The presence of CO mediates coupling of the BK_{Ca} channels, increased sensitivity to calcium of these channels and lastly CO induces more calcium sparks. These effects mediated by CO result in more BK_{Ca} channel activation, more efflux of potassium and thus more inhibition of the VGCC, resulting in less intracellular calcium which mediates vasorelaxation.

2.3 Hydrogen sulfide

Enzymatic production

Being the last discovered gaseous molecule to play a role in the regulation of vascular resistance, the entire production route of hydrogen sulfide is not completely revealed yet. So far there are four different H_2S producing enzymes identified, whereas NO or CO are produced by only one enzyme. The four identified H_2S producing enzymes are Cystathionine-β-synthase (CBS), Cystathionine-ϒ-lyase (CSE), 3-mercaptopyruvate sulfur-transferase (3MST), cysteine aminotransferase (CAT)[1]. CBS is the main source of H_2S, while in the vascular smooth muscle cells CSE is the predominant source of H_2S. CSE is expressed in both the endothelial and smooth muscle cells[7,8,17]. Like CO, H_2S mediated effects are not necessarily dependent on the endothelial cells, whereas NO is only produced in the endothelial cells. 3MST is also found in both the endothelial and smooth muscle cells, CAT is only expressed in the vascular endothelial cells[1]. However, the role of 3MST and CAT in regulation of the blood pressure is yet to be discovered[7].

CSE is able to produce H_2S by catalyzing an elimination reaction, forming from L-cysteine pyruvate, NH_3 and H_2S. The catalyzation of this reaction mediated by CSE is only possible with the following

cofactors and substrates. Firstly the cofactor pyrodoxal-5'-phosphate, better known as vitamin B6, which binds CSE on each of the four monomers[18]. Furthermore the endogenous activator s-adenosyl methionine (SAM) induces a more active state of the enzyme[8]. And lastly, the substrate L-cysteine is crucial for the reaction catalyzed by CSE.

Enzyme regulation

SAM is mentioned before as an endogenous inducer of CSE activity. SAM is also able to induce CBS activity, through changing the C-terminal inhibitory domain of the enzyme, the activity of CBS is increased. Hypothetically via this mechanism SAM could also induce activity of CSE[1]. In addition, the availability of the substrate L-cysteine is influencing CSE activity as well. High concentrations of L-cysteine allow binding between L-cysteine and PLP to take place, removing the cofactor of the enzyme and thus leading to decreased enzyme activity[1]. Furthermore, increases of calcium concentrations in the cells expressing CSE is required to initiate formation of the calcium-calmodulin complex, which is inducing a more than twofold activity of CSE. Calcium or calmodulin alone does not alter CSE activity[19].

Signaling mechanisms

Similar to CO and NO, also H_2S is able to induce vasorelaxation via the cGMP mediated signaling pathway. In contrast with CO and NO, H_2S does not alter sCG in activity but inhibits the degradation of cGMP through inhibition of phosphodiesterase (PDE). Inhibited degradation of cGMP results in higher cGMP levels for a longer time. Inducing once again decreasing calcium levels, preventing phosphorylation of the myosin chains and thus inhibiting contraction. And thus vasorelaxation is induced[1].

Another similarity between the signaling mechanism of CO and H_2S is the activation of potassium channels. The induced potassium efflux mediates membrane hyperpolarization, which results in closing of the voltage gated calcium channel (VGCC). The decreasing calcium concentration allows vasorelaxation to take place via inhibition of myosin light chain kinase[8].

Like NO, H_2S also includes posttranslational protein modification in its signaling mechanisms. H_2S is able to perform sulfhydration, which adds an -SSH group to a protein. This reversible process mostly increases activity of the sulfhydrated proteins. Which is in contrast with NO-nirtosylation, since this mostly reduces protein activity[4].

Distinctive of the other gasotransmitters, H_2S is able to influence adenylyl cyclase (CA). CA synthesizes cyclic adenosine monophosphate (cAMP), which then activates cAMP dependent protein kinase (PKA). PKA phosphorylates myosin light chain kinase making it inactive, which results in vasodilatation. However, inhibition of this cAMP/PKA pathway is ensuring vasoconstriction, which is exactly what low concentrations of H_2S might be doing. Researchers of the National university of Singapore have presented that low concentrations of the H_2S donor NaHS (≤100 µM) were able to induce vasoconstriction, higher concentrations showed vasodilatation. The study did not (yet) prove an inhibitory role of H_2S, however they've proven that this constrictive effect is at least partially mediated through the cAMP signaling pathway[17]. The mechanism of H_2S mediated contraction/dilatation via cAMP/PKA is schematically presented in figure 5.

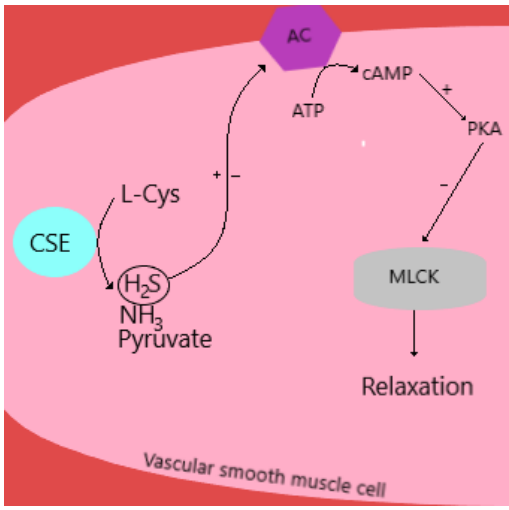

Figure 5: A schematic overview of how H_2S changes the vascular resistance via the cAMP/PKA signaling pathway according to researchers of the National university of Singapore. H_2S is produced in the vascular smooth muscle cells, high concentrations of H_2S are able to induce adenylyl cyclase (AC), resulting in more cAMP production. cAMP stimulates PKA activity which phosphorylates MLCK, preventing MLCK mediated contraction. Thus vasorelaxation is induced. On the contrary, low concentrations of H_2S mediate inhibition of AC, providing the exact opposite effect, contraction of the smooth muscle cells.

3. Cross communication

3.1 Nitric oxide & carbon monoxide

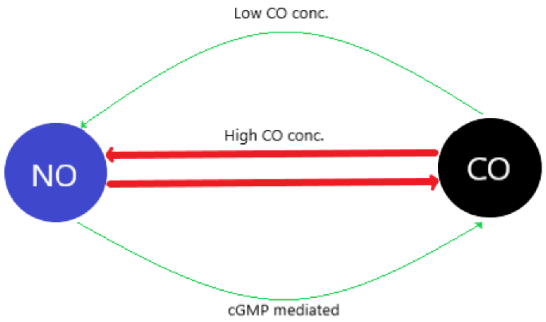

Figure 6: Cross communication between NO and CO in their production pathways. NO is able to directly inhibit CO by preventing HO-2 to produce CO. High concentrations similarly inhibit NO production. Low CO concentrations are able to prevent scavenging of NO, indirectly keeping NO levels higher. Additionally, NO is able to indirectly stimulate CO production via the cGMP mediated signaling mechanism.

Regulatory roles

The gasotransmitter NO and CO interact with each other in multiple ways. Firstly is NO able to directly inhibit HO-2 mediated CO production. This through binding of NO to the heme regulatory motif on the enzyme HO-2[3]. Relatively high concentrations of CO mediates the same direct inhibitory effect over eNOS, which decreases NO production[15]. In figure 6 alongside the effects of both these gasotransmitters on each other is shown. This mechanism probably prevents that high concentrations of both of these gasotransmitters are present at the same time. Both gasses mediate similar processes and high concentrations could induce to much vasodilatation, resulting in dangerous low blood pressures. On the contrary, low concentrations of CO do not influence eNOS at all, low CO concentrations compete with NO for NO-scavengers with heme groups[15]. Both NO and CO are able to bind heme, but if CO competes for the heme groups more NO remains free in the cell. Which means that the NO levels remain higher under the influence of low CO concentrations. Another contradiction is that NO is indirectly able to stimulate HO-2 mediated CO production, via the cGMP mediated signaling mechanism[3,4].
These regulatory mechanisms potentially compose a negative feedback mechanism. NO is capable of both inducing and inhibiting CO production, however, the inhibitory effect of CO on NO is concentration dependent. Thus if NO levels increase, the stimulatory effect of NO on CO can increase as well. If more CO is produced, the higher levels of CO mediate inhibition of NO, resulting in decreasing levels of NO. Since the stimulation of NO on the production of CO diminish with the decreasing NO levels, the CO concentration decreases as well. This way NO and CO are able to keep each other in balance.

Competitive roles

Both HO and NOS require the cofactors NADPH and molecular oxygen to catalyze their reactions, meaning that competition for these cofactors limit enzyme activity[4,15]. Low levels of CO specifically in the endothelial cells could also bind to the heme group present in eNOS, reducing the enzyme's activity[15]. Secondly, both gasses induce sCG, leading to increasing cGMP levels, activation of PKG and induction of vasorelaxation. This signaling mechanism is presented in figure 2, a simplified version is presented alongside, figure 7.
Because of this overlap in signaling mechanisms, competition for the substrates can take place. Meaning that both CO and NO feature a partial agonist role against the other gasotransmitter[20]. Additionally, since both activate the cGMP system the partial agonist role of the gasses prevent overstimulation of the cGMP vasorelaxation.
Similarly, NO and CO overlap in the signaling mechanisms in the activation of the BK_{ca} channels. In figure 8 is visible how all the three gasotransmitter overlap in the ability to induce the BK_{ca} channels. However, the signaling mechanism of how CO or NO mediate the activation of the BK_{ca} channels is not known. Thus it remains unclear if partial agonism is applied in this case.

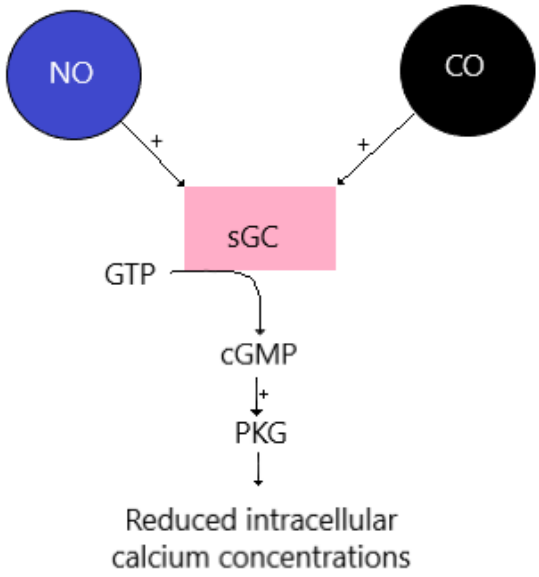

Figure 7: The overlapping sGC mediated signaling mechanism of CO and NO. Both are able to induce sGC, resulting in higher concentrations of cGMP. cGMP is able to induce PKG mediated calcium efflux, which results in vasorelaxation.

Substitutive roles

Presented in research of the University Health Sciences Center in New Orleans is that CO does not pursuit a strong role in the relaxation of the vascular resistance, when an intact NO system is in place. However, endothelial damage or an intact NO system reveals the dilatory function of the CO system[9]. Potentially acting as a protective mechanism to keep, even under dysregulated circumstances. To what extent CO is able to compensate the missing NO remains to be investigated. Additionally, the interplay between the NOS/NO pathway and the HO/CO pathway have shown to be age dependent in the arteries of pigs[3]. For this reason it is a possibility that CO is able to compensate for NO in adults, but during the development in younger individuals the absence of NO causes problems which CO is not able compensate.

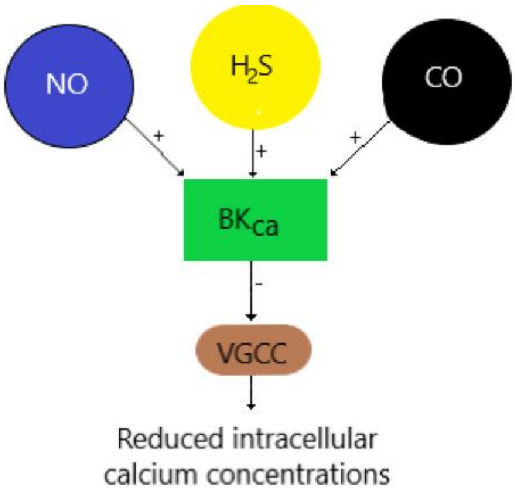

Figure 8: a schematic overview of how the three gasotransmitters all are able to induce the BK_{ca} channels. This activations mediates an efflux of potassium which results in membrane hyperpolarization. Voltage gated calcium channels (VGCC) are closing as a result of the change in membrane potential. Closing of the calcium channels means no influx of calcium and thus a decrease in calcium availability, leading to vasorelaxation.

3.2 Nitric oxide & hydrogen sulfide

Regulatory roles

NO is the only gasotransmitter which enhances the production of another, it enhances both the expression and the activity of CSE. In contrast, the precursor for NO, hydroxylamine, inhibits CSE activity[8].

H_2S is able to act as an NO-scavenger, forming nitrosothiols[17]. Thus H_2S causes decreasing levels of NO, and is in addition also able to inhibit eNOS activity. Presented in figure 9 are the effects of each of the gasotransmitters on each other or the enzymes responsible for gasotransmitter production. These findings suggest some kind of negative feedback mechanism between the two gasotransmitters. If the production of NO is high, a lot of the precursor is available. Meaning that the production of H_2S is inhibited, preventing H_2S production. But when the concentration of NO increases the positive effect of NO on CSE increases as well. Resulting in more H_2S production and with that an increasing inhibitory effect on both NO and eNOS. Providing inhibition of the eNOS/NO system until the stimulation of NO on CSE diminishes and the inhibitory effect of the precursor hydroxylamine returns, then the circle can start over.

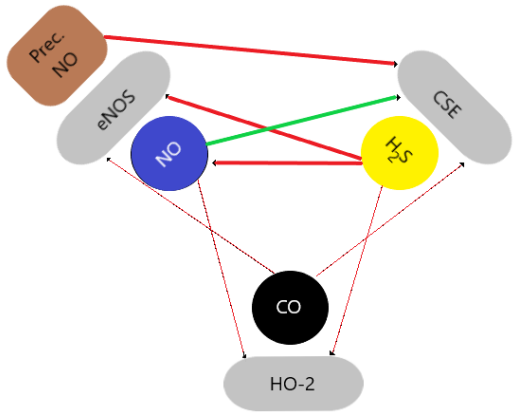

Figure 9: an overview of influence of one gasotransmitter on the other or on the enzymes CSE, eNOS or HO-2. Additionally, the precursor of NO, hydroxylamine, is able to inhibit the activity of CSE and is thus included in this figure. The cross communication is discussed in this chapter and thus emphasized in this figure. Visible is how the precursor is able to inhibit CSE, whereas NO is able to stimulate CSE. Furthermore is H_2S able to inhibit both eNOS and NO in activity. Meaning that in high concentrations NO is able to activate H_2S production which inhibits further production of NO and NO itself in activity. Creating a negative feedback circle.

Competitive roles

As mentioned in chapter 2 both NO and H_2S are able to mediate post translational protein modification. In this aspect both gasses could compete since they both target cysteine residues in proteins for nitrosulation or sulhydration, as presented in figure 10[1,4]. Which is interesting since nitrosylation induces reduced protein activity, while sulfhydration mediates increased protein activity. Perhaps both mechanisms are more complicated than now understood. For example, if both are somehow communicating, nitroxylation and sulfhydration could together turn various proteins on and of in activity.

Furthermore is mentioned in chapter 2 that H_2S able to inhibit the degradation of cGMP[7]. Meaning that both NO and H_2S are able to mediate vasorelaxation through cGMP, but do not direct overlap in their signaling mechanism. In addition is H_2S able to induce K_{ATP} channels to hyperpolarize the cell membrane, leading to closing of VGCC and thus decreasing calcium concentrations[1]. NO is able to

mediate hyperpolarization of the membrane as well, resulting in the same decreasing calcium concentration, only NO activates other ion channels. Thus both NO and H_2S mediate highly similar effects, there is no direct overlap between these mechanisms. Potentially these findings suggest that H_2S and NO could cooperate in inducing vasorelaxation.

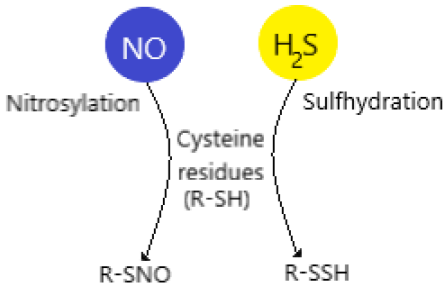

Figure 10: NO mediated nitrosylation and H_2S mediated sulfhydration compete for the same cysteine residues. An important difference is that nitrosylation is reducing the protein his activity, while sulfhydration increases the activity of the protein.

Substitutive roles

As mentioned, the mechanisms mediated by NO and H_2S do not directly overlap, substitution between the two gasotransmitters is therefore only indirectly. The gasses do however share that both increase cGMP availability. An important difference is that H_2S derived vasorelaxation is independent of the endothelial cells, meaning that incase of damaged endothelial H_2S derived vasodilatation is able to continue. Perhaps even compensating for the loss of dilatation without the intact eNOS/NO system.

3.3 Carbon monoxide & hydrogen sulfide

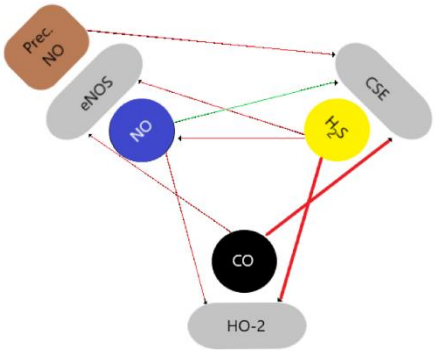

Figure 11: A copy of figure 9, however in this figure the cross communication between CO and H_2S and their producing enzymes is emphasized. Visible is that both gasotransmitters are directly able to inhibit the production of the other.

Regulatory roles

The gasotransmitters H_2S and CO are both directly inhibiting the production of each other[3]. Both compounds have shown to possess over heme binding moieties, and since both CSE and HO enzymes contain a heme group potentially via binding the heme groups the compounds are able to inhibit these enzymes. Next to inhibition of the production of H_2S, the H_2S mediated posttranslational sulfhydration is also inhibited by CO[4]. The inhibition of the H_2S production is thought to be an allosteric inhibitory mechanism. The binding between the enzymes and CO is however at a relatively slow rate, but this could allow filtration to avoid unproductive responses[5].

Gene transcription

One of the inducing mechanisms mediated by H_2S is via the induction of gene transcription. H_2S induces nuclear localization of the transcription factor NRF2, allowing the expression of HO-1 to increase. Resulting on the longer-term in increased CO production. However, as mentioned in chapter 2 the HO-1 mediated CO responses are slow. The enzyme needs to be made before CO is released by HO-1, which takes time. Thus this regulatory mechanism expands over several hours, other more direct influencing roles between CO and H_2S remain to be identified.

Competitive roles

The cGMP mediated signaling pathway does not overlap between CO and H_2S, as it did not between NO and H_2S. Additionally, CO is also not able to mediate protein modification like H_2S does. This means that the only overlapping signaling method between the two gasses is via activation of the BK_{ca} channels. Because the exact mechanism of how CO and H_2S are able to activate these channels is still unknown, little can be said about any competition between the two gasses. Though perhaps these gasses are able compensate one another in the activation of the BK_{ca} channels which would mediate some vasodilatory effect if one of the gasses were absent.
Furthermore, the enzymes HO and CSE are not responsive to the same substrates or cofactors. Meaning that regulation through competitivity is not recognized in research so far. However, since that H_2S is the latest discovered gaseous transmitter it's only logical that more research is needed to fully understand the entire signaling mechanisms. For example, CAT and 3MST are both H_2S producing enzymes, and are expressed in the vascular system. Both of those enzymes are yet to be understood, perhaps these interact more with the CO mediated signaling pathways.

Conclusion

NO is considered the main regulator of vascular resistance in our blood vessels. NO is produced in the endothelial cells mainly by eNOS, the physiological effect of NO is mediated in the smooth muscle cells where it is able to induce vasorelaxation via the cGMP signaling pathway. CO is mainly produced by HO-2, expressed in both the smooth muscle and endothelial cells of the vascular system. CO mediates vasodilatation mainly via activation of potassium channels inducing calcium efflux. And lastly H_2S is in the vasculature mainly produced by CSE, an enzyme also expressed in both the endothelial and smooth muscle cells of the vasculature.
The cross communication between the gasotransmitters is mediated either through regulatory roles of one gasotransmitter on the enzyme of another, through competitive roles for either substrates for enzymatic conversion, cofactors or substrates for protein modification. But CO also showed to mediate increased H_2S expression through transcription factor mediated DNA transcription.
So far it's clear that the gasotransmitters are able to compensate one another a bit. When NO production by eNOS is dysfunctional, nNOS is capable of maintaining some of the vasodilatation. Total dysregulated NO signaling could partially be taken over by H_2S or CO which are able to mediate partial dilatation completely independent of the endothelial cells. And lastly a cytochrome p450 mediated mechanism is found which releases NO form nitrite, potentially this mechanism is able to restore NO availability during endothelial dysfunction[13]. However all of these mechanism show partial substitution, indicating that the complete function of the gasotransmitters is not regained through substitution.

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