Abstract
Nitric oxide (NO) is produced in endothelial cells by nitric oxide synthase. This NO plays an important role in normal pathophysiology and homeostasis of human body. NO exerts a surfeit of biological effects in the cardiovascular system. Hydrogen sulphide (H\textsubscript{2}S) is synthesized naturally in the body from L-cysteine mainly by the activity of two enzymes, cystathionine-\(\gamma\)-lyase and cystathionine-\(\beta\)-synthetase. The H\textsubscript{2}S plays a vital role in vasorelaxation. Both NO and H\textsubscript{2}S works by different mechanism to produce the effect of vascular muscle relaxation. The present review focuses on the molecular enzymatic targets for NO and H\textsubscript{2}S for producing vascular muscle relaxation with recent advances and studies.

Key Words: Vasorelaxation, L-cysteine, cystathionine-\(\gamma\)-lyase, cystathionine-\(\beta\)-synthetase, (NO) nitric oxide and Hydrogen sulphide (H\textsubscript{2}S).

Introduction
Small molecular weight gasses are amongst the most studied biological mediators over the past twenty years. Gaseous mediators such as nitric oxide (NO) and hydrogen sulfide (H\textsubscript{2}S) are now accepted as a most important of such mediators in human body mediating blood flow, neurotransmission, immune reaction, mucosal integrity and intonation of inflammatory reactions. The most important physiological role of NO is in cardiovascular and inflammatory diseases. In cardiovascular system NO produces vasodilatation which is ligand mediated and flow dependent, inhibition of vasoconstriction by inhibition of angiotensin II and sympathetic vasoconstriction, anti-thrombotic by inhibition of platelet adhesion to the vascular endothelium, anti-inflammatory by inhibition of leukocyte to the vascular endothelium and anti-proliferative. NO is secreted as a pro-inflammatory mediator of arthritis in joints (1-7). In recent years, physiological roles of hydrogen sulfide (H\textsubscript{2}S) have been documented, and there is promising evidence that this endogenous gaseous substance can modulate inflammatory processes. Indeed, H\textsubscript{2}S donors have been exposed to reduce edema formation and leukocyte adherence to the vascular endothelium, and to inhibit pro-inflammatory cytokine synthesis. Moreover, an H\textsubscript{2}S donor also increases resistance of the gastric mucosa to injury and speed up repair. H\textsubscript{2}S probably works as vascular smooth muscle relaxant both in vitro and in vivo by opening vascular smooth muscle K\textsuperscript{+}\textsubscript{ATP} channels (8-10). There is a need to design more NO and H\textsubscript{2}S releasing drugs for the therapeutic application as vascular muscle relaxation potency which can be used in cardiovascular complications as well as pain management. The present review discusses the different molecular and enzymatic targets for designing new molecules for vascular muscle relaxation action.

cGMP mediated Soluble Guanylyl Cyclase: With cGMP signalling being as crucial as it is to
the physiologic functions of the heart and vasculature, in many cardiovascular diseases major factor is dysfunction at any level of the cGMP signalling.

Structure of guanosine 3', 5'-cyclic monophosphate: Because of endothelial cell dysfunctioning both systemic and pulmonary hypertension results; which further results in disorders like vascular smooth muscle dysfunction, systemic and pulmonary hypertensive and ischemic heart disease, cardiac myocyte dysfunction, hypertrophic and ischemic heart disease as well as cardiomyopathy and heart failure (Figure 2). Dysfunctional cGMP signaling has also been concerned in dysfunctional mitochondrial metabolism which is a new area that is now beginning to be explored and targeted for its role in heart disease.

Structure of Guanosine 3', 5' - cyclic monophosphate: Nitric oxide activates soluble guanylyl cyclase (sGC) by binding of NO to heme and non-heme sites of sGC. Two-step activation process of NO binding results in two distinct NO-bound forms of sGC which are characterized by low and high enzymatic activity. Initially NO forms an inactive but NO-responsive six coordinate nitrosyl intermediate by binding to the ferrous, five-coordinate heme moiety of sGC. Further NO binding results in conversion of nitrosyl intermediate sGC species into five-coordinate nitroxyl complex in the presence of magnesium, cGMP and pyrophosphate (11-13). This second NO-binding step accelerates the basal rate of conversion of GTP to cGMP by several hundred folds by breaking the bond between the heme iron and the protein histidine axial ligand results in a conformational change in the catalytic domain of the enzyme (Figure 1). However, in the absence of magnesium, cGMP, pyrophosphate, NO binding to the six-coordinate nitrosyl intermediate sGC species does not activate the enzyme. Thus, at low levels of NO, sGC remains in a low-activity state, whereas at high levels of NO and substrates/products, even the low-activity state sGC can be converted to the highly active state.

As initially suggested by the study that the rate of NO dissociation from sGC is much slower than the rate of sGC deactivation so NO can also activate sGC by binding to a non-heme site (14). Again the initial step of sGC activation involves NO binding to the ferrous heme moiety of sGC. However, the second NO binding episode involves binding of NO at a non-heme site, which ruptures the histidine-iron bond and completely activates sGC. At low NO levels, NO dissociates from the non-heme site to give a low-activity state of sGC. The above mechanism of non-heme NO-binding is responsible for cascade of activity by which rapid rise in cGMP production though acute increase in NO. At continual low levels of NO, sGC produces cGMP at long-lasting, low levels. So in this regard the compounds having ability
to release NO, which will bind to the heme or nonheme part of the enzyme is very important therapeutic application to cure vascular muscle related disorders. It was observed that cGMP-Dependent Protein Kinase I may be involved for smooth muscle relaxation (15). The compounds such as organic nitrates, nitrates and COX-inhibiting nitric oxide donators (CINODs) are in clinical trial (16). Following are some examples of compounds, releases NO and causes vasorelaxation.

NO donor moieties

Phosphodiesterases: Vascular smooth muscle responses to cGMP-dependent vasodilatory stimuli are regulated by the activity of vascular smooth muscle phosphodiesterase, which catalyzes hydrolyzation of cGMP to inactive products.

There are 12 isozymes of phosphodiesterases have been identified in mammalian tissues. Out of these type 5 phosphodiesterase (PDE5) is the predominant isozyme that contributes to regulation of cGMP content in vascular smooth muscle (18, 19). Sildenafil is a specific inhibitor of PDE5 that has been approved for the management of erectile dysfunction in humans (20–22). There is less information about the effect of PDE5 inhibition with sildenafil on endothelium-dependent vasodilation in patients with heart failure.

Nitric oxide (NO) is constitutively produced in the lung by NO synthases from vascular endothelium and the airway epithelia (23 - 24). Depending on alveolar ventilation local NO production regulates pulmonary perfusion to assure optimized ventilation distribution (25 - 26). Nitric oxide synthase activity is regulated on transcriptional and posttranslational redox-based modulation level (27). NO, prostaglandins and natriuretic peptides activates common signalling pathway of endogenous vasodilators such as cyclic adenylate monophosphate [cAMP] and cyclic guanylate monophosphate [cGMP]).

Phosphodiesterases (PDEs) represent a superfamily of enzymes, with PDE-1 through PDE-12 being currently known, that inactivate cAMP and cGMP, with different tissue distribution and substrate specificities (28-29). Due to the stabilization of these second messengers, PDE inhibitors differentially regulate levels of cAMP and/or cGMP, depending on their selectivity profile. Therefore, they might offer as therapeutic tools to boost and prolong prostanoid- and NO-related vascular effects. The efficacy of this approach has been proven in several experimental studies (30, 31). Interestingly, the major cGMP-degrading PDE and PDE5 are abundantly present in lung tissue (29). PDE5A was the first cGMP-selective PDE to be discovered and is also activated by cGMP, which binds to its GAF regulatory domain. It is expressed in vascular smooth muscle, endothelium, and fibroblasts.

Molecular Targets for Vascular Muscle Relaxation
In this regards, there is a need to investigate more molecules specifically targeting PDE/PDE5/PDE5A for its inhibitory potential to obtain a precise structure and phenomenon for vasorelaxation of smooth muscle. Inhibition of PDE5A is the main target for binding of drug molecule to produce smooth muscle relaxation potency.

**Calcium Channel:** Calcium channel blockers are widely prescribed drugs for the management of cardiovascular disease and have been approved by regulatory authorities for the treatment of hypertension and symptomatic relief of angina pectoris. Congestive heart failure is an increasingly common syndrome that continues to be a major cause of morbidity and mortality despite current therapy. Many patients with heart failure are treated with calcium blockers as hypertension and coronary disease are the two most common causes of heart failure.

Calcium channels can be divided into two principal subtypes: voltage-activated channels of the sarcolemma and calcium release channels of the sarcoplasmic reticulum. The voltage-activated channels comprise L, T, N, P, Q and R subtypes. The N, P, Q and R type channels are largely found in the nervous system. Voltage-activated L-type calcium channels are present in abundance in myocardial cells and vascular smooth muscle. Muscle contraction happens by entry of calcium through the L-channel. This channel is made up of five subunits termed α1, α2, β, γ and δ. α1 subunit contains the calcium channel conducting pore and binding site for calcium channel blockers. It consists of four repeating hydrophobic motifs (I-IV), each consisting of six segments (S1-S6) that are very similar and span the membrane. Each of the four motifs, which are folded in on them contributes to the calcium channel (32 - 33). Beta-adrenergic stimulation promotes phosphorylation of the α1 subunit intracellularly, increasing the probability of channel opening (34).

**Binding Sites for Calcium Antagonists:** Each of the three classes of calcium antagonist has a...
different binding site, located on the alpha subunit. For dihydropyridines, it is on the extracellular loop of the S6 segments (35). Diltiazem also binds on the S6 segments, but not in the extracellular loop (36) and verapamil blocks the calcium channel from the intracellular side (37). The dihydropyridine receptor is the best defined, and numerous dihydropyridine analogues have been synthesized such as nitrendipine or nicardipine. These drugs can activate the receptor and have positive inotropism at low doses, but negative inotropism at high doses (38). Analogues of verapamil, such as anipamil and tiapamil, do not have the same intrinsic mechanisms of action as verapamil (39 - 40). The different binding sites of the three classes help explain their differing tissue selectivities. Because the binding sites of verapamil and diltiazem are intracellular, they only gain access when the channel is open (41).

Immunocytochemical and in situ hybridization studies have made known the presence of the Ca2+:calmodulin dependent NO synthase (NOS) in gonadotrophs and folliculostellate cells of the rat anterior pituitary gland. This fact is also similar in normal and neoplastic human pituitaries (42 - 44). A local regulatory function of NO has been recommended, as it can inhibit in vitro prolactin release and moderately mediate the inhibitory effect of dopamine and GABA on prolactin secretion (45). Furthermore, NO also modulates LH, growth hormone and ACTH release (46). NO has also been shown to induce intracellular calcium changes in different cells such as endothelial cells, intestinal epithelial cells, pulmonary artery and smooth muscle cells.

Keeping in view the findings reported above there is a scope to target calcium channel blocking action by nitric oxide donor drug moieties. There is need to develop correlation of pharmacological action between the different subunits of calcium channel and NO for smooth muscle relaxation potency.

**Potassium Channel:** Potassium channels, which are the main determinants of resting membrane potential (RP), have been emerged as possible mediators of NO-evoked hyperpolarisation and vasodilatation (47 - 48). Vascular smooth muscle cells and endothelial cells have been found to express at least five types of K+ channels depending on voltage dependence, activation and inactivation kinetics, sensitivity to regulating ions, toxins or other chemical factors. They include a voltage-dependent ATP-sensitive channel (KATP), a depolarization-activated channel (KV), a fast inactivating channel both blocked and an inwardly rectifying channel (Ki), a Ca2+-activated channel (KCa) and apamin (49-51).

There is a contradiction in different reports about the type of K+ channels activated by NO is heterogeneous with vascular beds and animal species. For instance, NO activates K_Ca and relaxes the vascular smooth muscles of rabbit aorta and rat mesenteric arteries (52 - 53). But the K_Ca is in fact not involved in vasodilatation responses of the newborn pig pia arterioles to NO donor or hypoxia (54). It is also reported that

---

**Fig. 3.** Vasodilatory effect of H$_2$S in vascular smooth muscle and tissues.
NO hyperpolarizes the vascular smooth muscles by opening of $K_{ATP}$ channels in rabbit and rat mesenteric arteries (55). In rat small mesenteric artery, NO activates both $K_{Ca}$ and $K_{ATP}$ (56), in rat aorta, the NO donor failed to activate $K_{ATP}$ (57).

$H_2S$ relaxes vascular smooth muscle both in vitro and in vivo probably by opening vascular smooth muscle $K_{ATP}$ channels through Kv1 (58-59). The figure 3 explains the mechanism of $H_2S$ release in the smooth muscle and its therapeutic implications (60). In rats with experimentally induced hypoxic pulmonary hypertension the reduced expression and activity of Cystathionine-γ-lyase CSE coupled with a decrease in plasma $H_2S$ concentration was observed in lung tissue recently (61).

**Conclusion**

The above information and recent studies have clearly shown that the gaseous mediators such as nitric oxide(NO) and hydrogen sulphide ($H_2S$) are to be the next molecular targets for vascular muscle relaxation action. The potency and role of NO and $H_2S$ as a vasodilator in several clinical conditions makes them an attractive therapeutic target for many conditions. These molecular targets are to be studied and further research is needed to characterize the molecular mechanisms by which NO and $H_2S$ exerts these actions, including their interaction with other molecules in the cell. There is need to investigate $H_2S$ as a vasodilator in humans. The new molecules having a potency to donate NO and $H_2S$ should be synthesized and preclinical and clinical study should be performed. It is hoped that these molecular targets would help to address several unanswered questions in health and diseases.

**Acknowledgments**

The author would like to acknowledge grant support from All India Council for Technical Education (AICTE), New Delhi as a Research Promotion Scheme (RPS) (file no. 8023/RID/RPS/20/2011-12). Authors are also thankful to Principal Bharati Vidyapeeth College of Pharmacy, Kolhapur for providing all necessary facilities to carry out above work.

**References**


Caused by Ischemic Preconditioning in the Rat Heart and Cardiac Myocytes. The J pharmaco and exp thera, 316: 670- 678.


