Abstract

Nitric Oxide has been implicated as a toxic chemical throughout much of its history only until recently, where it has been shown to be a very important biological messenger molecule in our bodies. It has been shown that nitric oxide can regulates many vital functions in our bodies, including gastrointestinal digestion provide immunity defense. The medicinal community has jumped on this newly found discovery, researching new drugs and methods to get nitric oxide to benefit our bodies when its levels become unregulated. Because of its earlier history, however, nitric oxide could be fatal in certain amounts, and the medicinal community should exercise caution when testing these new drugs on humans. Its biological impact has only been unearthed in fairly recent years, and nitric oxide's ambivalent character only becomes more pronounced as research continues.

Introduction

Nitric Oxide (NO) research had emerged in the past few decades as a result of the increasingly disastrous effects of automobile combustion and other combustible processes. Outside of our bodies, nitrogen reacts with oxygen only in extreme temperatures such as through that of lightning (15). Concentrations of NO had begun to rise rapidly, forming acid rain and heavy smog as a result (See Equation 1) (3).

$$\mathbf{2NO + O_2 \dashrightarrow 2NO_2}$$

$$\mathbf{3NO_2 + H_2O \dashrightarrow 2HNO_3 + NO}$$

Equation 1. Formation of Nitric Acid from Nitric Oxide (16)

It catalyzes ozone destruction by converting ozone to dioxygen (16). NO's early correlation as an air pollutant had led to the assumption that it was solely a toxic chemical. It

wasn't until the late 1980s that NO has been discovered to play a very important role in biological chemical signaling in mammals (3).

Nitric Oxide is a free radical diatomic molecule which is soluble in saline solutions (7). Despite its unpaired electronic structure, it is a very unreactive molecule (3). It possesses a slightly sweet odor, is colorless, and is a gas at room temperature (13). NO's recent discoveries have led it to be the instigator behind a number of very crucial biological processes in mammals. It is now understood to directly maintain homeostasis, regulate blood pressure, provide immune defense, and regulate the digestive tract (3).

With all of the crucial benefits NO provides, it also has the ability to react with certain molecules to form much more reactive free radicals, which in turn leads to many cytotoxic effects (3). External administration of NO has incited hesitation in the therapeutic community as a number of drugs helping to regulate concentrations of NO in the body have been approved by the FDA. The complete elucidation of NO's purposes have yet to be fulfilled, as this recent biological breakthrough still leaves many questions unanswered (3). The most crucial question that needs to be answered before medicines become approved is: what concentrations of NO form predominately reactive free radicals reactions and cause damage to the body? The answer has never been clear cut in modern research, and NO's most prominent link to modern medicine has been through the recently approved Erectile Dysfunction (ED) drugs such as Viagra, Cialis, and Levitra, which are really popular drugs and make their respective companies billions of dollars every year (14). While NO's role in facilitating an erection is now understood, it is too soon to approve anything that can regulate NO in the body, because much of NO's action still remains a mystery.

Background

Nitric Oxide (NO) is produced in vivo by enzymes reacting with the amino acid l-arginine (3). These enzymes are specifically called Nitric Oxide Synthase (NOS) enzymes, and there are three different types: neuronal, endothelilal, and inducible. Endothelial Nitric Oxide Synthase (eNOS) is activated by released Calcium ion (Ca^{2+}) attaching to calmodulin in the endothelial, or blood vessel lining, layer. The NO that is produced in the endothelilal layer by eNOS is directly and indirectly responsible for many of NO's smooth muscle relaxing and vasodilating properties. Neuronal Nitric Oxide Synthase (nNOS) is produced in much the same manner (3), but it is mostly found in the nervous system. NO is carried throughout the blood stream either by binding to Fe in a heme protein complex or by binding to Fe present in hemoglobin, where it can then subsequently react at various sites throughout the body (7). NO, once produced, can activate soluble guanylate cyclase enzyme (sGC), which then increases the concentration of cyclic guanosine monophosphate (cGMP). cGMP reacts with protein kinases (PKG) to reduce levels of calcium, resulting in vasodilation of the endothelium and thus leading to increased blood flow through smooth muscle relaxation (See Figure 1) (2).

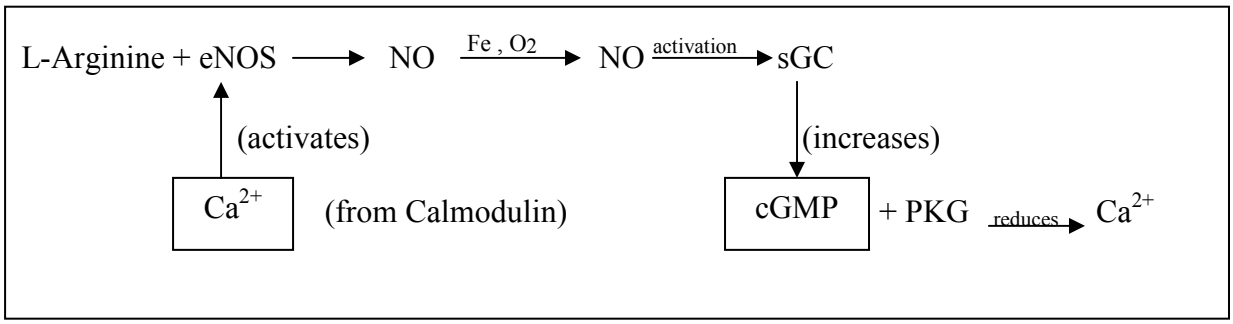

Figure 1. Nitric Oxide Reactions in Smooth Muscle Relaxation

Due to its small size and high lipophilicity, NO can travel through many biological membranes and activate target molecules as far as 300 micrometers away (3). It has been

implicated in a number of beneficial tasks, including the registering of olfactory senses, where the NO-cGMP levels are raised in the olfactory bulb of mammals during scent detection. It also helps formation of memories, specifically in reminding us what foods are inedible (7). While many of NO's direct interactions are beneficial to the body, indirect interactions can have disastrous consequences. At high concentrations, NO can react with superoxide (O_2^-) and free oxygen gas to form peroxynitrite and dinitrogen trioxide, respectively (1). This can lead to nitration and nitrosation reactions, forming very reactive free radicals which can target and can potentially inhibit a number of protein sites (See Figure 2).

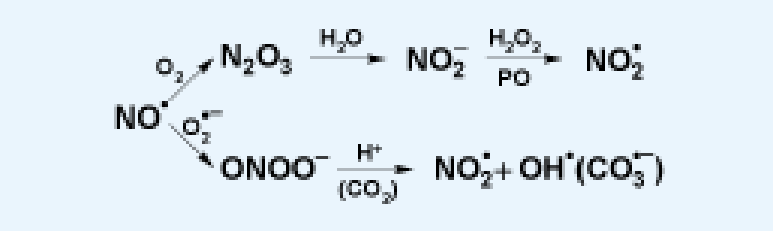

Figure 2. Nitrosation and Nitration reactions to form reactive free radicals (3)

Unlike nitric oxide, these reactive free radicals increase oxidative stress within the vascular walls and lead to significant tissue damage to the endothelial layer (9). It also has been shown that patients with high blood pressure suffer from a dysfunction in their NO-cGMP pathway in their endothelial layer. This dysfunction also applies to the very common problem of erectile dysfunction (ED) (4). Drugs like Sildenafil Citrate (Viagra) help to block inhibitors that disrupt the NO cGMP pathway, thereby helping to dilate the blood flow into the corpus cavernosum in the penis and provide an erection. With the indirect effects NO has on the body, there should be caution exercised when evaluating and approving any sort of medicine that promotes NO production. Its recent biological breakthrough should not overshadow and dismiss

its prior findings to have the potential to be extremely toxic to human beings, especially when it comes to its promotion through external therapeutic administration of drugs such as sildenafil citrate. The specific effects, mechanisms, and risks of NO production in the corpus cavernosum will be reviewed in the following report.

Focus

Endothelial Nitric Oxide Synthase's (eNOS) production of nitric oxide (NO) is directly responsible for maintaining penile erection (5). Nitric Oxide in the endothelial layer is directly produced from L-arginine and eNOS. eNOS contains a reductase structure, called Nicotinamide Adenine Dinucleotide (NADPH), which acts as a reducing agent to the terminal nitrogen atom on the doubly bonded amine group of l-arginine in the presence of dioxygen and water (See Figure 3) (3). eNOS is calcium ion dependent throughout this reaction, and L-Citrulline is also formed as a product.

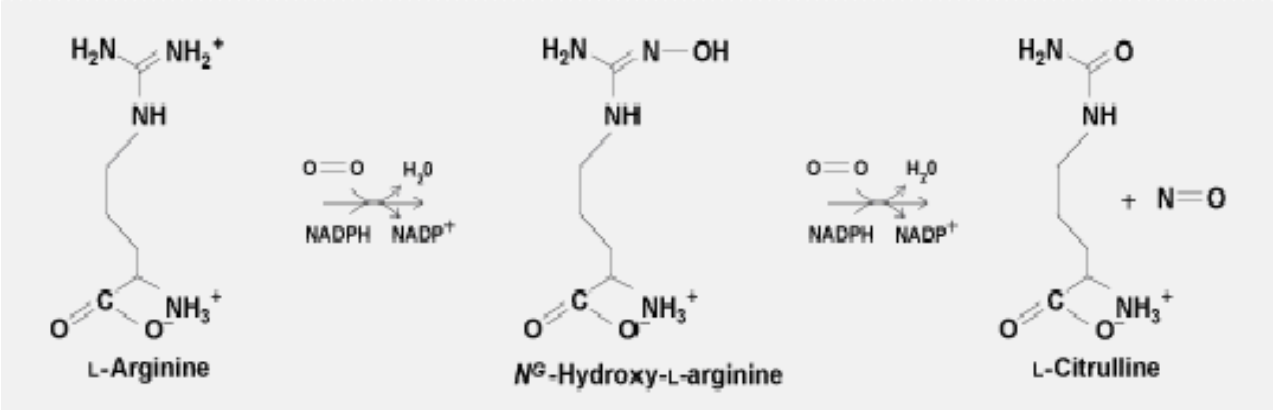

Figure 3. Formation of Nitric Oxide from L-Arginine and eNOS (3).

After formation of NO, it then diffuses across many biological membranes rapidly. It can form a covalent bond by iron bound in heme groups such as guanylyl cyclase or by iron present in hemoglobin in various sites throughout the body (7). When it bonds to soluble guanylyl cyclase, it activates it, which helps to catalyze the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) by dephosphorylation, a process which involves hydrolysis, forming pyrophosphate as a byproduct (See Figure 4) (3).

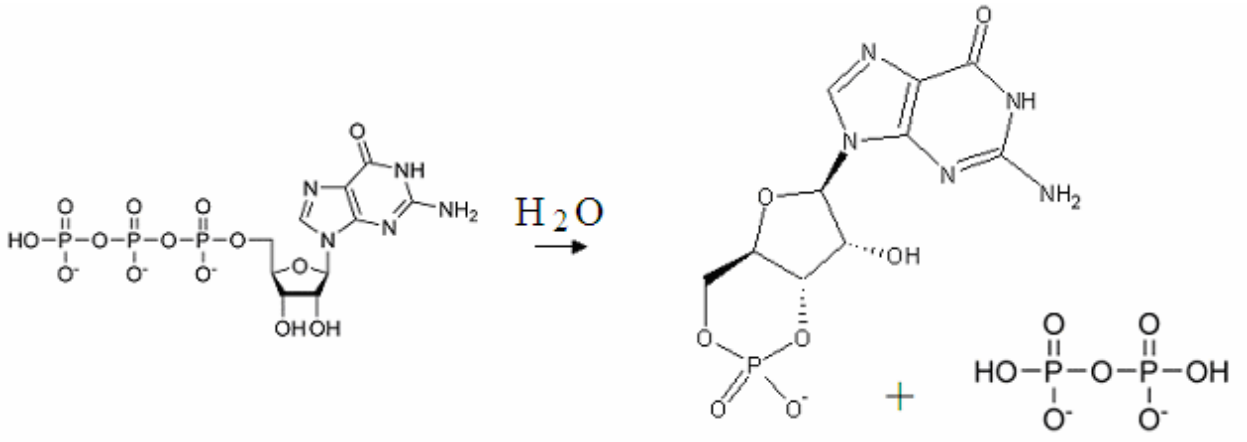

Figure 4. Conversion of GTP to cGMP by dephosphorylation (8)

cGMP then activates protein kinase G, a specific protein that phosphorylates calcium transporters, reducing calcium ion concentrations in the endothelial layer and moving them back to the smooth muscle cells. This reduction in calcium ion concentration allows the smooth muscles to finally relax, since this ion is responsible for its contraction (3).

Endothelial dysfunction results in a marked decrease in endothelial nitric oxide synthase production. Damage to the endothelium can happen as a result of smoking, diabetes, or hyperlipidemia (obesity) (9). This damage can lead to cardiovascular problems as well as erectile problems. Erectile dysfunction (ED) is directly correlated with endothelial dysfunction,

and thus ED can be the first noticeable biological indicator of cardiovascular problems such as hypertension, stroke and heart failure. Nitric Oxide's role in the endothelial layer is affected greatly as a result of these problems, as it cannot be produced in sufficient quantities to support adequate smooth muscle relaxation and contraction (6). This NO deficiency disrupts the NO-cGMP pathway, lowering intracellular levels of cGMP. These lowered levels lack the ability to reduce calcium ion by means of transportation to its original site in the smooth muscle, thus preventing vasodilation. Drugs like sildenafil (Viagra), vardenafil (Cialis), and tadalafil (Levitra) help to increase the efficiency of cGMP, which is constantly being degraded in our bodies. Sildenafil was discovered by Pfizer in England, and was originally intended to be a medicine to treat hypertension or angina, but in clinical trials it was very ineffective. Penile erections, however, were easily inducible with the drug, and the FDA decided to approve the drug for erectile dysfunction in 1998 (14). cGMP is constantly being degraded by phosphodiesterase 5 enzyme (PDE5). Phosphodiesterase is a family of enzymes that break down a phosphodiester bond. Specifically, phosphodiesterase 5 is responsible for the degradation of cGMP's phosphodiester bond before it reaches the endothelial layer of the corpus cavernosum (11). It converts cGMP to guanosine 5'monophosphate (GMP) enzyme (See Figure 5) (10).

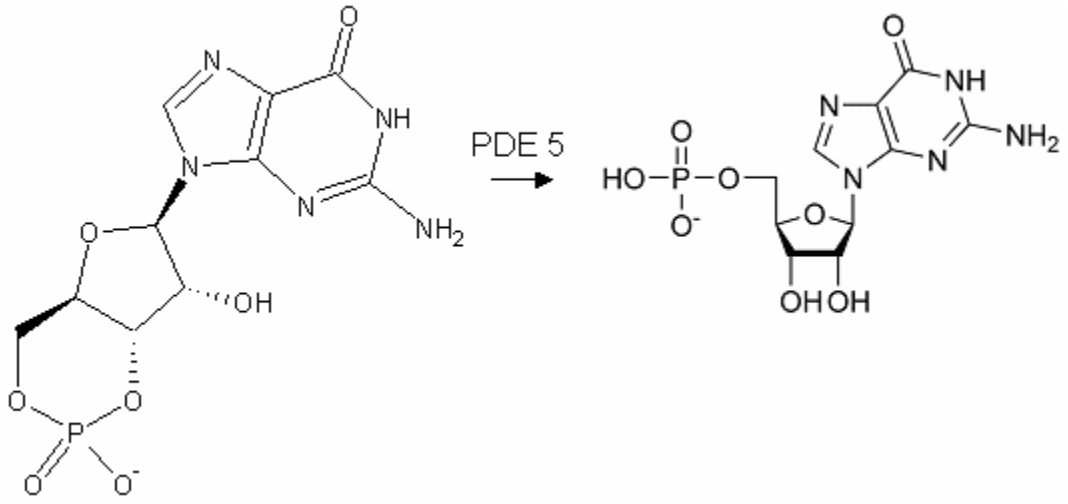

Figure 5. Conversion of cGMP to 5'-GMP by PDE5 (8)(10)(11)

This conversion to guanosine monophosphate renders it useless in the process of smooth muscle relaxation. Thus, the three drugs for erectile dysfunction mentioned above act as phosphodiesterase type 5 (PDE5) inhibitors, blocking the cGMP-GMP pathway, thus increasing the efficiency of cGMP by making up for its decreased production. This is how all three drugs act in order to provide penile erections to those with erectile dysfunction. Commonly labeled side effects of these drugs include hypotension, change in vision and priapism, which is an erection lasting more than 4 hours (11). However, a recent study indicates that PDE5 inhibitors actually help to regulate erectile function through PDE5 control and normal penile erections. The assumption for the study was that PDE5-inhibitors would assume a role in balancing adequate levels of cGMP needed to sustain an erection. This has worked successfully for a number of patients with long-term recurring priapism (12). A PDE inhibitors' efficacy has been shown to cross over to the other families of phosphodiesterase enzymes. While sildenafil, vardenafil, and tadalafil are designed to selectively inhibit phosphodiesterase type 5, it also inhibits the other 10 families with varying degrees of efficacy. PDE6 enzyme, which lies in the retina, is inhibited to 1/10 the degree that PDE5 is when the ED drugs are used. This explains

why altered vision is commonly reported as a side effect in ED drug users; retina activity is being altered as a direct result of using the drug (11).

PDE-inhibitor activity ratio to PDE5				
PDE	6	1	3	2-4, 7-11
Ratio	1:10	1:80	1:4000	1:700+

Table 1. PDE5 Inhibitors' Inadvertent Cross-Selectivity Ratios Relative to PDE5

Also, PDE3 enzyme is responsible for contraction of the cardiovascular system. Although the cross-selectivity for PDE3 inhibitor activation is very minute, long term PDE5 therapy could be implicated in vasodilation of the cardiovascular system. This is why one of the side effects listed for the drug is, in rare cases, hypotension (abnormally low blood pressure) (14). Since many men with ED are on the path to or are currently having cardiovascular problems take PDE5 inhibitors, this cross-selectivity is an increased benefit for them. Also, reversibly taking statin drugs such as Lipitor to lower cholesterol levels increase vascular NO activity through increased production of eNOS, and could be implicated in reversing the effects of ED (6).

Conclusions

Although Nitric Oxide (NO) has been the perpetrator of increased acid rain and a major contributor to heavy smog in our environment, NO is a very important biological signaling molecule in our bodies, and the benefits of having NO regulate our immune systems and digestive tracts far outweigh the detriments. In the corpus cavernosum, specifically, there have been no case reports found of any serious complications with taking phosphodiesterase type 5

(PDE5) inhibitors. In fact, it is actually PDE5 inhibitors which help to regulate NO production once again to a normal, healthy level. If ED goes untreated along with its impending cardiovascular problems, then these unstable levels of NO react very differently, and could be the underlying cause of why cardiovascular problems usually get worse if they go untreated. However, medicinally speaking, NO regulation has done a greater good for many men suffering from erectile dysfunction (ED).

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