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Nitroglycerine

by

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Abbreviations:

cGMP: cyclic guanosine monophosphate
•NO: nitric oxide
GMN: glyceryl mononitrate
GTN: nitroglycerine or glyceryl trinitrate
FMN: flavin monucleotide
GDN: glyceryl dinitrate
GST: glutathione S-transferse
GTP: guanosine triphosphate

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Abstracts

Nitroglycerine (GTN), which is also known as glyceryl trinitrate, was introduced to treat angina pectoris over 100 years ago and remains to be the first choice drug for relief of angina pectoris today. GTN is often prescribed in the treatment of a variety of heart and vascular diseases because of its ability to relax vascular smooth muscle. Although the clinical effect of GTN has been known for over a century, the mechanism of relaxation of the smooth muscle cell became known only in the late 1970's. GNT is reductively metabolized to release nitric oxide ($^{\bullet}NO$); $^{\bullet}NO$ then activates guanylate cyclase, which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). Increased level of cGMP can decrease the concentration of cytosolic calcium ions and thereby relax the vascular smooth muscle cells. Although the reductively produced $^{\bullet}NO$ is responsible for the biological effect of GTN, the metabolic pathway to generate $^{\bullet}NO$ has yet to be established. This paper will briefly discuss GNT about its bioconversion, physical and chemical properties and mechanism of tolerance.

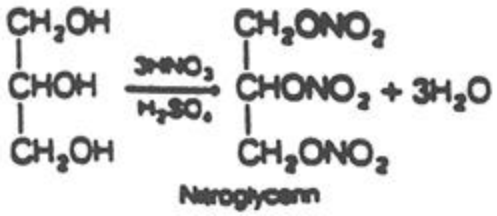
Introduction

Nitroglycerine (GTN) was first made by Ascanio Sobrero in 1846 [1]. It is an explosive liquid that is used to make dynamite [1]. GTN is also a nitrate drug used in treatment of angina. It is rapidly absorbed and short acting. In 1977, Ferid Murad demonstrated that vasodilating effect of nitrate esters (including GTN) was due to the fact that they release nitro oxide *in vivo* [2], $^{\bullet}NO$, in turn, leads to activation of guanylate cyclase, which catalyzes the conversion of GTP to cyclic GMP [2, 3]. The increased cGMP level then leads to smooth muscle relaxation [3]. Though it is already established that GTN exerts its vasodilatation effect through releasing $^{\bullet}NO$, the mechanism of generating $^{\bullet}NO$ from GTN *in vivo* is still controversial. Enzyme conversion is widely accepted. Chung and Fung proposed that GTN releases $^{\bullet}NO$ through a cellular surface membrane-associated enzyme system [4]. Others have proposed that cytochrome P-450 and glutathine S-transferases (GST) systems are also involved in GTN bioconversion [5,6]. In this paper, nitrate tolerance will also be reviewed.

Physical and chemical properties

Nitroglycerine is a colorless oil when it is pure. It is soluble in alcohols but insoluble in water. The molecular formula of GTN is $C_3H_5(ONO_2)_3$. It has a high nitrogen content (18.5%) and contains more than enough oxygen atoms to oxidize the carbon and hydrogen atoms when nitrogen is liberated, so it is one of the most powerful explosives [1].

In 1846, Ascanio Sobrero first made nitroglycerine by treating glyceryl with a mixture of nitric and sulfuric acid. This reaction (1) shown below is highly exothermic, and will result in an explosion if the mixture is not cooled down.



(1)

Nitroglycerine is extremely sensitive to shock and in the early days, when impure GTN was used, it was very difficult to predict under which conditions GTN would explode. Alfred Nobel worked hard to improve GTN as an explosive that could be used in blasting rock and in mining. He made one of his most important discoveries when he found that by mixing oily GTN with silica, the mixture could be turned into a paste. He called his paste dynamite and went on to develop a blasting cap which could be used to detonate dynamite under controlled conditions [1]. The GTN explosive reaction (2) is shown below [1]:

$$4C_3H_5(ONO_2)_3 \rightarrow 12CO_2 + 10H_2O + 6N_2 + O_2 \quad (2)$$

GTN can also undergo hydrolysis and reduction. Reaction 3 shows the hydrolytic reaction, and reaction 4 shows the reductive reaction [7].

$$C_3H_5(ONO_2)_3 + 5KOH \rightarrow KNO_3 + 2KNO_2 + HCOOK + CH_3COOK + 3H_2O \quad (3)$$

$$C_3H_5(ONO_2)_3 + 2GSH \rightarrow C_3H_5(ONO_2)_2OH + GSSG + HNO_2 \quad (4)$$

The metabolism and bioconversion

Nitroglycerine has a very short life-time (about 2 minutes) *in vivo*. It is widely accepted that $^{\bullet}NO$ is responsible for the vasodilation effect of GTN, but the mechanism of converting GTN to $^{\bullet}NO$ is yet to be established. Evidence suggests that the conversion of GTN into its vasodilator metabolites is an intracellular enzymic process [8]. One enzyme system being considered is the glutathione S-transferases (GST) [10]. GST is a family of enzymes present in the cytosol of most cells. The mammalian enzymes can be classified into four groups: Alpha, Mu, Pi and Theta based on their properties and functions [9].

When GTN is absorbed, it is rapidly denitrated into water-soluble 1,2- glyceryl dinitrate (GDN), 1,3-glyceryl dinitrate, 1- and 2-mononitrates (GMN), and glycerol by more than one enzyme systems. In 1989, Servent *et al.* studied GTN denitration in rat liver tissue; they found that the main metabolic transformation of GTN by liver microsomes is a cytochrome P-450-dependent reduction by NADPH leading to di- and mono-nitrates, GDN and GMN, and $^{\bullet}NO$ which is able to bind to cytochrome P-450 (Reaction 5) [10]. But they pointed out that its importance *in vivo* remains to be established because the above reactions could be inhibited by oxygen [10]

$$RO\text{-}NO_2 + P\text{-}450\text{-}Fe(II) \xrightarrow{NADPH} ROH + P\text{-}450\text{-}Fe(II)\text{-}NO \qquad (5)$$

In 1993, Harrison *et al* studied GTN metabolism in dog caroid arteries. Though there is no doubt that GSTs are involved in converting GTN into glyceryl dinitrate and NO_2^-, GSTs are not involved in the bioactivation of GTN [9]. The denitration reactions are shown in reaction (6) and (7) [9].

$$RONO_2 + 2GSH \xrightarrow{GSH\text{-}S\text{-}transferase} GSSG + ROH + NO_2^- \qquad (6)$$

$$H^+ + GSSG + NADPH \xrightarrow{GSH\text{-}reductase} 2GSH + NADP^+ \qquad (7)$$

In1996, McCurie reported that GTN bioactivation could be mediated by a flavin protein [11]. Fukuto further examined the intimate chemistry among flavin, thiol, and GTN [12]. In their model, they found that GTN could be reduced by two electrons to form the corresponding nitrite ester in the presence of flavin protein and NADPH (reaction 8).

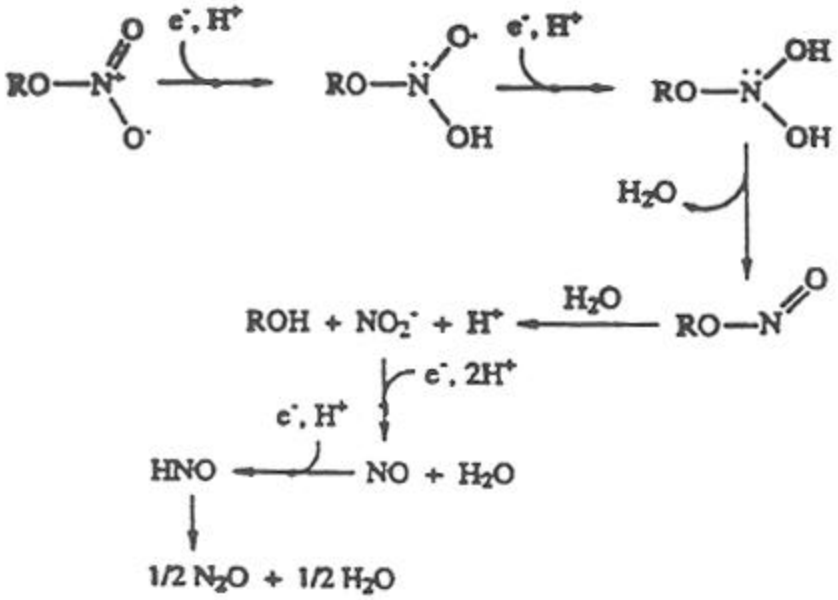
$$R\text{-}O\text{-}NO_2 + 2e^- + 2H^+ \rightarrow R\text{-}O\text{-}NO + H_2O \qquad (8)$$

Hydrolysis of nitrite ester generates NO_2^-, which is then further subjected to 2-electron reduction to HNO. Dimerization of HNO can produce N_2O, which can easily be detected in the experiments. Scheme 1 shows this pathway [12].

Nitrite esters can also react with glutathione (GSH) to form GSNO; this reaction is probably catalyzed by GST. GSNO reacting with flavin monucleotide (FMN)/NADH can release $^{\bullet}NO$ (Reaction 9, 10). The presence of FMN can increase $^{\bullet}NO$ release dramatically [12].

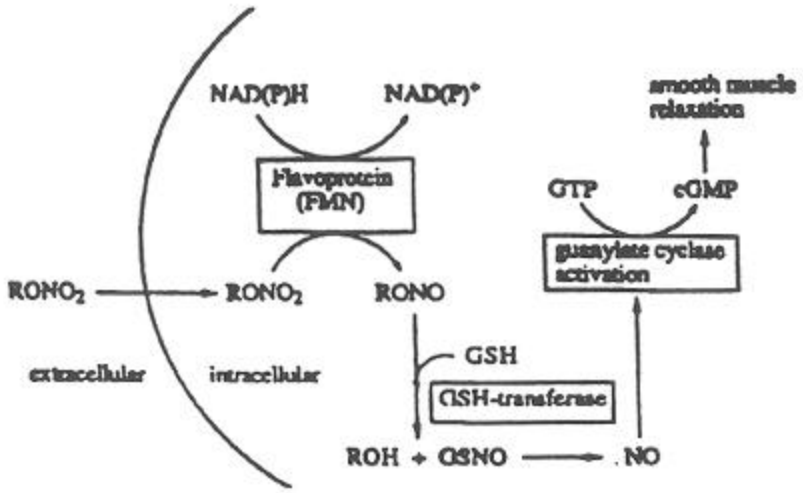
$$GSNO + NADH \rightarrow NAD^+ + GS^- + HNO \quad (9)$$

$$GSNO + HNO \rightarrow GSN(OH)NO \rightarrow GSH + 2^{\bullet}NO \quad (10)$$



Scheme 1: Reduction of organic nitrate esters. Adapted from [12].

In summary, scheme 2 describes a possible mechanism for bioactivation of organic nitrate esters. First, organic nitrate esters are up taken by the smooth muscle cells; then a flavin protein (a possible membrane-bound protein) reduces it into an organic nitrite ester; nitrite ester further reacts with endogenous GSH to form GSNO in the presence of GST; GSNO will eventually release $^{\bullet}NO$ by various mechanisms [12]. Scheme 2 shows this pathway [12].



Scheme 2: Possible reaction pathway for $RONO_2$ reduction to $^{\bullet}NO$. Adapted from [12]

Nitrate tolerance

A major limitation of the use of GTN is the nitrate tolerance, which develops shortly after onset of treatment. The mechanism of this phenomenon is still not clear. A number of hypotheses have been proposed.

Sulfydryl-depletion hypothesis has been studied extensively. It states that nitrate tolerance is due to depletion of reduced sulfhygryl groups that are necessary for biotransformation of nitrate to nitric oxide. Although sulfhydryl donors can enhance the vascular effects of GTN, the work of Fung *et al.* suggested that the augmentation of vascular responses might occur by a mechanism independent of nitrate tolerance [13].

According to the neurohormonal hypothesis, nitrate administration is associated with reflex release of several vasoconstrictor hormones that reduce the vasodilating effect of nitrate. The plasma-volume-expansion hypothesis states that nitrate-induced expansion of plasma volume reverses the effects of nitrates on ventricular preload. Some attempts based on these hypotheses to prevent tolerance have not achieved consistent results [14].

In animals, the tolerance of GTN is associated with increased production of superoxide anion and can be reversed by addition of an antioxidant into tolerant vascular tissue [14]. The mechanism of generating increased amount of superoxide is unclear. Recent evidence suggests angiotesin II may be involved [15]. Interestingly, hydralazine, which can inhibit membrane-bound oxidases, has been reported to prevent the development of tolerance to the hemodynamic effects of GTN in both animals [16]. Based on the fact that superoxide reacts with nitric oxide to remove $^{\bullet}$NO, free radical hypothesis seems very promising in nitrate tolerance.

Summary

Nitroglycerine is an important drug for treatment patient with angina, and it is also a powerful explosive. Alford Nobel studied its physical and chemical properties carefully, and made it safe as dynamite. About Two decades ago, it is found out that $^{\bullet}$NO is responsible for vasodilating effect of GTN. Unfortunately, how GTN is converted into $^{\bullet}$NO *in vivo* is still unclear; therefore, understanding nitrate tolerance is also hard. Recent studies suggest enzyme bioconversion is the major pathway to produce $^{\bullet}$NO *in vivo.* A number of hypotheses of nitrate tolerance have been proposed; some modifications based on these hypotheses have achieved good effect against nitrate tolerance. I believe further study will make GTN a better drug for heart and vascular diseases.

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