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Nitroglycerin: Exploding Biology

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Abbreviations:

1,2-GDN	1,2-glycerol dinitrate
1,3-GDN	1,3-glycerol dinitrate
GTN	glyceryl trinitrate or nitroglycerin
mtALDH	mitochondrial aldehyde dehydrogenase
$NO^{\bullet}$	nitric oxide
NO_2	nitrite
$NO_2^{\bullet}$	nitrogen dioxide radical
$O_2^{\bullet -}$	superoxide
$ONOO^-$	peroxynitrite

Table of Contents

Abstract

Nitroglycerin (GTN) is a simple molecule and a highly effective drug that has been used in medicine for over 130 years. Nitroglycerin was originally used as an explosive, but was later found to have medicinal benefits. Here we examine the explosive topics of bioactivation and mechanism of nitrate tolerance. Biologically, the reduction of GTN leads to the production of nitric oxide (NO•) and 1,2-glycerol dinitrate. The resulting NO• serves as a vasodilator in cardiovascular disease. The exact mechanism of this bioactivation is still under debate but it is believed to involve the protein, mitochondrial aldehyde dehydrogenase (mtALDH). Data have also shown that constant exposure to GTN leads to nitrate tolerance which induces a loss of biological function for NO• as a vasodilator.

Introduction

Pure nitroglycerin, also known as glyceryl trinitrate (GTN), is a colorless, oily liquid that is highly explosive. Ascanio Sobrero, an Italian chemist, initially prepared GTN in 1846, but it was the Swedish physicist Alfred Nobel in 1867 that captured its explosive power and generated a profitable precursor to modern day dynamite [1]. In early forms, GTN was mixed with gun-powder and marketed as "Swedish blasting oil"[1].

Even though GTN is highly destructive, it was noticed that Nobel's factory workers who suffered from angina experienced relief during the week when working, but chest pain returned over the weekend. Also, workers complained of headaches on Monday mornings that also disappeared over the weekend.

As a consequence of these observations, GTN was prescribed for angina but the mechanism of its relief was not known. During the early 1980s, it was discovered that nitric oxide ($NO^{\bullet}$), which is released from GTN, produces vasodilatation in smooth muscle. This finding, which earned the Nobel Prize, did not however explain the mechanism of $NO^{\bullet}$ release from GTN. The following will highlight physical properties of GTN, its explosive action, the physiological mechanism of $NO^{\bullet}$ release from GTN, and the biological consequences of GTN.

Physical Properties of GTN

Nitroglycerin, structure shown in **Figure 1**, is obtained by nitrating glycerol and is a heavy, colorless, poisonous, and oily liquid. When GTN decomposes at acidic pH, it becomes yellowish[1]. Nitroglycerin is shock-sensitive (*i.e.*, physical shock can induce explosions) and over time naturally degrades to more stable forms. Nitroglycerin can be "desensitized" by cooling to its freezing temperature of 5 – 10°C. Thawing of the solidified crystals nevertheless can be extremely

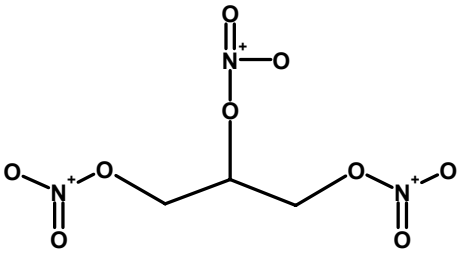

Figure 1. Structure of Nitroglycerin.

[1] *http://www.en.wikipedia.org/wiki/Nitroglycerin*. Accessed February 17, 2005

dangerous and must be done slowly. The temperature of pure GTN should not exceed 30°C otherwise there is a risk of explosion.

Explosive Power of GTN

An explosion is fundamentally a very fast combustion and combustion requires fuel and an oxidant. Nitroglycerin contains both of these components. During detonation, the initial burn creates a pressure gradient *via* a shock wave that pre-ignites unshocked material. This generates a fast moving transition zone that can detonate any appropriately combustible material that the wave encounters. In essence, this creates a self-propagating, hyper-instantaneous pressure induced combustion that grows exponentially[1]. Detonation is then solely dependent on the material's ability to self-propagate the pressure wave. In the case of GTN if it were detonated under pressure, it explodes in the form of hot gas to a thousand times more volume, rxn 1. The enthalpy of this reaction is -2842 kJ under constant pressure.

$$2\ C_3H_5(NO_3)_3\ (l) \rightarrow 3\ N_2\ (g) + 1/2\ O_2\ (g) + 6\ CO_2\ (g) + 5\ H_2O\ (g) \qquad \text{(rxn 1)}^2$$

$$\Delta H^\circ_{rxn} = \Sigma n\ \Delta H^\circ_f\ (\text{products}) - \Sigma m\ \Delta H^\circ_f\ (\text{reactants})$$

$$\Delta H^\circ_{rxn} = -2842\ \text{kJ}$$

Mechanism of NO• Release from GTN

The production of NO• from GTN is highly dependent on the specific tissue and cell type as well as the dosage of GTN. The reduction of GTN yields either 1,2-glyceryl dinitrate (1,2-GDN) or 1,3-glycerol dinitrate (1,3-GDN) and nitrite (NO_2) in different amounts [2], rxn 2 and rxn 3.

$$GTN + E_{red} \rightarrow \text{1,2-GDN} + NO_2^- + E_{ox} \qquad [2]\ \text{(rxn 2)}$$

$$GTN + E_{red} \rightarrow \text{1,2-GTN} + NO_2^- + E_{ox} \qquad [2]\ \text{(rxn 3)}$$

The NO_2 is then reduced to produce NO•, rxn 4.

[2] http://jan.ucc.nau.edu/~bjc/151f04/handouts/enthalpykey.pdf, accessed on March 9, 2005.

$$NO_2 + 2\ H^+ + e^- \rightarrow NO^\bullet + H_2O \qquad \text{(rxn 4)}^3$$

Mitochondrial aldehyde dehydrogenase (mtALDH) was found by Chen *et al.* (2002) [2] to be responsible for this bioactivation. Human mtALDH uses a thiol cofactor as a one electron reductant to convert GTN to 1,2-GDN.

Using aortic ring bioassay to measure vessel dilation, Chen *et al.* (2002) demonstrate role of mtALDH in GTN conversion and their ability to induce vessel dilation, **Figure 2**. Nitroglycerin reduction releases $NO^\bullet$ which can induce vessel dilation *via* guanylate cyclase. When the vessels were pre-incubated with choral hydrate or cynamide (both competitive inhibitors of mtALDH) and acetaldehyde (a competitive inhibitor of $NO^\bullet$), a decrease in vessel relaxation is observed. The addition of $NO^\bullet$ directly to the vessels induced relaxation in the vessels (data not shown by Chen *et al.* (2002)).

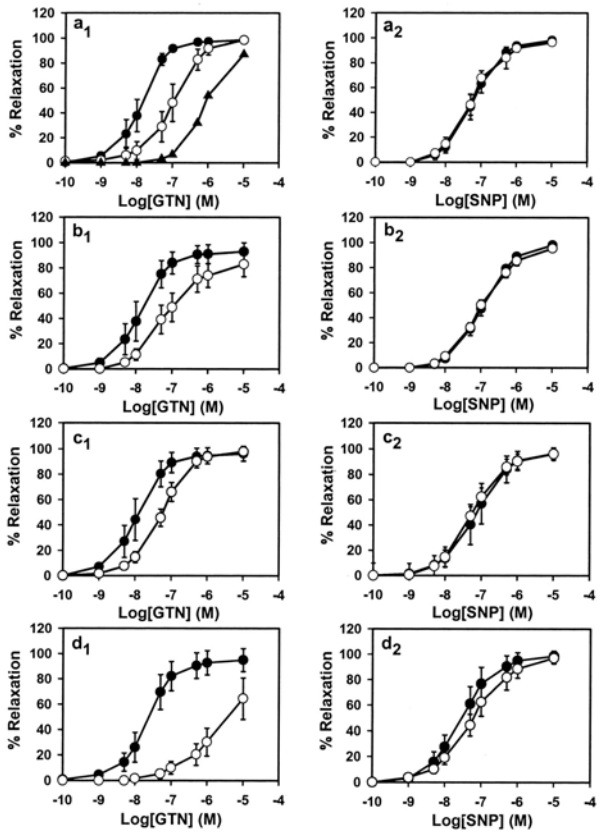

Figure 2. Effects of inhibitor treatment and GTN tolerance on GTN-induced (*Left*) and SNP-induced (*Right*) relaxation in rabbit aorta. The dose-response curves of control rings are shown as ●, and inhibitor-treated rings (1 mM) are shown as ○. (*a*) Chloral hydrate dose-response (○, 1 mM; ▲, 5 mM). (*b*) Cyanamide. (*c*) Acetaldehyde. (*d*) GTN tolerant. From Chen *et al.* (2002) [2].

The data indicates that mtALDH can metabolize GTN, and that its activity could be reduced due to nitrate tolerance. No data exist to compare the activity of this enzyme relative to other enzymes that have also been reported to metabolize GTN, such as cytochrome P450 and glutathione S-transferase. Other studies have confirmed that mtALDH converts GTN to $NO^\bullet$-linked species that activate soluble guanylyl cyclase [3]. The inhibitors used by Chen *et al.*(2002), chloral hydrate and cyanamide, may not be uniquely specific toward mtALDH, and may inhibit other sulfhydryl enzymes [2]. Consequently,

[3] http://www.biologie.uni-hamburg.de/lehre/bza/1nir/enire.htm, accessed March 9, 2005.

mtALDH may not be the only enzyme that reduces GTN to GDN and $NO^{\bullet}$. Currently, researchers are still struggling to understand the bioactivation mechanism of GTN by mtALDH and other enzymes [3].

Biological Application and Effects of GTN

For over 130 years, GTN has been used to treat angina. The principle mechanism of smooth muscle relaxation for treatment of angina is the activation of the intracellular enzyme, soluble guanylyl cyclase. Activation of guanylyl cyclase leads to elevation of the cyclic guanosine-3',-5'- monophosphate levels [4]. Research has shown that the release of $NO^{\bullet}$ from GTN causes the phosphorylation of the vasodilator-stimulated phosphoprotein at serine-239 which is a reliable marker vasorelaxation [4].

Due to the explosive instability of GTN, there are several pharmacological analogs currently used (*i.e.*, Nitrol®, an ointment containing 2% GTN). Drugs, such as Nitrol are not intended to stop acute angina attacks rather they are designed to prevent attacks *via* vessel relaxation. The mechanism of action for these modern medicines is similar to that of GTN directly, while the induction of side-effects such as headaches are limited.

Overproduction of $NO^{\bullet}$ from GTN can have dire consequences. For example, $NO^{\bullet}$ can react with superoxide ($O_2^{\bullet -}$) which leads to the production of peroxynitrite ($ONOO^-$).

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^- \qquad k_5 = 1.9 \times 10^{10}\ M^{-1}\ s^{-1} \qquad [5] \qquad \text{(rxn 5)}$$

Peroxynitrite can oxidize and nitrate DNA, which could potentially cause single-strand DNA breaks of the sugar-phosphate backbone. Nitrosation of primary amines (*i.e.*, DNA bases) could also lead to the formation of diazonium ions, subsequent deamination, and DNA crosslinks [6].

Chronic nitrate administration may have a harmful effect on endothelial function, such as an inability to undergo $NO^{\bullet}$ induced vasodilation [7]. The exact pathways by which nitrates may exert these seemingly contrasting actions have not been elucidated, however several mechanisms have been proposed.

One proposed mechanism is the Needleman hypothesis where the oxidation of –SH groups in the "nitrate receptor" is responsible for the phenomenon of nitrate tolerance. The primary supporting evidence is that oxidation and alkylation of cellular sulfhydryl groups has to diminished vascular GTN response, and that replenishment of reduced –SH can reduce or reverse tolerance [8].

Another popular theory is based on the observation that tolerance may involve a reduction of nitrate metabolism (both mechanism-based and clearance-based). Funk and Poliszcuk (1986) showed that rat aortas produced less 1,2-GDN, but not 1,3-GDN, after tolerance development [9]. Similarly, reduced $NO^{\bullet}$ production accompanied nitrate tolerance [10]. The mechanism of this theory is not clear, however, it is believed that the diminished production of $NO^{\bullet}$ is a function of reduced GTN bioactivation is the result of desensitization to GTN.

Discussion

Nitroglycerin is a simple molecule and a highly effective drug that has been used in medicine for over 130 years. Only recently however have we begun to understand its biochemical activation through the discovery of mtALDH. And as this pathway remains under debate, we must simultaneously examine the relationship between GTN and tolerance development. Once again, we are forced to question what the exact mechanism might be. Only a few facts are clear, the first being that nitroglycerin has shaped our current society whether it is as a medicine or as an explosive. Secondly, GTN is clearly capable of assuaging the symptoms associated with angina. And thirdly, the bio-mechanism of GTN will remain an explosive topic in research for years to come.

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