Oximes Induce Erection and Are Resistant to Oxidative Stress

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ABSTRACT

Introduction. Because of their nitric oxide (NO)-donating capacities, oxime derivatives have shown to offer some therapeutic perspective for the treatment of erectile dysfunction (ED) as well as cardiovascular diseases. However, to date the in vivo effect of these oximes on erectile function remains unknown. In many disease states oxidative stress occurs, impairing NO-mediated relaxations. Hence the in uence of oxidative stress on oxime-induced effects is also of interest.

Aims. This study aimed to evaluate the in vivo effect of formaldoxime (FAL) and formamidoxime (FAM) on blood pressure and intracavernosal pressure (ICP); and to examine the role of soluble guanylyl cyclase (sGC) and the in uence of oxidative stress on the FAL and FAM responses.

Methods. Blood pressure and ICP were monitored in vivo after resp. intravenous or intracavernosal injection of FAL and FAM. Moreover isometric tension was measured in vitro on isolated mice corpora cavernosa (CC), thoracic aorta, and femoral artery in organ baths. The role of sGC was investigated using transgenic mice lacking the alpha 1 subunit of sGC.

Main Outcome Measures. Mean arterial pressure (MAP) and ICP were measured after FAL/FAM injection. In vitro relaxation of CC strips was evaluated in response to addition of FAL/FAM.

Results. In vivo both FAL and FAM elicit a dose-dependent lowering of blood pressure (maximal MAP: 33.66 ± 4.07 mm Hg [FAL] and 20.43 ± 2.06 mm Hg [FAM]) as well as an increase of ICP (maximal increase of ICP/MAP: 70.29 ± 2.88% [FAL] and 52.91 ± 8.61% [FAM]). The FAL/FAM effect is significantly lower in knockout vs. wild-type mice. Oxidative stress has an inhibitory effect on corporal NO-mediated relaxations induced by electrical field stimulation, acetylcholine, and sodium nitroprusside whereas the responses to 8-(4-chlorophenylthio)-guanosine 3 ,5 -cyclic monophosphate sodium salt, FAL and FAM were not in uenced.

Conclusions. Oximes induce erection which is mediated by sGC. The oxime-induced relaxations are resistant to oxidative stress, which increases their therapeutic potential for the treatment of ED. **Pauwels B, Boydens C, Brouckaert P, and Van de Voorde, J. Oximes Induce Erection and Are Resistant to Oxidative Stress. J Sex Med 2015;12:906–915.**

Key Words. Corpora Cavernosa; Erectile Function; Oxime; Soluble Guanylyl Cyclase; Oxidative Stress

Introduction

The nitric oxide (NO)/soluble guanylyl cyclase (sGC)/cyclic guanosine monophosphate (cGMP)-pathway plays a pivotal role in the normal regulation of penile erection as well as in the complex balance of vascular tone. Consequently, any profound lowering in NO production and/or decreased response to endogenously formed NO can result in erectile dysfunction (ED) and/or various cardiovascular diseases [1]. NO donors have been used for several years to compensate for lost NO bioavailability. Recently, oxime compounds such as formaldoxime (FAL) and formamidoxime (FAM) (Figure 1) have been presented as a new class of NO-donating molecules

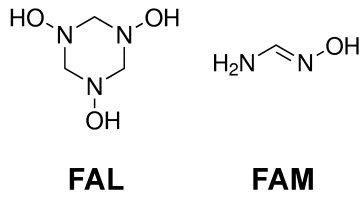

Figure 1 The chemical structure of formaldoxime (FAL) and formamidoxime (FAM).

because of their structural resemblance with N-hydroxy-L-arginine, a stable intermediate in the endogenous NO production. These oximes relax vascular tissues as well as mice corpora cavernosa (CC) through metabolization within the tissue [2–4]. Furthermore, oxime derivatives show a distinct blood pressure-lowering effect in rats after inhibition of the endogenous NO synthesis [5]. However, so far, the in vivo effect of oximes on intracavernosal pressure (ICP) and their ability to induce erection is not yet studied.

The lowered NO functioning in ED often results from an increased production of reactive oxygen species, diminishing the effective NO concentration for cavernosal smooth muscle relaxation [6]. Therefore, oxidative stress associated with hypertension [7], diabetes [8,9], aging [10,11], hypercholesterolemia [12], and radiation damage [13], can lead to ED. It was reported that oxidative stress impairs NO-mediated relaxations of mice CC [14]. Hence, we were interested if oxidative stress also has an in uence on the FAL-/FAM-induced corporal relaxations, as an impairment would limit their therapeutic relevance.

Aims

In our study, we examined the in vivo effect of FAL and FAM on blood pressure and ICP. The importance of sGC in the FAL-/FAM-induced effects was characterized both in vitro and in vivo. Moreover the in uence of oxidative stress on (NO-mediated) corporal relaxation was evaluated in vitro.

Materials and Methods

Animals

For our in vitro and in vivo experiments, mature (8–14 weeks) male sGC alpha 1 knockout ($sGC\alpha_1^{-/-}$) mice and $sGC\alpha_1^{+/+}$ mice (129SvJ) were used [15]. All animals were bred in the SPF facility of the Department of Molecular Biomedical Research (VIB, Ghent, Belgium) and were treated in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. This study was also approved by the local Ethical Committee for Animal Experiments (Faculty of Medicine and Health Sciences, Ghent University, Belgium).

In Vitro Study

Tissue Preparations and Mounting

Thoracic aorta, femoral artery as well as CC were isolated from the animals after cervical dislocation. The tissues were then mounted into 10-mL organ baths from a myograph for isometric tension measurements as previously described [16,17]. The mounted tissues were left to equilibrate for 30 minutes in Krebs-Ringer bicarbonate (KRB) solution at 37°C (pH 7.4) and continuously bubbled with carbogen gas (95% O_2 – 5% CO_2).

Preliminary Protocol

In order to obtain maximal, stable contractions and relaxations during the actual experiments, each tissue was subjected to a slightly different preliminary protocol as previously described [16–18]. Functionality of the endothelium was tested by addition of 10 μmol/L (arteries) or 1 μmol/L (CC) acetylcholine (ACh) to the organ baths after contraction with 5 μmol/L (aorta and CC) or 10 μmol/L (femoral artery) norepinephrine (NOR). Afterwards, the preparations were rinsed extensively until they returned to their basal tension level.

Experimental Protocol

In a first series of experiments, both the effect of FAL and FAM was tested in $sGC\alpha_1^{-/-}$ and compared with their effect in the wild-type control mice ($sGC\alpha_1^{+/+}$). All preparations were contracted with NOR and when a stable plateau was obtained, cumulative concentration–response curves for FAL and FAM (100 nmol/L–1 mmol/L) were established. In a second series of experiments the in uence of oxidative stress on corporal relaxations was evaluated. CC were incubated with the superoxide generator/free radical scavenger hydroquinone (50 μmol/L) and the superoxide dismutase inhibitor diethyldithiocarbamic acid (DETCA) (8 mmol/L) for 20 minutes. After reaching a stable contraction with phenylephrine (5 μmol/L), electrical field stimulation (EFS; train duration 40 seconds; frequency 1, 2, 4, and 8 Hz; pulse duration 5 ms; 80 V) as well as cumulative concentration response curves for ACh, sodium