NO-Donating Oximes Relax Corpora Cavernosa Through Mechanisms Other than Those Involved in Arterial Relaxation

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ABSTRACT

Introduction. Erectile dysfunction (ED), as well as many cardiovascular diseases, is associated with impaired nitric oxide (NO) bioavailability. Recently, oxime derivatives have emerged as vasodilators due to their NO-donating capacities. However, whether these oximes offer therapeutic perspectives as an alternative NO delivery strategy for the treatment of ED is unexplored.

Aims. This study aims to analyze the influence of formaldoxime (FAL), formamidoxime (FAM), and cinnamaldoxime (CAOx) on corporal tension and to elucidate the underlying molecular mechanisms.

Methods. Organ bath studies were carried out measuring isometric tension on isolated mice corpora cavernosa (CC), thoracic aorta, and femoral artery. After contraction with norepinephrine (NOR), cumulative concentration–response curves of FAL, FAM, and CAOx (100 nmol/L–1 mmol/L) were performed.

Main Outcome Measures. FAL-/FAM-induced relaxations were evaluated in the absence/presence of various inhibitors of different molecular pathways.

Results. FAL, FAM, and CAOx relax isolated CC as well as aorta and femoral artery from mice. ODQ (soluble guanylyl cyclase-inhibitor), diphenyliodonium chloride (nonselective flavoprotein inhibitor), and 7-ethoxyresorufin (inhibitor of CYP450 1A1 and NADPH-dependent reductases) substantially blocked the FAL-/FAM-induced relaxation in the arteries but not in CC. Only a small inhibition of the FAM response in CC was observed with ODQ.

Conclusions. This study shows for the first time that NO-donating oximes relax mice CC. Therefore, oximes are a new group of molecules with potential for the treatment of ED. However, the underlying mechanism(s) of the FAL-/FAM-induced corporal relaxation clearly differ(s) from the one(s) involved in arterial vasorelaxation. **Pauwels B, Boydens C, Decaluwé K, and Van de Voorde J. NO-donating oximes relax corpora cavernosa through mechanisms other than those involved in arterial relaxation. J Sex Med 2014;11:1664–1674.**

Key Words. Corpora Cavernosa; Oxime; Formaldoxime; Formamidoxime; Cinnamaldoxime; Erectile Function

Introduction

Over the past decades, nitric oxide (NO) has evolved as an important (patho)physiological signaling molecule with therapeutic potential. The endogenous production occurs via NO synthases (NOS) that hydroxylates L-arginine to the stable intermediate N-hydroxy-L-arginine (L-NOHA). L-NOHA is further oxidized to L-citrulline and NO [1]. NO acts as the principle mediator of penile erection and is also the best known vasodilator in the cardiovascular system. Several disease states such as erectile dysfunction (ED) and hypertension are associated with impaired NO-mediated relaxations due to loss of endothelial and/or neuronal production of and/or response to NO [2,3]. To compensate for this loss, some oxime derivatives have been presented as new NO donor molecules, mimicking the action of endogenous NO by their bioconversion to NO or NO-related compounds [4,5]. In the past, the NO-donating capacities of oximes were demonstrated both by direct (electron

paramagnetic resonance spectroscopy) and indirect (NO-scavenging with PTIO) strategies [4–6]. In rat aorta, both formaldoxime (FAL) and formamidoxime (FAM) have been shown to induce a substantial vasorelaxant effect [4]. Moreover, participation of the NO pathway in the cinnamaldoxime (CAOx)-induced relaxation has been demonstrated in rat mesenteric artery [5]. As proven vasodilators of arteries, oximes could also be of value in treatment of ED. However, no studies support this hypothesis yet.

Aims

In our study, we examined the ability of FAL, FAM, and CAOx to relax isolated mice corpora cavernosa (CC). Furthermore, the mechanisms underlying this effect were examined and compared with the mechanisms involved in the relaxation of mice aorta and femoral artery.

Materials and Methods

Animals

Mature (age 8–12 weeks) male Swiss mice were obtained from Janvier (Saint-Berthevin, France). Food and water were provided ad libitum, and all animals 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 approved by the local Ethical Committee for Animal Experiments.

Tissue Preparation and Mounting

After cervical dislocation thoracic aorta, femoral artery and CC were carefully isolated, displaced in cold Krebs-Ringer bicarbonate (KRB) solution, mounted into 10-mL organ baths from a myograph for isometric tension measurements as previously described [7,8], and left to equilibrate for 30 minutes in KRB solution (37°C; pH 7.4, bubbled with 95% O_2–5% CO_2).

Preliminary Protocol

Before starting with the actual experiments, each tissue was subjected to a slightly different preliminary protocol in order to obtain maximal, stable contractions and relaxations as previously described [7–9]. To test the functionality of the endothelium, 5 μmol/L (aorta and CC) or 10 μmol/L (femoral artery) norepinephrine (NOR) was used to induce contraction, and when a stable plateau was reached, 10 μmol/L (arteries) or 1 μmol/L (CC) acetylcholine chloride (ACh) was added. Thereafter, preparations were washed until basal tone was obtained.

Experimental Protocol

All preparations were contracted with NOR, and when a stable plateau was obtained, cumulative concentration–response curves for FAL, FAM, and CAOx (100 nmol/L–1 mmol/L) were established. The molecular pathways involved in these effects were tested using different inhibitors. In some experiments, the endothelium of aortic or femoral artery segments was removed by gently rubbing the intimal surface with a rough polyethylene tube or small hair respectively, while the CC were carefully squeezed between two fingers for 30 seconds. The absence of functional endothelium was evaluated by the loss of response to ACh.

Drugs, Chemicals, and Reagents

The experiments were performed in a KRB solution with the following composition (mmol/L): NaCl, 135; KCl, 5; $NaHCO_3$, 20; glucose, 10; $CaCl_2$, 2.5; $MgSO_4$, 1.3; KH_2PO_4, 1.2; and EDTA, 0.026 in H_2O. NOR bitartrate, dimethylsulfoxide (DMSO), ACh, FAL trimer hydrochloride, FAM, CAOx, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), diphenyliodonium chloride (DPI), 4′-hydroxy-3′-methoxyacetophenone (apocynin), 7-ethoxyresorufin (7-ER), miconazole nitrate, Nω-Nitro-L-arginine (L-NNA), tetraethylammoniumchloride (TEA), apamin, SQ 22,536, and compound C were obtained from Sigma-Aldrich (St. Louis, MO, USA). DPI, apocynin, 7-ER, miconazole, and compound C were dissolved in DMSO and ODQ in ethanol. All other drugs were dissolved in distilled water. Incubation with ODQ, 7-ER, and L-NNA elicited a significant increase in the NOR-induced contraction, whereas DPI and miconazole evoked a substantial decrease. However, these changes had no influence on the conclusions drawn from our results. The final concentrations of vehicle solution in the organ bath never exceeded 0.1%.

Data Analysis and Statistical Procedures

Data are presented as mean values ± SEM; N represents the number of preparations. The relaxations are expressed as the percentage decrease in contraction level. Statistical significance was evaluated by using the Mann–Withney *U*-test (aorta and femoral artery) and Wilcoxon test (CC)