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Regulation of vascular tone in rabbit ophthalmic artery: Cross talk of endogenous and exogenous gas mediators

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ABSTRACT

Nitric oxide (NO), carbon monoxide (CO) and hydrogen sulphide (H_2S) modulate vascular tone. In view of their therapeutic potential for ocular diseases, we examined the effect of exogenous CO and H_2S on tone of isolated rabbit ophthalmic artery and their interaction with endogenous and exogenous NO. Ophthalmic artery segments mounted on a wire myograph were challenged with cumulative concentrations of phenylephrine (PE) in the presence or absence of N^G-nitro-L-arginine (LNNA) to inhibit production of NO, the CO-releasing molecules CORMs or the H_2S-donor GYY4137. The maximal vasoconstriction elicited by PE reached 20–30% of that induced by KCl but was dramatically increased by incubation with LNNA. GYY4137 significantly raised PE-mediated vasoconstriction, but it did not change the response to PE in the presence of LNNA or the relaxation to sodium nitroprusside (SNP). CORMs concentration-dependently inhibited PE-induced constriction, an effect that was synergistic with endogenous NO (reduced by LNNA), but insensitive to blockade of guanylyl cyclase by 1*H*-[1,2,4]oxadiazolo[4,3,-α]quinoxalin-1-one (ODQ). In vascular tissues cyclic GMP (cGMP) levels seemed reduced by GYY4137 (not significantly), but were not changed by CORM. These data indicate that CO is able *per se* to relax isolated ophthalmic artery and to synergize with NO, while H_2S counteracts the effect of endogenous NO. CO does not stimulate cGMP production in our system, while H_2S may reduce cGMP production stimulated by endogenous NO. These findings provide new insights into the complexities of gas interactions in the control of ophthalmic vascular tone, highlighting potential pharmacological targets for ocular diseases.

1. Introduction

Nitric oxide (NO), carbon monoxide (CO) and hydrogen sulphide (H_2S) are gaseous molecules that have long been known as environmental pollutants and highly toxic gases [1]. Since the seminal discovery of the endothelium-derived relaxing factor (EDRF) by Furchgott [2], later identified as NO [3], CO [4] and H_2S [5] have also been found to be endogenously produced and to act as signalling molecules in vertebrates. Nowadays these molecules are studied not only as endogenous regulators, but also as potentially exploitable therapeutics. In fact, although NO was initially characterized as the main mediator of endothelium-dependent vasodilatation, CO and H_2S have also been shown to affect vascular tone among other important biological actions. Collectively, these three signalling gases have become known as gasotransmitters [6]. While a large body of studies during the last three decades has focused on the molecular and cellular mechanisms of NO and its functions in different biological systems, including the eye, much less is known about CO and H_2S, for which the mechanisms underlying their pharmacologic effects are still mostly unclear.

Interestingly, CO, NO and H_2S are endogenously produced in vertebrate retinas and have been studied in several non-clinical and clinical ocular paradigms [7–19].

A number of chemicals able to deliver CO and H_2S to tissues in a controlled manner have been developed and could potentially be used in human therapy alongside NO-donors, which are approved drugs used to relieve acute cardiac ischemia and hypertension [20].

A potential cross talk between the three gas mediators has been hypothesized but poorly examined. Thus, the aim of the present study was to investigate the effects of exogenous CO and H_2S on

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tone of isolated rabbit ophthalmic artery and their interaction with endogenous and exogenous NO. Furthermore, in order to gain insight on the mechanism/s whereby these gases exert the regulation of vascular tone, we measured cyclic GMP (cGMP) levels in rabbit ophthalmic arteries.

2. Materials and methods

2.1. Preparation of ophthalmic arteries

Animal use was approved by the subcommittee for research and animal care at the University of Catania according to guidelines from Italian Ministry of Health. Male New Zealand rabbits (Charles River, Calco, Italy; 200–250 g) were euthanatized with a single injection of zoletil® 100 in the peripheral vein of the hear. Ophthalmic arteries from both eyes were dissected, immersed in physiological salt solution (PSS, composition in mM: NaCl, 118; KCl, 4.6; $NaHCO_3$, 25; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 1.2; glucose, 10; EDTA, 0.025, pH 7.4 at 37 °C), cut in segments (2 mm length) and mounted on the 40 μm diameter stainless steel wire of a wire myograph (610 M, Danish Myo Technology, Aarhus, Denmark) for isometric record of contractile force. After mounting, each preparation was equilibrated unstretched for 30 min in PSS (37 °C, aerated with 95% O_2–5% CO_2, pH 7.4). The normalized passive resting force and the corresponding diameter were then determined for each preparation from its own length–pressure curve, as previously described [21,22]. The normalized internal diameter of the preparations was 613 ± 23 μm (range: 342–925 μm). Contractile responses were recorded into a computer by using a data acquisition and recording software (Myodaq and Myodata, Danish Myo Technology).

2.2. Analysis of vascular responses

After normalization and 30-min equilibration in PSS, the preparations were stimulated with isotonic depolarizing KCl solution, in which part of NaCl had been replaced by an equimolar amount of KCl (composition in mM: NaCl, 22.6; KCl, 98.8; $NaHCO_3$, 25; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 1.2; glucose, 10; EDTA, 0.025, pH 7.4 at 37 °C). After washout and 30-min recovery, in the presence or in the absence of the inhibitor of NO synthase N^G-nitro-L-arginine (LNNA, 0.1 mM), the preparations were exposed to phenylephrine (PE, 10 nM–10 μM); once the contractile response had reached a steady state, cumulative concentrations of acetylcholine (ACh 1 nM–10 μM) were added to the organ bath to ensure endothelium-dependent relaxation. After washout and 30-min recovery, the preparations were incubated for 30 min in the presence or absence of LNNA and/or the CO-releasing molecules CORM A1/CORM 371 or the H_2S donor GYY4137, followed by cumulative concentrations PE. Finally, following incubation with LNNA in the presence or absence of GYY4137, the vasodilatation to cumulative concentrations of the NO donor sodium nitroprusside (SNP, 1 nM–10 μM) was assessed.

2.3. Determination of vessel cyclic GMP

Cyclic GMP production in the conditions used in myograph experiments was measured in ophthalmic arteries following incubation for 30 min in PSS bubbled with 95% O_2–5% CO_2 (pH 7.4, 37 °C) and for additional 30 min in the presence or absence of 1 mM GYY4137, 0.1 mM CORM 371 and 10 μM SNP. At the end of the incubation, ophthalmic arteries were put into 0.2 ml of an ice-cold solution containing 0.1 M HCl and manually homogenized at 4 °C with a glass potter, by alternating 2 times of a 30-s strike. The homogenates were centrifuged for 10 min at 1500 × *g* and 100 μl of the supernatant was used for cGMP determination. Protein concentration in the supernatant was determined with the Bradford assay [23]. Cyclic GMP was determined by using an ELISA kit (acetylation protocol, Enzo Life Sciences, Farmingdale, NY, USA) according to manufacturer's instructions.

2.4. Drugs and reagents

Sodium boranocarbonate ($Na_2[H_3BCO_2]$), here termed CORM A1, is a water-soluble compound that spontaneously releases CO in aqueous solutions (Fig. 1). CORM 371 is a new water-soluble manganese-containing CO-releasing molecule (Fig. 1). These two CO-donors were synthesized as previously described [24] and, despite marked differences in their chemical structure, liberate CO with similar rates. GYY4137 (morpholin-4-ium-4-methoxyphenyl(morpholino) phosphinodithioate) is a slow-releasing H_2S donor (Fig. 1). The molecular mechanism of H_2S release from GYY4137 seems related to a protonation of the sulphide group to form a sulfhydryl moiety followed by hydrolysis to release H_2S [25]. PE, LNNA, ACh, SNP and GYY4137 were purchased from Sigma (Saint Louis, MO, USA). All compounds were dissolved at 10 mM in aqueous stock solutions, except GYY4137 that was dissolved as a 0.1 M stock solution. ODQ (Enzo Life Sciences, NY, USA) was dissolved at 10 mM in dimethyl sulfoxide (DMSO). CORM A1 and CORM 371 were, respectively, dissolved as 10 mM and 5 mM stock solutions in water. All stock solutions were further diluted directly to the final concentration in PSS.

2.5. Statistical analysis

Data in concentration-contraction curves were reported as a percentage of K^+-induced vasoconstriction against a log molar

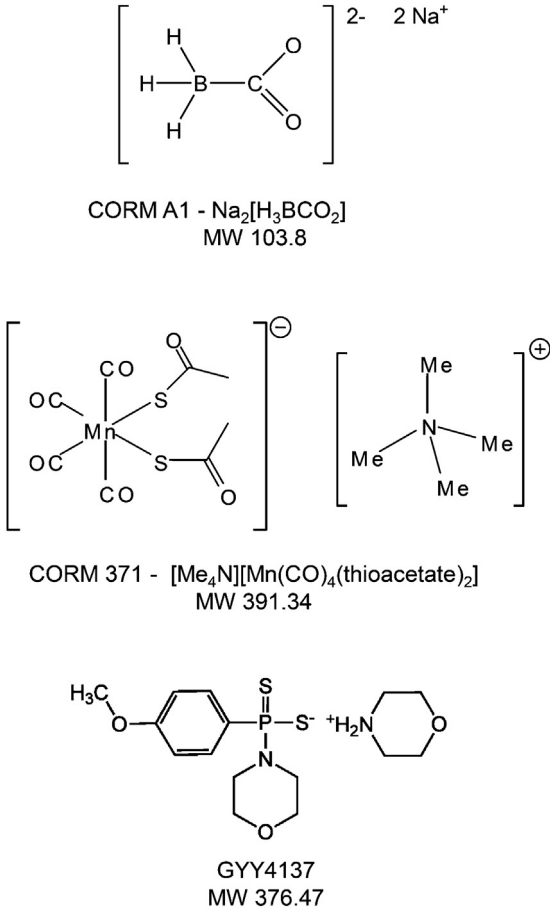

Fig. 1. Chemical structures of GYY4137, CORM A1 and CORM 371.