RELAXANT EFFECT OF A WATER SOLUBLE CARBON MONOXIDE-RELEASING MOLECULE (CORM-3) ON SPONTANEOUSLY HYPERTENSIVE

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Relaxant Effect of a Water Soluble Carbon Monoxide-Releasing Molecule (CORM-3) on Spontaneously Hypertensive Rat Aortas

Paola Failli · Alfredo Vannacci · Lorenzo Di Cesare Mannelli · Roberto Motterlini · Emanuela Masini

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Abstract
Purpose Both carbon monoxide (CO) and nitric oxide (NO) are two gaseous molecules performing relevant functions in mammals. In order to better understand their actions in the cardiovascular system, we have investigated the effects of CORM-3, (tricarbonylchloro(glycinato)ruthenium(II), a water soluble CO-releasing molecule and SNAP (S-nitroso-N-acetyl-DL-penicillamine, a well known NO-releasing molecule) on aortas of normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR).
Methods The isometric contraction of angiotensin II (AT-II) and endothelin-1 (ET-1) was evaluated in endothelium-denuded aortic strips.
Results In control conditions, AT-II induced a similar concentration-dependent contraction in both WKY and SHR, while ET-1 was more effective in SHR aortic strips. CORM-3 or SNAP (10^{-7}–3×10^{-4} M) reduced the contraction induced by AT-II or ET-1 in a concentration-dependent way. Whereas the median inhibitory concentration of SNAP was significantly lower in WKY than in SHR, CORM-3 had a similar effect in both strains. The scaffold compound *i*CORM-3 was ineffective. Pretreatment with an inhibitor of soluble guanylyl cyclase (ODQ, 3×10^{-6} M) marginally reduced CORM-3 relaxation in both strains, whereas it reduced relaxation induced by SNAP in WKY and, to a lesser extent, in SHR. The benzylindazole derivative YC-1 (10^{-6} M), a sensitizer of soluble guanylate cyclase to the action of NO, significantly increased the relaxant effect of SNAP in AT-II precontracted aortic strips. The blocker of calcium-activated potassium channels, charybdotoxin (10^{-8} M), reduced the relaxation induced by CORM-3 in both strains.
Conclusions Different mechanisms seem to be implicated in CO- and NO-mediated vascular relaxation. Since the relaxant properties of CO are conserved in SHR aortas, CORM-3 could be a new potential agent for the treatment of hypertension, when NO donors show sub-optimal or absent responses.

Key words Angiotensin-II · Endothelin-1 · Soluble guanylyl cyclase · Calcium-activated potassium channels

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Abbreviations

AT-II	Angiotensin-II
CHTX	Charybdotoxin
CORM-3	Tricarbonylchloro(glycinato)ruthenium(II)
ET-1	Endothelin-1
ODQ	1H-(1,2,4)-oxadiazolo(4,2-a)quinoxalin-1-one
SHR	Spontaneously hypertensive rats
SNAP	S-nitroso-N-acetyl-DL-penicillamine
WKY	Wistar Kyoto rats
YC-1	3-(5′-hidroxymethyl-2′-furyl)-1-benzylindosole

Introduction

Mammalian cells constantly generate carbon monoxide (CO) gas via the endogenous degradation of heme by a family of constitutive (HO-2 and HO-3) and inducible (HO-1) heme oxygenase (HO) enzymes [1].

CO is a versatile signalling molecule with essential regulatory roles in a variety of physiological and pathological processes [2–4] In the cardiovascular system, CO exerts an important relaxant effect [5] which is shared with the endothelium-derived relaxing factor nitric oxide (NO) generated by the constitutive isoform of endothelial NO synthase (eNOS) [6]. CO, like NO, activates soluble guanylate cyclase [7], although its effect is less potent than that exerted by NO [8, 9]. Although this mechanism contributes to the relaxant effect of CO, it has been demonstrated that CO can directly activate big-conductance calcium-activated potassium channels (BK channels) [10], resulting in membrane hyperpolarization and relaxation in precontracted arteries.

These findings have prompted many researchers to consider CO as a potent therapeutic tool in the treatment of vascular dysfunction, leading to the synthesis and characterization of CO-releasing compounds, among which are transition metal carbonyl compounds capable of carrying and delivering CO into biological systems and possessing vasodilatory properties [11].

Systemic hypertension impairs vascular tone, increases arterial blood pressure and is a major risk for patients with cardiovascular diseases. While the physiological and pharmacological role of NO in hypertension is well established, that of CO in this pathological condition is less clear.

Thus, the purpose of this study was to assess the effect of the water soluble carbon monoxide-releasing molecule CORM-3 [12, 13] on aortas of control normotensive Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats in comparison with S-nitroso-N-acetyl-DL-penicellamine (SNAP), an NO donor. Angiotensin-II (AT-II) and endothelin-1 (ET-1) are very potent contractile agonists of the vasculature system, both of them implicated in human hypertension. Their contractile action on vascular smooth muscle cells is mediated via an increase in cytosolic Ca^{2+}. However, mechanisms of this process are different between the two agents.

Endothelin-1 increases intracellular Ca^{2+} by stimulating influx through Ca^{2+} channels [14] and is known to produce a much more sustained contraction by the vascular smooth muscle cells, whereas angiotensin II elicits a biphasic response that is generated primarily by mobilization of Ca^{2+} from intracellular stores followed by Ca^{2+} entry [15]. Given the different mechanisms for smooth muscle contraction of these agonists, we decided to investigate the relaxant effect of NO and CO on preparations precontracted with AT-II or ET-1.

Methods

Synthesis of CORM-3 and other chemicals

CORM-3 was synthesized as described previously [12, 13] and was prepared as a 10 mM stock by dissolving the compound in pure distilled water. It was kept at −20 °C and defrosted before each experiment. Final concentrations of CORM-3 were prepared immediately before their use.

Inactive CORM-3 (*i*CORM-3) was obtained by leaving CORM-3 in phosphate buffer saline (PBS), pH 7.4 at room temperature overnight to permit the release of all available CO from the molecule [12]. This treatment produces an inactive carrier molecule that no longer releases CO (*i*CORM-3), which was used as a negative control [13].

Angiotensin II (AT-II), endothelin-1 (ET-1) and charybdotoxin (CHTX) were purchased from Sigma Chemical Co. (Milan, Italy), S-nitroso-Nacetyl-DL-penicillamine (SNAP) from Tocris Cookson Ltd (Bristol, UK), 1H-(1,2,4)-oxadiazolo(4,2-a)quinoxalin-1-one (ODQ) from Calbiochem (Milan, Italy) and 3-(5′-hidroxymethyl-2′-furyl)-1-benzylindosole (YC-1) from Alexis Corporation (Nottingham, UK). All other chemicals used in the study were from Sigma Chemical Co. (Milan, Italy), unless otherwise specified.

Detection of carbon monoxide (CO) release from CORM-3 and *i*CORM-3

The amount of CO liberated by CORM-3 and iCORM-3 was measured with an ultra-trace gas detection system (RGA3, Reduction Gas Analyzer, SAES Getters, Milan, Italy) dedicated to CO detection as already described [16] with few modifications. Briefly, 2 ml of PBS solution (pH 7.4) containing different concentrations of CORM-3 and iCORM-3 (10 nM–1 mM) were incubated at 37 °C in sample vials. The gas developing in 2 min was collected in the headspace of the vial and transferred to the reduction gas analyzer via a two-needle assembly attached to the injection valve. This valve was controlled by a timer. The head space gas components were separated chromatographically within an isothermal mandrel-heating column oven at a temperature of 100 °C with CO-free air carrier gas (N_2 70 cc/min). Species eluting from the chromatographic column passed immediately into the detector. CO was quantified in the detector by means of A_{254} of Hg generated from the reaction of CO with HgO at 225 °C. A 10-mV recorder charted the detector response in terms of peak height at a paper speed of 10 mm/min. Peak heights and areas were used to correlate detector response between standards and unknowns. A CO standard curve was prepared immediately prior to sample analysis. Standard gas was obtained from a commercial source (Rivoira, Milan). The method allowed the

precise determination of the CO amount developed by the studied molecules.

Animals

All animal manipulations were carried out according to the European Community guidelines for animal care (DL 116/92, application of the European Communities Council Directive 86/609/EEC). The ethical policy of the University of Florence conforms with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication No. 85-23, revised 1996; University of Florence assurance number: A5278-01). Formal approval to conduct the experiments described was obtained from the animal subject review board of the University of Florence. For all experiments described herein, 12–14-week-old male normotensive WKY (310.5±6.95 g) and age-matched SHR (311.7±9.26 g) were used (Charles River, Italy). Four rats were housed per cage (size 26×41 cm); animals were fed standard laboratory diet and tap water ad libitum, and kept at 23±1 °C with a 12 h light/dark cycle, light at 7 a.m. Animals were killed by cervical dislocation.

Functional studies on rat thoracic aortas

Thoracic aortas were isolated from WKY and SHR and, after removing the endothelium by rubbing the cell layer with a cotton swab, were mounted in an organ bath of 2 ml volume [17].

The strips were perfused with pre-warmed (37 °C) Krebs-Henseleit solution, (pH 7.4) of the following composition (mM): 110 NaCl, 25 $NaHCO_3$, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 11 (+) glucose, 2.5 $CaCl_2$, and gassed with 5 % CO_2 in O_2. The resting tension applied to the aorta preparation was 0.7 g.

After 60 min of stabilization, the absence of endothelium was tested as previously reported [18], and cumulative concentration-response curves of AT-II or ET-1 were performed in different aortic preparations. Then, strips were extensively washed, re-stabilized and precontracted using the maximal contracting dose of AT-II or ET-1 (100 % of contractile response). Cumulative concentration-response curves of either CORM-3 or SNAP were performed in control conditions or after the preincubation (30 min, 37 °C) with 3×10^{-6} M ODQ or 10^{-8} M CHTX.

The effects of CORM-3 and SNAP on vascular relaxation were also assessed in the presence of the soluble guanylate cyclase stimulator, YC-1 (10^{-6} M, which was added 30 min before AT-II or ET-1).

Determination of cyclic GMP levels

Wiped, frozen arteries from WKY and SHR obtained from the different experiments were homogenized in 6 % trichloroacetic acid (TCA) and the samples were centrifuged at 2,000 × g for 15 min. at 4 °C. The precipitate was discarded and TCA was removed from the supernatant by extraction with 5 ml of ether (three times). Residual ether was removed by heating the samples to 70 °C for 5 min.

Cyclic GMP (cGMP) concentration was determined using a competitive enzyme immunoassay kit (Cayman Chemicals, Ann Arbor, MI, U.S.A.). The cGMP content of each sample was divided by tissue wet weight and expressed as $pmol.mg^{-1}$ tissue.

Data handling and statistical analysis

Data are reported as means ± s.e.m. of at least six different experiments per group. Sigmoidal concentration-response curves were plotted with OriginPro® (version 8.1) program and compared with one-way ANOVA followed by Bonferroni's *t* test. The EC50 and IC50 were calculated using the OriginPro® (version 8.1) program in each single curve and compared with one-way ANOVA followed by Bonferroni's *t* test. A $P\leq0.05$ was considered significant.

Results

Detection of CO release from CORM-3 and *i*CORM-3

The release of CO from CORM-3 solution at different concentrations (pH=7.4, 37 °C) is reported in Fig. 1. As shown, the CORM-3 solution spontaneously released CO in a concentration-dependent way in the range from 10 nM to 10 mM.

Predictably, *i*CORM-3 did not release CO at all the tested concentrations, as reported in Fig. 1.

Effect of ET-1 and AT-II on isolated aortas

We tested the contractile effect of AT-II and ET-1 on endothelium denuded aortas isolated from WKY and SHR. The

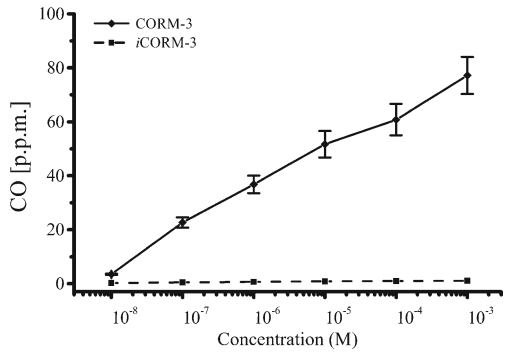

Fig. 1 Release of carbon monoxide (p.p.m.) from CORM-3 and *i*CORM-3 solutions at different concentrations, pH 7.4, 37 °C. The values are means ± s.e.m. of six determinations performed in duplicate

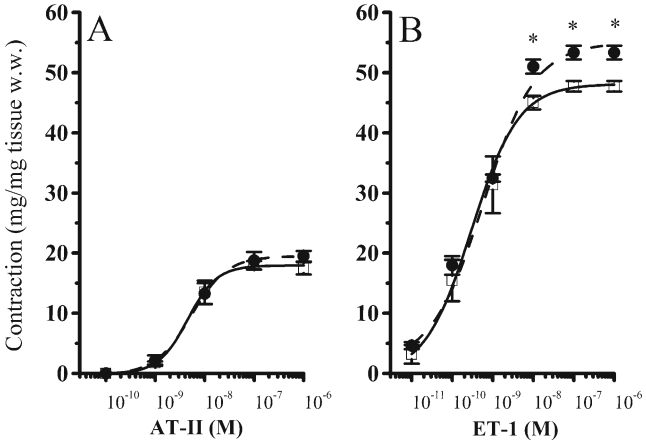

Fig. 2 Concentration-response curves of angiotensin-II (AT-II, panel **a**) and endothelin-1 (ET-1, panel **b**) on endothelium–denuded rat aortic strips isolated from WKY (□) and SHR (•). The values are means ± s.e.m. of 6 different preparations. *$P<0.001$ vs WKY preparations, one way ANOVA followed by Bonferroni's test

cumulative concentration-response curves of AT-II were similar in WKY and SHR preparations in the full range (10^{-10}–10^{-6} M, Fig. 2a), the maximal contractile effect being obtained at 10^{-7} M AT-II. ET-1 was more active than AT-II in evoking contraction in both strains, its effect being significantly higher ($p<0.001$) in aortas isolated from SHR than WKY, even if ET-1 full contractility was obtained at 10^{-8} M in both strains (Fig. 2b).

Vasodilatatory effects of CORM-3 and SNAP in aortic rings isolated from WKY and SHR rats

It has been shown that CORM-3 promotes rapid and significant relaxation in rat aortic rings precontracted with phenylephrine, its effect being mediated by CO [15]. Therefore, we tested the effect of CORM-3 on aortic strips isolated from WKY and SHR and precontracted with 10^{-7} M AT-II or 10^{-8} M ET-1. CORM-3 relaxed precontracted aortic preparations from both strains, its maximal effect occurring at 10^{-4} M when AT-II was used as contractile agonist (Fig. 3a) and 3×10^{-4} M (the maximal concentration usable in our protocol) in ET-1 contraction (Fig. 3b). The CORM-3 concentration-dependent curves were similar at all concentrations tested, independently of the rat strain and contractile agonist used. This comparable effectiveness of CORM-3 in WKY and SHR was also reflected by the median inhibitory concentrations (IC50s) on 10^{-7} M AT-II, and in 10^{-8} M ET-1 precontracted preparations (Table 1). In parallel experiments, AT-II or ET-1 precontracted aortic strips were also treated with 300 μM *i*CORM, the inactive compound which was totally ineffective (data not shown).

Compared to the NO releaser SNAP, CORM-3 showed reduced activity in relaxing WKY aortic preparations, but a similar effectiveness in SHR at all concentrations tested (Fig. 3, panel A and B). To note that SNAP was significantly less potent in SHR than WKY preparations: indeed, the IC50s were about 9 and 6 fold higher in SHR than in WKY in AT-II and ET-1 precontracted preparations respectively (Table 1). On the other hand, CORM-3 showed a similar potency in SHR and WKY preparations and, in SHR aortic strips, its IC50s tending to be lower than those calculated for SNAP in the same strain.

CO-mediated vasorelaxation by CORM-3: possible mechanisms of action

It is well established that CO, endogenously generated from HO-1, exogenously applied as CO gas or delivered by CORMs, behaves by activating several intracellular targets and these actions are shared with NO. For example, activation of soluble guanylyl cyclase [7] and potassium channels [10] has been demonstrated for both CO and NO. Therefore, we performed experiments in order to ascertain the possible multiple mechanisms in the relaxation induced by CO and NO of aortic strips precontracted with AT-II and ET-1.

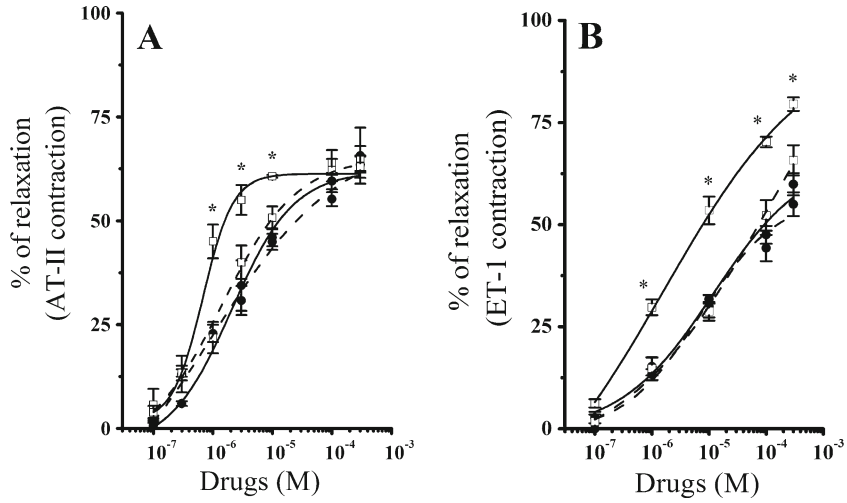

Fig. 3 Concentration-response curves of CORM-3 (*bold symbols* •) and SNAP (*open symbols* □) on endothelium-denuded rat aortic strips isolated from WKY (*solid line*) and SHR (*dot line*) pre-contracted with 10^{-7} M AT-II (panel **a**) and 10^{-8} M ET-1 (panel **b**). The values are means ± s.e.m. of at least 6 different preparations. *$P<0.001$ one way ANOVA followed by Bonferroni's test SNAP in WKY preparations vs all other treatments in SHR preparations

Table 1 Median inhibitory concentrations (IC50%) of SNAP and CORM-3 in aorta preparations isolated from WKY and SHR rats precontracted with AT-II or ET-1

	CORM-3	SNAP
	10^{-7} M AT-II	
WKY	2.6±0.48 μM	0.4±0.05 μM
SHR	1.9±0,73 μM	3.0±0.31 μM *
	10^{-8} M ET-1	
WKY	13.8±1.26 μM	3.5±0.45 μM
SHR	15.6±1.75 μM	21.3±2.88 μM *

The values are the means ± s.e.m. of 6 different preparations. IC50%s were calculated for each different preparation from its concentration-response curve. *$P<0.001$ vs WKY SNAP, one way ANOVA followed by Bonferroni's test

We found that the soluble guanylate cyclase inhibitor ODQ (3×10^{-6} M) reduced the vasodilatation determined by the highest doses (10^{-4}–$3\cdot10^{-4}$ M) of CORM-3 in preparations isolated from both strains and precontracted either with 10^{-7} M AT-II (Fig. 4, panels A and B) or 10^{-8} M ET-1 (Fig. 5, panels A and B). On the other hand, ODQ was more effective in inhibiting SNAP than CORM-3 relaxation (Fig. 4, panels C and D, AT-II contraction; Fig. 5, panels C and D, ET-1 contraction). Particularly in WKY aortic strips precontracted with AT-II or ET-1, ODQ shifted the concentration-dependent curve to the right in the full range of SNAP concentrations. In SHR, ODQ reduced SNAP efficacy at high SNAP concentrations only (10^{-5} M–3×10^{-4} M).

To further investigate the involvement of soluble guanylyl cyclase in mediating the effect of CO and NO, we evaluated the levels of cGMP in aortic preparations isolated from WKY and SHR and preincubated with 3×10^{-4} M CORM-3 or 10^{-5} M SNAP (Table 2). The level of cGMP in control conditions was significantly higher in WKY than in SHR preparations. Nevertheless, the treatment with 3×10^{-4} M CORM-3 increased cGMP contents to a similar value in WKY and SHR. Also 10^{-5} M SNAP significantly increased cGMP up to 6 times in WKY and up to 5.86 times in SHR preparations as compared to their respective control values (Table 2).

The benzylindazole derivative, YC-1 is known to sensitize soluble guanylate cyclase to the action of NO [19]. When aorta preparations were pre-incubated with 10^{-6} M YC-1 and precontracted with AT-II, the relaxing effect of SNAP was significantly increased at all concentrations tested in preparations isolated from WKY and, to a lesser extent, in SHR (Fig. 4, panels A and B); on the contrary the sensitizing action of YC-1 on the relaxing effect of CORM-3 was practically absent in preparations isolated from both strains (Fig. 4).

The BK channel blocker CHTX (10^{-8} M) reduced CORM-3 relaxation in WKY and SHR preparations precontracted with AT-II or ET-1 and shared a similar or higher effectiveness than ODQ in reducing CORM-3 relaxation (Figs. 4 and 5, panels A and B). However, CHTX was less effective than ODQ in reducing SNAP relaxation of WKY precontracted with AT-II (Fig. 4, panel C), but it was similarly effective when the contraction was induced by ET-1 (Fig. 5, panel C). Moreover, SNAP relaxation was reduced by CHTX more efficiently in WKY than in SHR aortic strips precontracted with both agonists (Figs. 4 and 5, panels C and D).

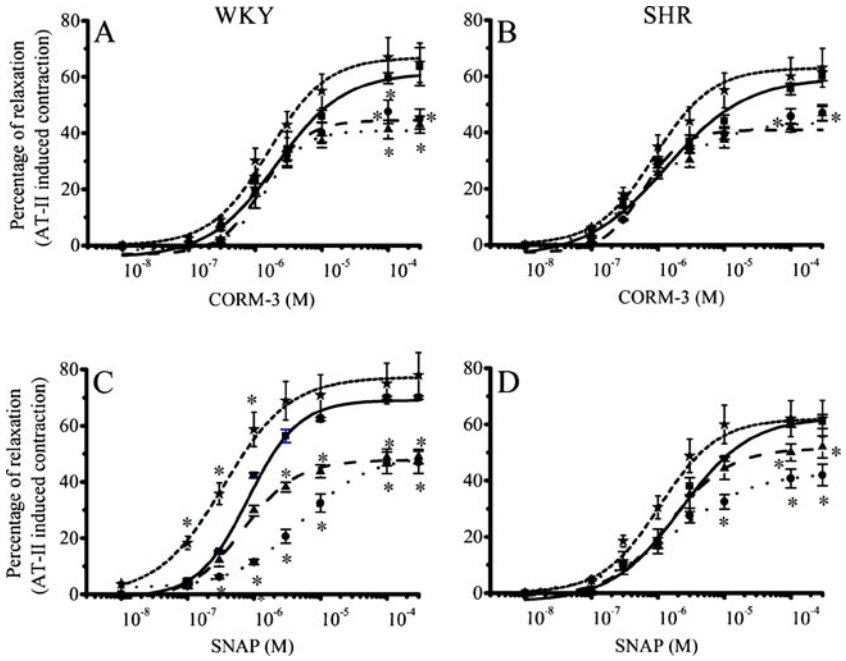

Fig. 4 Concentration-response curve of CORM-3 (WKY panel **a**, SHR panel **b**) or SNAP (WKY panel **c**, SHR panel **d**) in control condition (■, *solid line*), in the presence of 3×10^{-6} M ODQ (•, *dot line*), 10^{-8} M CHTX (▲, *dash line*), 10^{-6} M YC-1 (✳, *short dash line*) on endothelium-denuded rat aortic strips isolated from WKY (panels **a** and **c**) and SHR (panels **b** and **d**) and precontracted with 10^{-7} M AT-II. Aortic preparations were pretreated with ODQ or CHTX for 30 min before contraction with 10^{-7} M AT-II. Values are means ± s.e.m. of 6 preparations. *$P<0.05$ one way ANOVA followed by Bonferroni's test, vs control

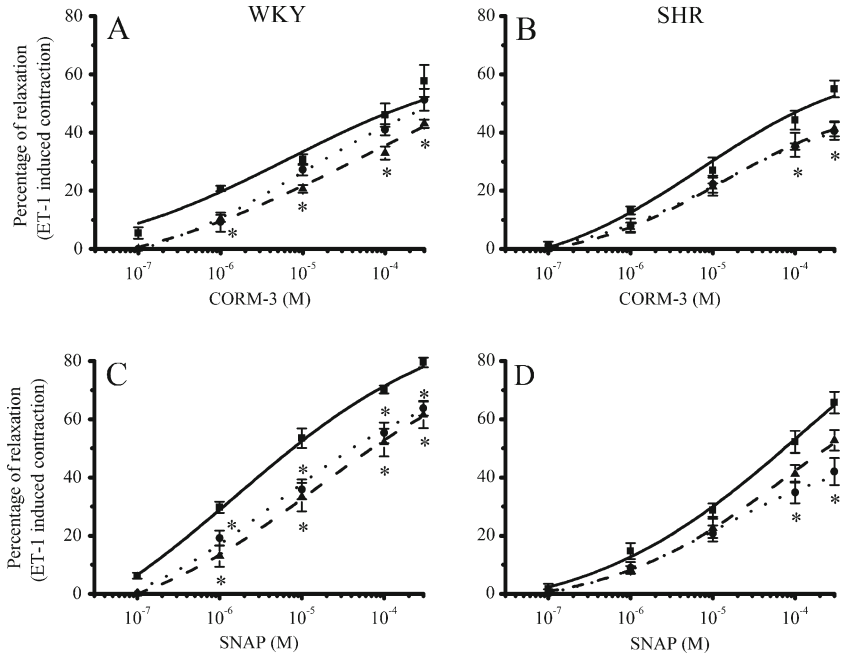

Fig. 5 Concentration-response curve of CORM-3 (WKY panel **a**, SHR panel **b**) or SNAP (WKY panel **c**, SHR panel **d**) in control condition (■, *solid line*), in the presence of 3×10^{-6} M ODQ (•, *dot line*), 10^{-8} M CHTX (▲, *dash line*) on endothelium-denuded rat aortic strips isolated from WKY (panels **a** and **c**) and SHR (panels **b** and **d**) and precontracted with 10^{-8} M ET-1. Aortic preparations were pretreated with ODQ or CHTX for 30 min before contraction with 10^{-8} M ET-1. Values are means ± s.e.m. of 6 preparations. *$P<0.05$ one way ANOVA followed by Bonferroni's test, vs control

Discussion

The present study shows that tricarbonylchloro(glycinato) ruthenium(II), CORM-3 promotes significant relaxation in aortas isolated from WKY and SHR and precontracted with AT-II or ET-1.

CORM-3 as already demonstrated is a highly water soluble, rapid CO releaser molecule [12, 13]. As measured by reduction gas analyzer, CORM-3 can release CO in a concentration-dependent fashion. Although the CO quantity detected is lower than the CORM-3 concentration used, this measurement allows to perform concentration-response curves with CORM-3 on biological preparations.

Like NO, CO is a relaxing factor which induces relaxation of vascular muscle cells by several mechanisms including the stimulation of soluble guanylate cyclase/cGMP/cyclic dependent protein kinase I pathway and various types of potassium channels [3, 8]. All these mechanisms can contribute in a different way to the final relaxation according to the different vascular districts, contractile agents used and patho-physiological experimental models.

Table 2 Effect of CORM-3 and SNAP on cGMP levels of isolated WKY and SHR aortic strips

Cyclic guanosyl monophosphate (cGMP) levels (pmol/mg tissue w.w.)		
	WKY	SHR
Control	1.2±0.11	0.7±0.12*
3×10^{-4} M CORM-3	5.4±0.72	5.5±0.95
10^{-5} M SNAP	7.2±0.82	4.1±0.51**

*$P<0.05$, one way ANOVA followed by Bonferroni's test, SHR vs WKY control, **$P<0.01$ one way ANOVA followed by Bonferroni's test, SHR vs WKY SNAP

Vascular tissue isolated from SHR is a widely used model of experimental hypertension, even if it represents a genetic form of hypertension and therefore it reflects only a subtype of human hypertension. On the other hand, several naturally-occurring cardiac and renal derangements in SHR, especially older ones, are very similar to those observed in human essential hypertension [20]. Therefore, studies in this hypertensive animal model can suggest useful information on the action of pharmacological agents. Moreover, early studies on 7-week old SHR demonstrate that a 4 day treatment with stannous chloride (SnCl2) can increase HO-1 activity in the kidney and decreases blood pressure [21]. However, the blood pressure is not modified by a similar treatment in 20-week old animals. These results suggests that CO may lower blood pressure.

In our study we tested the relaxing effect of CORM-3 and SNAP in endothelium denuded precontracted aortic preparations, isolated from SHR and WKY rats. It is well known that endothelium is the major source of NO [6]. Therefore, in its absence it is unlikely that endogenous produced NO can influence the effect of both exogenous vasodilators.

CORM-3 and SNAP relax aortic strips precontracted with both AT-II and ET-1 in a concentration-dependent way although the vasodilator responses induced by CORM-3 and SNAP are different in the four studied groups. In particular, both relaxing agents are less effective when aortas are contracted with ET-1 that, according to literature data [22], produces a much stronger contraction than AT-II as a result to its different Ca^{2+} mobilizing mechanisms [14, 15, 22]. Particularly in SHR, its maximal effect is significantly

higher than in WKY aortas. Therefore, the reduced effectiveness of both CORM-3 and SNAP in ET-1 precontracted preparations could be related to the stronger contraction induced by ET-1.

In preparations isolated from WKY rats, CORM-3 is less potent than SNAP in relaxing AT-II or ET-1 -precontracted preparations, but CORM-3 shows a similar effectiveness in SHR aortic strips. Whereas the SNAP-induced relaxation is significantly decreased in preparations from SHR as compared to WKY ones, the vasodilator response to CORM-3 is of the same extent in both strains as demonstrated by their IC50s.

The SNAP-induced relaxation is strongly decreased by ODQ and this effect is particularly evident in WKY where it decreases relaxation at all SNAP concentrations used. On the other hand, CORM-3 relaxation is only affected by ODQ at high CORM-3 concentrations (10^{-4}–3×10^{-4} M), suggesting that different pathways may predominate in SNAP and CORM-3 relaxation, at least at low CORM-3 concentrations. It seems that in our experimental conditions, the cGMP pathway is the prominent mechanism involved in the SNAP effect, but it plays a less important role in CORM-3 relaxation. The experiments performed by preincubating the aorta preparations with YC-1, a soluble guanylate cyclase sensitizing agent confirmed this hypothesis. It should also be remembered that CO activation of soluble guanylate cyclase differs by several orders of magnitude from that elicited by NO [8, 9]. In agreement with these data, our results show a significant increase in cGMP content after 3×10^{-4} M CORM-3 or 10^{-5} M SNAP treatment of isolated aortas, while NO, at a low concentration, was more effective than CO in increasing cGMP in WKY rats. This different sensitivity of soluble guanylate cyclase activation by NO and CO could explain why ODQ prevents relaxation only at a high CORM-3 concentration. In preparations isolated from SHR, the increase in cGMP induced by SNAP is significantly lower than in those isolated from WKY rats as well as the relaxation induced by SNAP of precontracted aortas. This result is not unexpected, since the cGMP pathway is impaired in SHR. Soluble guanylate cyclase and cGMP-dependent protein kinase expression are decreased in SHR aortas [23] and functionally, SNAP and the membrane-permeable dibutirryl cyclic GMP do not regulate intracellular calcium concentrations in aortic smooth muscle cells isolated from SHR [24].

CHTX is at least similar or more effective than ODQ in reducing CORM-3 relaxation in both SHR and WKY aortic strips, but less active in inhibiting SNAP relaxation, suggesting that potassium channels are chiefly involved in CO relaxation, but represent a secondary pathway in NO relaxation. CO and NO can activate potassium channels with different modalities [25]. In particular, the stimulatory effects of CO depends on specific interaction with the α-subunit of the calcium activated potassium channel (KCa,α), while NO activation relies on β-subunit (KCa,β), although it can also bind the α-subunit [25]. Pioneer studies on aortas and aortic isolated smooth muscle cells of SHR [26, 27] describe an upregulation of BK that can represent a compensatory mechanism in the hypertension setting. Therefore, CO that targets potassium channels in cardiovascular pathologies, such as hypertension, can provide alternative vasodilator therapies to the traditional agents acting via direct or indirect NO production. Moreover, a recent report suggests the involvement of ATP-dependent (K_{ATP}) potassium channels as part of the dilatatory mechanisms exerted by CORM-3 [13]. However, other authors report the central role of the KCa,α in CO effect [25, 28, 29].

In conclusion, an agent which can relax vasculature independently of cGMP-dependent protein kinase could represent a suitable pharmacological approach in clinical situations where the response to NO is sub-optimal. In this perspective, although less effective than SNAP in physiological conditions, CORM-3 could be a good candidate when the NO/cGMP/cGMP-dependent protein kinase pathway is impaired.

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