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Venodilator action of an organotransition-metal nitrosyl complex

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Abstract

Nitrovasodilators, such as nitroglycerin, cause endothelium-independent dilatation of arterial and capacitance vessels via the release of nitric oxide (NO). This study examined the venodilator effect of $CpCr(NO)_2Cl$ (organotransition-metal nitrosyl complex) relative to that of nitroglycerin in conscious, unrestrained rats. Organotransition-metal nitrosyl complexes have releasable NO directly attached to metal centres. The dose–response effects of $CpCr(NO)_2Cl$ and nitroglycerin on the mean arterial pressure and the mean circulatory filling pressure (index of the body venous tone) were obtained in rats continuously infused with either normal saline or noradrenaline. The results show that both $CpCr(NO)_2Cl$ and nitroglycerin reduced the mean arterial pressure in rats with normal or elevated vasomotor tone. However, maximum depressor response of $CpCr(NO)_2Cl$ was greater than that of nitroglycerin. In vehicle-treated rats, both compounds increased the mean circulatory filling pressure. In rats with elevated vasomotor tone through the infusion of noradrenaline, both agents reduced the mean circulatory filling pressure. These results show that $CpCr(NO)_2Cl$ is an efficacious depressor and venodilator agent.

Keywords: Nitroglycerin; Nitric oxide (NO); Metal nitrosyl complex; Capacitance vessel; Vasodilator; MCFP

1. Introduction

Nitrovasodilators such as nitroglycerin and sodium nitroprusside cause dilatation of resistance and capacitance vessels through the release of nitric oxide and these two drugs have been clinically used for decades for the management of angina pectoris and hypertensive emergencies, respectively. Over the years, other types of nitric oxide donors such as the nucleophile/nitric oxide adduct (Maragos et al., 1991; Ng and Pang, 1998), *S*-nitrosothiols (Ignarro et al., 1981; Ng and Pang, 1998), furoxan (Bohn et al., 1995), zwitterionic diamine/nitric oxide adduct (Zhang et al., 1996), and iron–sulphur–nitrosyls (Flitney et al., 1992, 1996) have also been investigated as alternative vasodilator agents.

The organotransition-metal nitrosyl complexes are a series of nitric oxide-containing compounds that are chemically distinct from the aforementioned drugs (Richter-Addo and Legzdins, 1988, 1992). These compounds contain nitric oxide directly attached to the metal centres via M–NO (metal–nitric oxide) linkages and the rate of release of nitric oxide from these compounds depend on the electron density of the metal centres and the nature of the ancillary ligands. Organotransition-metal nitrosyl complexes such as chloro (η^5-cyclopentadienyl)dinitrosylchromium [$CpCr(NO)_2Cl$] and chloro(η^5-cyclopentadienyl)dinitrosylmolybdenum [$CpMo(NO)_2Cl$] have been shown to cause endothelium-independent relaxation of aortic rings in vitro (Wang et al., 2000). Unlike nitroglycerin, in vitro experiments show that preexposure to $CpCr(NO)_2Cl$ for 1 h does not attenuate subsequent relaxation response to the compound, suggesting that tolerance does not develop readily to $CpCr(NO)_2Cl$. Furthermore, i.v. bolus injections of $CpCr(NO)_2Cl$ and $CpMo(NO)_2Cl$ cause dose-dependent reductions of the mean arterial pressure. The hypotensive potency of $CpCr(NO)_2Cl$ is higher than that of $CpMo(NO)_2Cl$ (by approximately 300-fold) and nitroglycerin (40-fold).

It is unclear if organotransition-metal complexes cause venodilatation in vivo. This study examined the effects of an organotransition-metal nitrosyl complex, relative to those of nitroglycerin, on the mean arterial pressure, and the mean circulatory filling pressure in conscious rats. Mean circulatory filling pressure is the equilibrium pressure that would exist following the circulatory arrest and instantaneous redistribution of blood throughout the circulation (Guyton et al., 1973). A change in the mean circulatory filling pressure

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at a constant blood volume reflects a change in the body venous tone (Tabrizchi and Pang, 1992; Rothe, 1993; Pang, 2000, 2001).

2. Materials and methods

2.1. Animal preparation

Male Sprague–Dawley rats (300–400 g) were anesthetized with halothane. A polyethylene cannula (PE50) was inserted into the left iliac artery for the recording of the mean arterial pressure, by a pressure transducer (P23DB, Gould Statham, CA, USA). PE50 cannulae were also inserted into the right iliac vein for the administration of drugs or vehicle, and the inferior vena cava, via the left iliac vein, for the measurement of the central venous pressure by another pressure transducer (P23DB). A saline-filled, balloon-tipped catheter was inserted into the right atrium via the right external jugular vein. The proper location of the atrial balloon was tested by injecting saline into the balloon and obtaining a simultaneous decrease of the mean arterial pressure to 20–25 mm Hg and an increase of the central venous pressure within 5 s of circulatory arrest. All cannulae were filled with heparinized (25 I.U./ml) normal saline, tunneled subcutaneously along the back, and exteriorized at the back of the neck. The rats were used at 6–8 h after surgery. In our experience, 6 h is a sufficient time for the recovery of the effects of brief halothane anesthesia and minor surgery on hemodynamics. Afterwards, the conscious rats were allowed to wander freely in a small cage. Upon the commencement of experiments, the mean arterial pressure and the central venous pressure were monitored continuously. Heart rate was electronically derived from the upstroke of the arterial pulse pressure by a tachograph (Grass, Model 7P4G).

2.2. Measurement of mean circulatory filling pressure

The method for measuring the mean circulatory filling pressure in rats has been described in detail (Pang, 2000). Briefly, steady-state readings of the mean arterial pressure and the central venous pressure were noted at 4–5 s after the circulatory arrest via inflation of the implanted balloon. The difference between steady state and the baseline central venous pressure (before balloon inflation) was referred to as the venous plateau pressure. To avoid rapid equilibration of the arterial and venous pressures during circulatory arrest, the arterial pressure contributed by the small amount of trapped arterial blood was corrected by the following equation: $MCFP = VPP + 1/60\ (FAP - VPP)$, where MCFP, VPP, and FAP represents the mean circulatory filling pressure, the final arterial pressure, and the venous plateau pressure, respectively, obtained within 5 s of the circulatory arrest, and 1/60 represents the ratio of arterial to the venous compliance.

2.3. Experimental protocol

Rats were randomly assigned into two main groups that received a continuous i.v. infusion of either noradrenaline (71 nmol/kg min) or the vehicle (2.5 μl/min). Noradrenaline was used to increase the vasomotor tone to enable the examination of the venodilator effect of a compound. At 20 min after the start of infusion of noradrenaline or vehicle, the rats within each main group received infusions of various doses of either $CpCr(NO)_2Cl$ (2×10^{-8} to 2×10^{-6} mol/kg min, $n = 5–6$) or nitroglycerin (2×10^{-7} to 10^{-5} mol/kg min, $n = 5–6$), at intervals of 10 min and recovery periods of 5 min/dose. The mean arterial pressure and mean circulatory filling pressure readings were taken at 8 and 9 min following the start of each infusion, respectively.

2.4. Drugs

$CpCr(NO)_2Cl$ was provided by Dr. Peter Legzdins at the Department of Chemistry, the University of British Columbia. Nitroglycerin injection was purchased from David Bull Lab. (Victoria, Australia). Noradrenaline bitartrate was obtained from Sigma (St. Louis, MO, USA). All drugs were dissolved in normal saline (0.9% NaCl solution).

2.5. Calculations and statistical analysis

All results were expressed as the mean ± standard error of the mean (S.E.M.) and analyzed by the analysis of variance followed by Duncan's multiple range test, with $P < 0.05$ selected as the criterion for the statistical significance.

3. Results

Baseline group values of the mean arterial pressure and the mean circulatory filling pressure in all groups of rats

Table 1
Baseline values (mean ± S.E.M.) of the mean arterial pressure (MAP), the mean circulatory filling pressure (MCFP), and the heart rate (HR) in four groups of conscious, unrestrained rats ($n = 5–6$ per group) to be treated with either $CpCr(NO)_2Cl$ or nitroglycerin

Treatment	MAP (mm Hg)	MCFP (mm Hg)	HR (beats/min)
$CpCr(NO)_2Cl$: saline pretreatment	109 ± 2	5.4 ± 0.4	387 ± 13
$CpCr(NO)_2Cl$: noradrenaline pretreatment	152 ± 4[a]	7.5 ± 0.3[a]	383 ± 18
Nitroglycerin: saline pretreatment	103 ± 3	6.3 ± 0.3	380 ± 15
Nitroglycerin: noradrenaline pretreatment	159 ± 3[a]	9.2 ± 0.5[a]	394 ± 13

These rats were pretreated with a continuous infusion of either saline (0.9% NaCl; 2.5 μl/min) or noradrenaline (71 nmol/kg min).

[a] Significantly different ($P < 0.05$) from the corresponding saline infusion group.

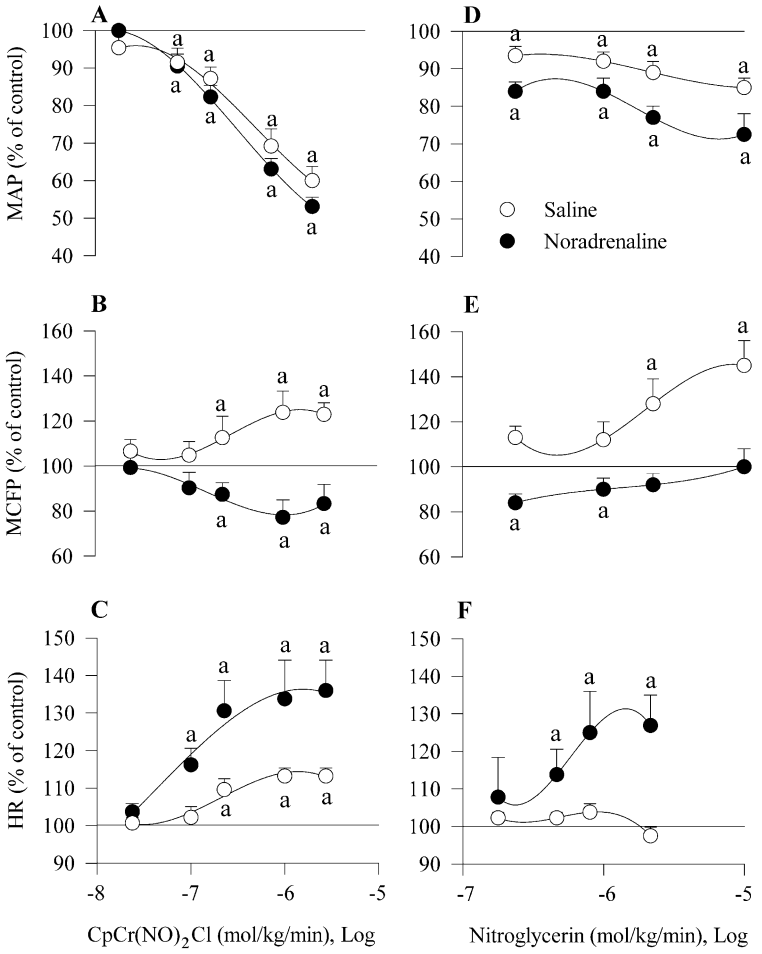

Fig. 1. Dose–response curves (mean ± S.E.M.; $n = 5–6$ per group) of the effects of $CpCr(NO)_2Cl$ and nitroglycerin on the mean arterial pressure (MAP; A and D), the mean circulatory filling pressure (MCFP; B and E), and the heart rate (HR; C and F) in four groups of conscious, unrestrained rats ($n = 5–6$ per group) continuously infused with either saline (0.9% NaCl; 2.5 μl/min) or noradrenaline (71 nmol/kg min). [a]Significantly different from the corresponding baseline measurement.

continuously infused with noradrenaline were significantly higher than those infused with saline (Table 1). Heart rate was similar among all the groups.

In vehicle-treated rats, i.v. infusion of $CpCr(NO)_2Cl$ dose-dependent decreased the mean arterial pressure, and increased the mean circulatory filling pressure, as well as the heart rate (Fig. 1). The infusion of nitroglycerin in vehicle-treated rats also significantly decreased the mean arterial pressure, maximum depressor response to nitroglycerin was, however, significantly less than that to the highest dose of $CpCr(NO)_2Cl$ (Fig. 1A and D). Nitroglycerin caused similar increases in the mean circulatory filling pressure as $CpCr(NO)_2Cl$ (Fig. 1B and E) but unlike $CpCr(NO)_2Cl$, nitroglycerin did not alter the heart rate (Fig. 1C and F).

In rats pretreated with noradrenaline, $CpCr(NO)_2Cl$ caused dose-dependent decreases in the mean arterial pressure, as well as in the mean circulatory filling pressure (Fig. 1). Nitroglycerin also decreased the mean arterial pressure and the mean circulatory filling pressure in noradrenaline-treated rats. Relative to $CpCr(NO)_2Cl$, nitroglycerin caused significantly less maximum depressor response (Fig. 1A and D) but a similar maximum decrease in the mean circulatory filling pressure (Fig. 1B and E) and similar tachycardia (Fig. 1C and F) in rats treated with noradrenaline. However, unlike $CpCr(NO)_2Cl$, there was no apparent dose-dependent mean circulatory filling pressure relationship obtained with the infusion of nitroglycerin.

4. Discussion

The results show that both $CpCr(NO)_2Cl$ and nitroglycerin reduced the mean arterial pressure in rats pretreated with vehicle or noradrenaline, maximum depressor response to nitroglycerin was, however, less than those of $CpCr(NO)_2Cl$. Reflex tachycardia was observed with $CpCr(NO)_2Cl$ but not with nitroglycerin which caused markedly less hypotension. Both compounds significantly increased the mean circulatory filling pressure, an index of body venous tone (Tabrizchi and Pang, 1992; Pang, 2000, 2001). Increase in the mean circulatory filling pressure also occurred following the administration of vasodilators such as Ca^{2+} channel antagonist (e.g.,

verapamil and nifedipine; Waite et al., 1988), nitrovasodilators (e.g., nitroglycerin and sodium nitroprusside; D'Oyley et al., 1989), potassium channel activator (e.g., pinacidil; Waite et al., 1995), or calcitonin gene-related peptide (Abdelrahman and Pang, 1992). These increases were previously shown to be due to the hypotension-induced reflex sympathetic activation.

In rats continuously infused with noradrenaline, both $CpCr(NO)_2Cl$ and nitroglycerin reduced the mean arterial pressure, as well as the mean circulatory filling pressure and increased the heart rate. Maximum depressor response to nitroglycerin in the noradrenaline-treated rats was again less than that of $CpCr(NO)_2Cl$. The reductions in the mean circulatory filling pressure in response to $CpCr(NO)_2Cl$ was dose-dependent but the response to nitroglycerin was apparently not dose-dependent.

These results show that $CpCr(NO)_2Cl$, an organotransition-metal nitrosyl complex, is an efficacious dilator of arterial and capacitance vessels. Organotransition-metal nitrosyl compounds are potential nitrovasodilators for the management of cardiovascular diseases.

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