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Cardiovascular effects of a novel, potent and selective phosphodiesterase 5 inhibitor, DMPPO: *in vitro* and *in vivo* characterization

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1 The aim of this study was to investigate the cardiovascular effects of a novel, potent and specific phosphodiesterase 5 (PDE 5) inhibitor, 1,3 dimethyl-6-(2-propoxy-5-methane sulphonylamidophenyl)-pyrazolo[3,4-d]pyrimidin-4-(5H)-one (DMPPO) in phenylephrine-precontracted rat aortic rings and different *in vivo* rat preparations.

2 DMPPO elicited a concentration-dependent relaxation of rat aortic rings with functional endothelium. DMPPO-induced relaxation was abolished by endothelium removal or pretreatment with the soluble guanylate cyclase inhibitor, methylene blue (10 μM).

3 In aortic rings without endothelium, the potency ($pD_2 = -\log_{10} EC_{50}$) of atrial natriuretic peptide (ANP) to induce relaxation increased from 8.13 ± 0.05 in the absence of DMPPO to 8.32 ± 0.05 and 8.52 ± 0.08 in the presence of 30 nM and 100 nM DMPPO, respectively. Similarly, the potency of sodium nitroprusside (SNP) in inducing relaxation increased from 7.38 ± 0.07 in the absence of the PDE 5 inhibitor to 8.07 ± 0.11 and 8.15 ± 0.08 in the presence of 30 nM and 100 nM DMPPO, respectively. In contrast, relaxation to the adenylate cyclase activator, forskolin, was unchanged by DMPPO (100 nM).

4 In rings without endothelium, DMPPO (100 nM) increased by 2.5 fold intracellular levels of guanosine 3':5'-cyclic monophosphate (cyclic GMP). Moreover, DMPPO (100 nM) potentiated the increases in cyclic GMP levels induced by ANP (30 nM) by 3 fold and SNP (30 nM) by 2.7 fold. Adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels were not modified by DMPPO.

5 In anaesthetized normotensive or spontaneously hypertensive rats (SHR), DMPPO (2 and 5 mg kg^{-1}, i.v.) lowered blood pressure without affecting heart rate. Similarly, in conscious SHR, orally administered DMPPO (5 mg kg^{-1}) induced a 25 mmHg decrease in blood pressure for at least 7 h without modifying heart rate. Meanwhile, urinary cyclic GMP was increased by 50% whereas cyclic AMP remained unchanged.

6 In normotensive anaesthetized rats, sodium nitroprusside (SNP) (i.v. bolus) induced a decrease in blood pressure which rapidly returned to baseline. In DMPPO (1 mg kg^{-1}, i.v.)-treated rats, the hypotensive effects of SNP (10 to 100 μg kg^{-1}) were prolonged over time whereas the peak effect was unchanged.

7 In pithed rats, phenylephrine (i.v. bolus) induced dose-dependent increases in blood pressure. Pretreatment with DMPPO (5 mg kg^{-1}, i.v.) partially inhibited the pressor response to phenylephrine (0.3 to 100 μg kg^{-1}).

8 In conclusion, the potent and selective PDE 5 inhibitor, DMPPO, produces relaxation in isolated vessels in the presence of a cyclic GMP drive and reduces blood pressure of intact animals. Its high oral bioavailability and long duration of action should make it a useful tool to study the role of cyclic GMP in various biological systems.

Keywords: Phosphodiesterase 5; cyclic GMP; PDE inhibitor; DMPPO; vasorelaxation; aortic ring; hypertension

Introduction

Guanosine 3':5'-cyclic monophosphate (cyclic GMP) is a second messenger implicated in signal transduction in a wide variety of cell types. In vascular smooth muscle cells (VSMC), a large body of evidence indicates an essential role of cyclic GMP in modulating vascular tone (Ignarro & Kadowitz, 1985; Murad, 1986). In VSMC, two forms of guanylate cyclase (EC 4..6.1.2.) catalyse the synthesis of cyclic GMP from GTP (Fulle & Garbers, 1994); one is a plasma-membrane associated enzyme which is activated by natriuretic peptides (Garbers, 1989; Anand-Srivastava & Trachte, 1993). The other is soluble and can be stimulated physiologically by endothelium-derived nitric oxide (NO) (Gerzer *et al.*, 1981; Luscher, 1991; Ignarro, 1992) and carbon monoxide (Morita *et al.*, 1995; Christodoulides *et al.*, 1995), and, pharmacologically, by NO donors such as sodium nitroprusside (SNP).

Degradation of cyclic nucleotides by hydrolytic cleavage of the 3'-ribose-phosphate bond is catalyzed by cyclic nucleotide phosphodiesterases (PDE, EC 3.1.4.17.) (Beavo, 1990). PDEs have been classified into at least seven different isozyme families (Manganiello *et al.*, 1995) depending on the nucleotide being preferentially hydrolysed and the regulatory properties of the enzyme. In VSMC, three types of PDEs hydrolysing cyclic GMP are present: PDE 1 (Ca^{2+}-calmodulin-dependent PDE), PDE 3 (cyclic GMP-inhibited PDE) and PDE 5 (cyclic GMP-specific PDE) (Komas *et al.*, 1991; Saeki & Saito, 1993). Recently, Coste & Grondin (1995) described a new potent and selective PDE 5 inhibitor, DMPPO. This compound (1,3 dimethyl-6-(2-propoxy-5-methane sulphonylamidophenyl) pyrazalo [3,4-d] pyrimidin-4(5H)-one) is a competitive inhibitor with respect to cyclic GMP ($K_i = 0.003$ μM) and displays high selectivity for PDE 5 when compared to other PDE isozymes ($K_i = 1$ μM, 3 μM, 10 μM and 22 μM, respectively for PDE 1, 2, 3, and 4). In cultured rat aortic smooth muscle cells, DMPPO is capable of increasing SNP and atrial natriuretic peptide

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(ANP)-stimulated cyclic GMP levels. Comparative experiments performed with non selective PDE inhibitors and with the selective PDE 5 inhibitor, DMPPO, allowed Coste & Grondin (1995) to conclude that PDE 5 is the main PDE involved in the regulation of cyclic GMP levels in VSMC. Therefore, selective inhibition of this enzyme would lead to an increase in intracellular cyclic GMP content and subsequently to vasorelaxation and decrease of arterial blood pressure. Two mechanisms may be involved: potentiation of cyclic GMP-stimulating vasodilators such as NO or atrial natriuretic peptide (ANP) and/or functional antagonism of endogenous vasoconstrictors.

Zaprinast has widely been used in cells and tissues as a reference PDE 5 inhibitor (Harris *et al.*, 1989; McMahon *et al.*, 1989; Merkel *et al.*, 1992). However, this compound is not very potent and lacks specificity when compared to DMPPO (Coste & Grondin, 1995). Recently, two others inhibitors of cyclic GMP catabolism, WIN 58237 and E4021, have been described (Silver *et al.*, 1995; Saeki *et al.*, 1995).

The aim of the present study was to characterize the functional effects of DMPPO in the cardiovascular system using *in vitro* preparations and animal models.

Methods

Preparation of rat aortic rings

Male Sprague-Dawley rats (350–450 g) were killed by cervical dislocation. The thoracic aorta was quickly removed and placed in a physiological salt solution (PSS) containing (mM):NaCl 117, KCl 5, $CaCl_2$ 1.5, NaH_2PO_4 1.1, $NaHCO_3$ 25; $MgSO_4$ 1.2; glucose 11.5. The aorta was cleaned of fat and connective tissue and cut into 3 mm rings. Rings were mounted in 20 ml organ baths on stainless steel hooks and bathed with PSS maintained at 37°C and gassed with 95% O_2-5% CO_2. All experiments were carried out under an initial tension of 2 g. Tension development was measured isometrically with a Grass transducer (FT 03) connected to a Graphtec linearcorder (WR3310). Data acquisition and analysis were carried out with the JAD v1.2 programme (Notocord systems, France).

Rings were allowed to equilibrate for 1.5 h during which they were repeatedly washed with PSS. In some experiments endothelium was removed by gently rubbing the luminal surface of the vessel. Endothelium removal was confirmed by the lack of relaxation of 2 μM acetylcholine in rings precontracted with 1 μM phenylephrine. In contrast, acetylcholine (2 μM) relaxed, by more than 50%, phenylephrine precontracted rings with a functional endothelium. Relaxation was calculated as a percentage of the maximal contraction induced by phenylephrine 1 μM. Phenylephrine and acetylcholine were dissolved in distilled water and were freshly prepared before each experiment.

Direct effect of DMPPO on rat aortic rings

Vasorelaxant effects of DMPPO were measured in rat aortic rings with and without endothelium. In both types of preparation, DMPPO was added cumulatively (10 nM to 1 μM) every 15 min, after the contraction to phenylephrine (1 μM) had reached a plateau. In a different set of experiment, a dose-response to DMPPO was obtained in preparations with endothelium pretreated with the soluble guanylate cyclase inhibitor, methylene blue (10 μM). Methylene blue was dissolved in distilled water and added into the organ bath 30 min prior phenylephrine contraction. DMPPO was dissolved in 100% dimethylsulphoxide (DMSO). The final concentration of DMSO in organ baths never exceeded 0.1%.

Effect of DMPPO on ANP, SNP and forskolin-induced relaxation in rat aortic rings

These experiments were performed in endothelium-denuded rings to avoid interference of endothelial NO with the exogenously added NO donor (SNP) or particulate guanylate cyclase activator (atrial natriuretic peptide, ANP). Preparations were incubated for 30 min with DMPPO (30 nM and 100 nM) or its vehicle before addition of phenylephrine (1 μM). Once contraction to phenylephrine (1 μM) had become stable, a concentration-response curve to ANP (1 nM to 0.1 μM), SNP (1 nM to 1 μM) or forskolin (30 nM to 3 μM) was obtained. Relaxation caused by each addition of compound was allowed to develop its full effect before addition of the subsequent dose. ANP and SNP were dissolved in distilled water and solutions were freshly prepared before each experiment. Forskolin was dissolved in DMSO but final concentration of DMSO in organ baths never exceeded 0.1%.

Cyclic nucleotide measurements in rat aortic rings

Rat aortic rings without endothelium were equilibrated in PSS as described above except that they were not under tension. Since an effective removal of endothelium could not be functionally tested, L-NAME (300 μM), a NO synthase inhibitor, was added in all organ baths. Preparations were incubated with DMPPO (100 nM) or its vehicle for 30 min before addition of ANP (30 nM) or SNP (30 nM). Five min after ANP or SNP addition, rings were rapidly collected and frozen in liquid nitrogen to prevent degradation of cyclic nucleotides. Frozen tissue was homogenized in cold trichloroacetic acid (6%) and the homogenate centrifuged (2000 g for 15 min at 4°C). Protein content in the pellet was determined by the BCA protein assay reagent (Pierce). The supernatant was washed 4 times with 5 volumes of water saturated diethyl ether. The upper ether layer was discarded after each wash. The aqueous phase was then evaporated to dryness with a Speed-vac system and the dried extract dissolved in a suitable volume of assay buffer prior to analysis. Cyclic nucleotide content was determined by Scintillation Proximity assay (Amersham).

Blood pressure effects of DMPPO in anaesthetized normotensive and hypertensive rats

Male normotensive rats (CD, Charles River France) and spontaneously hypertensive rats (SHR, Charles River France) weighing 300 to 350 g were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). The left carotid artery and femoral vein were catheterized for blood pressure measurement and intravenous injections, respectively. Blood pressure and heart rate were monitored continuously and recorded on a Graphtec polygraph. After an equilibration time, DMPPO or its vehicle (5% NaOH 1N, 95% saline) were infused intravenously (100 μl min^{-1} over 5 min). Results are reported as changes in mean arterial blood pressure (MABP, mmHg) and heart rate (HR, beats min^{-1}).

Potentiation of SNP-dependent hypotensive effects by DMPPO in anaesthetized normotensive rats

Male normotensive rats (CD, Charles River France) were anaesthetized and catheterized as described above. An initial 3 μg kg^{-1} dose of SNP was administered i.v. (bolus 100 μl) to test vascular reactivity. When blood pressure returned to baseline value, DMPPO (1 mg kg^{-1}) or its vehicle (5% NaOH 1N, 95% saline) were infused i.v. (20 μl min^{-1} over 10 min). When blood pressure returned to the initial value, a dose-response to SNP (1 to 100 μg kg^{-1}) was obtained (i.v. bolus 100 μl). Blood pressure was allowed to recover between each SNP dose. Results are reported as changes in MABP (mmHg) and area under curve of the hypotensive effect (AUC, mmHg min^{-1}).

Inhibition of phenylephrine pressor effects by DMPPO in pithed rats

Male normotensive rats (CD, Charles River France) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.).

The trachea was cannulated for artificial ventilation and pithing was performed via the right orbit with a stainless-steel rod. The left carotid artery and femoral vein were catheterized for blood pressure measurement and drug administration, respectively. An initial 3 $\mu g\ kg^{-1}$ dose of phenylephrine was administered i.v. (100 μl bolus) to test vascular reactivity. When blood pressure returned to the baseline value, DMPPO (5 $mg\ kg^{-1}$) or its vehicle (5% NaOH 1N, 95% saline) were infused (20 $\mu l\ min^{-1}$, i.v. over 10 min). After blood pressure had recovered to its initial value, a dose-response curve to phenylephrine (0.3 to 100 $\mu g\ kg^{-1}$) was established (i.v. bolus, 100 μl). Blood pressure was allowed to recover between each dose. Results are reported as changes in MABP (mmHg).

Effects of orally administrated DMPPO on blood pressure of conscious SHR

These experiments were performed in hyptertensive rats (SHR, Charles River France) weighing 340 to 380 g. The day before the experiment, the left carotid artery was catheterized under pentobarbitone anaesthesia. The catheter was directed subcutaneously to the back of the neck and exteriorized. On the day of the experiment, animals were placed in individual plexiglas boxes. The arterial catheter was connected to a pressure transducer for blood pressure measurement. After an equilibration time of approximately 30 min, DMPPO 5 $mg\ kg^{-1}$ or its vehicle (10% NaOH 1N, 90% saline) were administered *per os* in a volume of 1 ml. Arterial blood pressure and heart rate were monitored continuously over 7 h and the results are expressed as changes in MABP and HR.

Another group of SHR was placed in metabolic cages after oral administration of DMPPO (5 $mg\ kg^{-1}$) or its vehicle. Urine was collected for 7 h and cyclic nucleotide content was determined by Scintillation Proximity Assay.

Statistical analysis

Data are presented as means ± s.e.mean. Comparisons were performed by unpaired two-tailed Student's *t*-test. Factorial two-way analysis of variance (ANOVA) was performed to test the interaction between treatments. Statistical significance was assumed when $P<0.05$.

Materials

L-Phenylephrine, acetylcholine bromide, SNP, ANP, forskolin, methylene blue and L-NAME were obtained from Sigma Chemical Co (St. Quentin Fallavier, France). DMPPO was synthesized according to Dumaître & Dodic (1995).

Results

Direct effect of DMPPO on rat aortic rings

In phenylephrine-precontracted rat aortic rings with endothelium, DMPPO induced a concentration-dependent relaxation which attained 100% at 1 μM (Figure 1). When rings with endothelium were preincubated with 10 μM methylene blue, or when endothelium was removed, DMPPO (up to 1 μM) was unable to relax phenylephrine-precontracted aortic rings (Figure 1).

Effect of DMPPO on ANP, SNP and forskolin-induced relaxation in rat aortic rings

In endothelium-denuded rat aortic rings, preincubation with DMPPO, 30 nM or 100 nM did not modify the magnitude of phenylephrine-induced contraction. In phenylephrine-precontracted rings, ANP induced a concentration-dependent relaxation (Figure 2a) which attained 100% for 100 nM ANP. The potency of ANP in the absence of the PDE 5 inhibitor ($pD_2 = 8.13 \pm 0.05$) was increased in the presence of 30 nM and 100 nM DMPPO ($pD_2 = 8.32 \pm 0.05$ and 8.52 ± 0.08), respectively.

Similarly, SNP produced a concentration-dependent vasorelaxation in phenylephrine-precontracted rat aortic rings (Figure 2b). Maximal relaxation (100%) was attained at 1 μM SNP. The potency of SNP in the absence of the PDE 5 inhibitor ($pD_2 = 7.38 \pm 0.07$) was greater in the presence of 30 nM and 100 nM DMPPO ($pD_2 = 8.07 \pm 0.11$ and 8.15 ± 0.08, respectively).

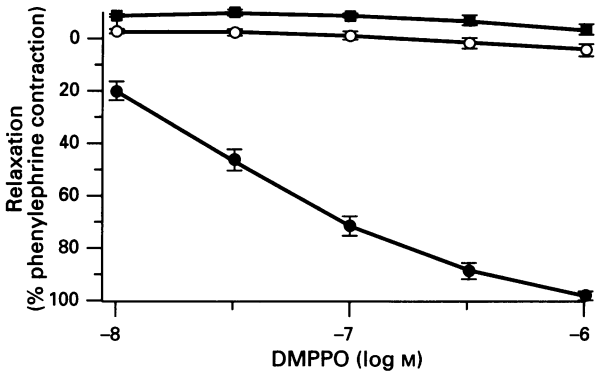

Figure 1 Concentration-response curve to DMPPO in phenylephrine (1 μM) precontracted rat aortic rings in the presence of a functional endothelium (solid symbols), either with (■) or without (●) 10 μM methylene blue, and in the absence of a functional endothelium (○). Initial contractions to phenylephrine were 3.46 ± 0.07, 2.9 ± 0.12 and 3.11 ± 0.23 g respectively. Values are mean ± s.e.mean for $n = 8-14$ rings.

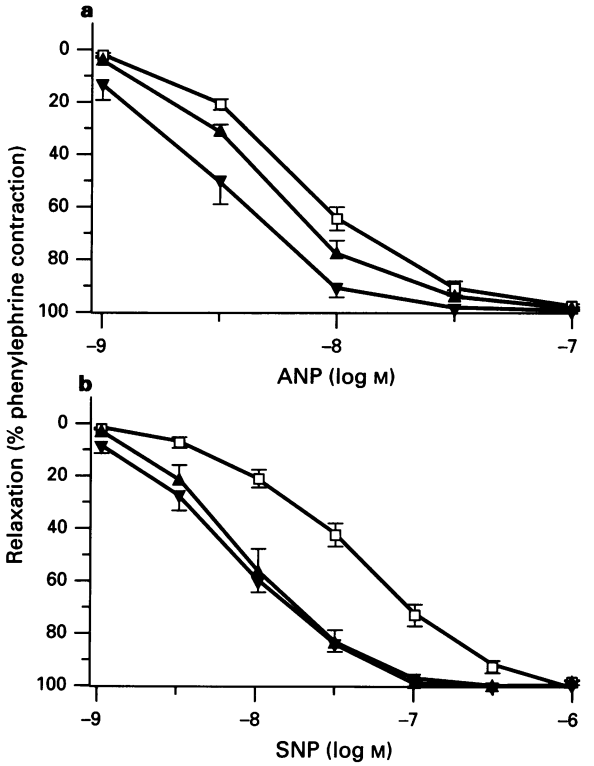

Figure 2 Potentiation of ANP (a) and SNP (b)-induced relaxation (□) by DMPPO 30 nM (▲) and 100 nM (▼) in endothelium-denuded rat aortic rings precontracted with phenylephrine (1 μM). In ANP experiments, initial contractions to phenylephrine were (g): 2.79 ± 0.17 in the control group (without DMPPO), 3.01 ± 0.26 in rings preincubated with DMPPO 30 nM and 2.70 ± 0.15 in rings preincubated with DMPPO 100 nM. In SNP experiments, initial contractions to phenylephrine were (g): 3.33 ± 0.22 in the control group (without DMPPO), 3.36 ± 0.14 in rings preincubated with DMPPO 30 nM and 2.89 ± 0.13 in rings preincubated with DMPPO 100 nM. Values are mean ± s.e.mean for $n = 6-12$.

In contrast, DMPPO (100 nM) did not modify the cyclic AMP-mediated concentration-dependent relaxation to forskolin (Figure 3).

Effect of DMPPO on cyclic nucleotides levels in rat aortic rings

In endothelium-denuded rat aortic rings, incubation with DMPPO, 100 nM for 30 min, induced a 2–3 fold increase in cyclic GMP content (Table 1). In the presence of ANP (30 nM) or SNP (30 nM), cyclic GMP levels were increased 3 and 5 fold, respectively. When the preparations were preincubated with DMPPO (100 nM), ANP and SNP increased cyclic GMP levels by 9 and 13 fold respectively, compared to basal levels. Factorial ANOVA showed a positive interaction ($P=0.02$) between ANP or SNP and DMPPO treatment. These results indicate that DMPPO has more than an additive effect on ANP- and SNP-increases in cyclic GMP levels and thus confirm a synergistic activity between ANP or SNP and DMPPO. In all the above experimental conditions, cyclic AMP levels were not significantly modified.

Effects of DMPPO on normotensive and hypertensive anaesthetized rats

In anaesthetized normotensive rats, initial MABP and HR were 127±4 mmHg and 416±15 beats min^{-1} respectively for the DMPPO-treated group ($n=6$) and 123±6 mmHg and 429±31 beats min^{-1}, respectively, for vehicle-treated group ($n=5$). DMPPO (5 mg kg^{-1}) infused intravenously over 5 min induced a rapid decrease in MABP (Figure 4): 2 min after starting the infusion, the effect of DMPPO was maximal and attained −30 mmHg relative to vehicle alone. At the end of the infusion, the decrease in MABP was 20 mmHg compared to vehicle-treated animals. However, a small but significant decrease in MABP (−10 mmHg) persisted until the end of experiment (30 min). Heart rate was unchanged by DMPPO throughout the experiment.

In anaesthetized spontaneously hypertensive rats, initial MABP were (mmHg): 174±8.3, 188±3.7 and 170±8.2, for vehicle ($n=8$), 2 mg kg^{-1} ($n=5$) and 5 mg kg^{-1} DMPPO ($n=7$)-treated group, respectively. Initial HR were (beats min^{-1}): 391±6.7, 382±22.9 and 386±12.1, for the vehicle, 2 mg kg^{-1} and 5 mg kg^{-1} DMPPO-treated groups, respectively.

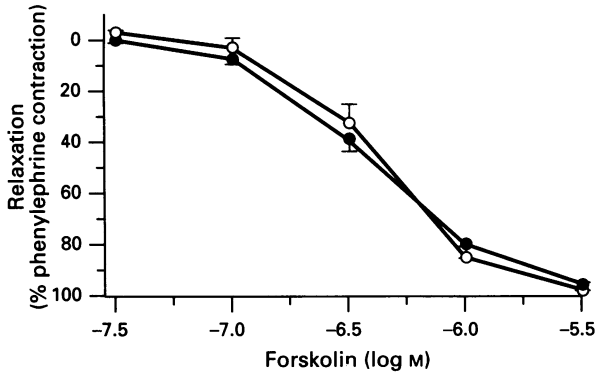

Figure 3 Concentration-response curve to forskolin in endothelium-denuded rat aortic rings, either in the absence (○) or in the presence (●) of DMPPO (100 nM). Initial contractions to phenylephrine were respectively (g): 2.09±0.12 and 2.25±0.16. Values are mean± s.e.mean for $n=6$.

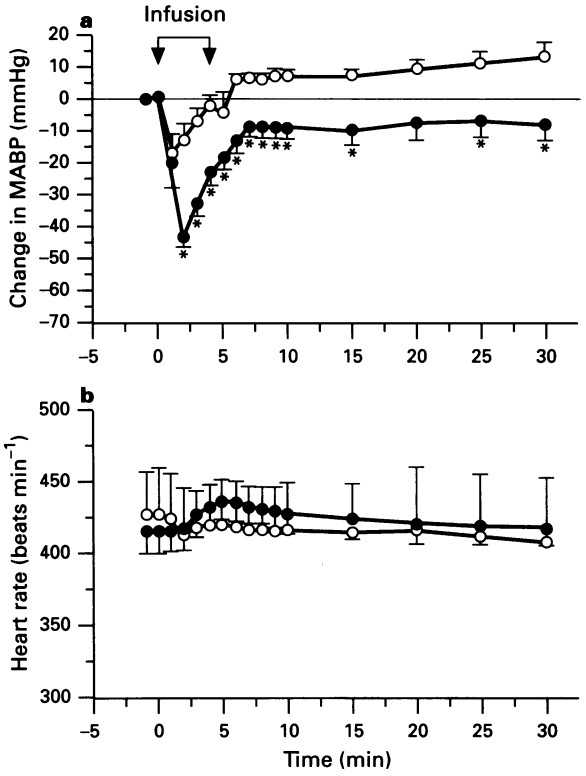

Figure 4 Blood pressure (a) and heart rate (b) effects of DMPPO 5 mg kg^{-1} (●, $n=6$) or vehicle (○, $n=5$) infused i.v. over 5 min to normotensive anaesthetized rats. An asterisk indicates statistically different from vehicle $P<0.05$.

Table 1 Effect of DMPPO on cyclic GMP and cyclic AMP levels in ANP (A) and SNP (B)-stimulated rat aortic rings without endothelium

Treatment	*Cyclic GMP* (pmol mg^{-1} protein)	P	*Interaction* (ANOVA)	*Cyclic AMP* (pmol mg^{-1} protein)
A				
None	0.07±0.02			1.95±0.19
DMPPO 100 nM	0.19±0.03	0.0064[a]		2.50±0.15[NS]
ANP 30 nM	0.21±0.02	0.0007[a]		1.79±0.18[NS]
ANP 30 nM + DMPPO 100 nM	0.62±0.11	0.0053[b]	$P=0.02$	1.79±0.25[NS]
B				
None	0.08±0.02			2.53±0.15
DMPPO 100 nM	0.18±0.01	0.0053[a]		2.62±0.16[NS]
SNP 30 nM	0.39±0.06	0.0008[a]		3.00±0.27[NS]
SNP 30 nM + DMPPO 100 nM	1.05±0.11	0.008[c]	$P=0.02$	2.85±0.24[NS]

[a] In comparison with control; [b] in comparison with ANP alone; [c] in comparison with SNP alone; [NS] not significantly different from control. Rings were incubated with DMPPO for 30 min at 37°C after which ANP or SNP were added for 5 min. Cyclic nucleotide content was determined by scintillation proximity assay as described in the experimental section. Values are mean±s.e.mean for $n=6$.

Infused intravenously over 5 min, DMPPO dose-dependently decreased MABP (Figure 5); the fall in MABP was maximum during the infusion period and attained −35 mmHg and −45 mmHg in the 2 and 5 mg kg^{-1} DMPPO-treated group, respectively. After the end of the infusion, a 15 mmHg and 30 mmHg fall in MABP still persisted until the end of experiment in the 2 mg kg^{-1} and 5 mg kg^{-1} DMPPO-treated animals, respectively. At 10 mg kg^{-1}, DMPPO displayed no greater hypotensive effects than at 5 mg kg^{-1} (data not shown). Heart rate was not affected by any of the tested doses of DMPPO.

Potentiation of SNP-dependent hypotensive effects by DMPPO in anaesthetized normotensive rats

This experiment was performed with a dose of DMPPO which did not significantly modify basal blood pressure (1 mg kg^{-1} infused i.v. over 10 min). Figure 6 represents a typical response obtained with a dose of 30 μg kg^{-1} SNP administered i.v. as a bolus injection. SNP produced a fall in MABP which attained a maximum (−80 mmHg) within 20 s and returned to the basal value after 2.5 min in vehicle-treated rats. In DMPPO-treated rats, the magnitude of the hypotensive effect of SNP was unchanged but the duration of the effect was prolonged to 6 min. Similar results (i.e. no change in maximum effect but change in duration) were obtained with the other doses of SNP tested. For this reason, we used the area under the change in MABP *vs* time curve as a parameter to take into account both the intensity and the duration of the fall in blood pressure. As shown in Table 2 DMPPO increased this area (AUC, area under the curve) singificantly for the highest doses of SNP tested (10, 30 and 100 μg kg^{-1}).

Inhibition by DMPPO of phenylephrine pressor effects in pithed rats

In pithed rats, DMPPO (5 mg kg^{-1}) did not modify basal blood pressure (data not shown). Baseline MABP was similar in vehicle (53±4 mmHg) and DMPPO-treated rats (51±2 mmHg). Therefore, a direct comparison of the pressor effect of phenylephrine between both groups was possible.

Administered i.v. as bolus injections, phenylephrine induced dose-dependent increases in MABP (Figure 7). After an infusion of DMPPO (5 mg kg^{-1}, infused i.v. over 10 min), the magnitude of the pressor effects of phenylephrine (0.3 to 100 μg kg^{-1}) was significantly reduced. The whole dose-response curve to phenylephrine was significantly displaced to the right ($P<0.001$) by DMPPO treatment.

Effect of orally administered DMPPO on blood pressure of conscious SHR

Given orally at a dose of 5 mg kg^{-1} to instrumented conscious SHR, DMPPO decreased MABP (Figure 8). A 25 mmHg fall

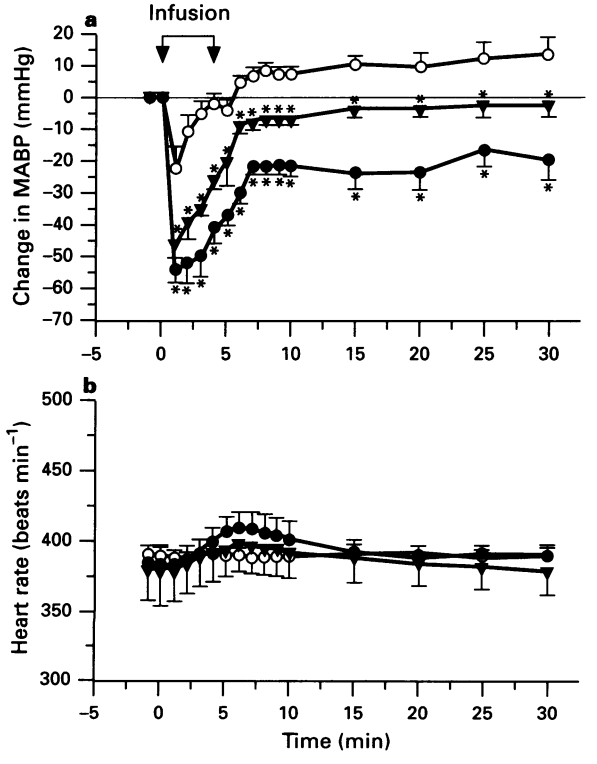

Figure 5 Blood pressure (a) and heart rate (b) effects of DMPPO 2 mg kg^{-1} (▼, $n=5$), 5 mg kg^{-1} (●, $n=7$) or vehicle (○, $n=8$) infused i.v. over 5 min to anaesthetized SHR. An asterisk indicates statistically different from vehicle $P<0.05$.

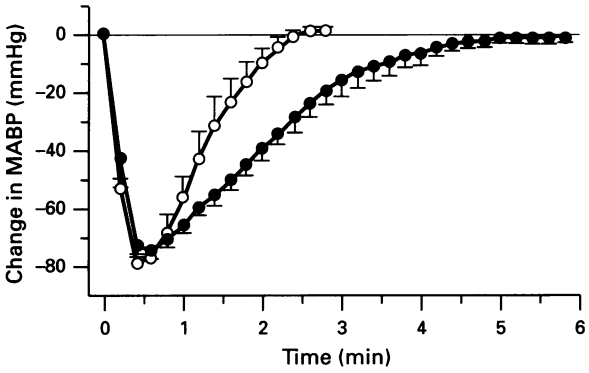

Figure 6 Effect of DMPPO (1 mg kg^{-1}, i.v.) (●) or vehicle (○) on the hypotensive response to SNP (30 μg kg^{-1}) in normotensive anaesthetized rats. Values are mean±s.e.mean for $n=5$.

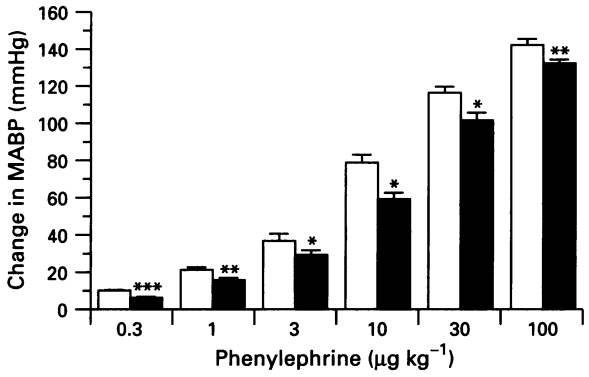

Figure 7 Effect of DMPPO 5 mg kg^{-1}, i.v. (solid columns) or vehicle (open columns) on the phenylephrine pressor response in pithed rats. Initial MABP were 53±4 mmHg and 51±2 mmHg for control and DMPPO-treated rats, respectively ($n=6$ for each group). Statistical difference from vehicle: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 2 Effect of DMPPO (1 mg kg^{-1}, i.v.) or vehicle on the SNP-induced change in MAPB

SNP (μg kg^{-1})	*AUC* (mmHg min^{-1}) *Vehicle*	*DMPPO*
1	18±7.2	18±4.5
3	35±6.6	50±9.7
10	57±8.1	94±13.7*
30	90±16.1	155±14.0*
100	157±2.6	222±18.4**

AUC is the area of the change in MABP vs time curve. Values are mean±s.e.mean for $n=5$. *$P<0.05$; **$P<0.01$ *vs* vehicle.

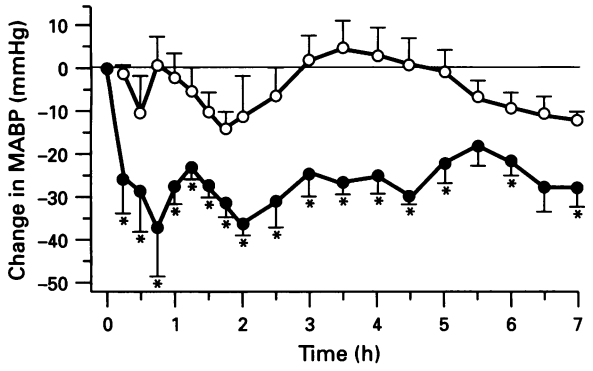

Figure 8 Effect of DMPPO, 5 mg kg^{-1} p.o., (●) or vehicle (○) on MABP of conscious SHR. Initial MABP were 191±5 mmHg (n=4) for control and 182±7 mmHg (n=6) for DMPPO-treated rats, respectively. An asterisk indicates statistically different from vehicle, $P<0.05$.

in blood pressure was observed 15 min after administration and persisted over 5 h; after that time the magnitude of the hypotensive effect decreased slightly but remained significant until the end of the experiment (7 h). Heart rate was unchanged by DMPPO throughout the experiment (data not shown). In addition, the amount of urinary cyclic GMP excreted during the 7 h of the experiment was significantly increased in DMPPO-treated rats (Table 3) whereas urinary cyclic AMP was unchanged.

Table 3 Effect of orally administered DMPPO (5 mg kg^{-1}) or vehicle on urinary cyclic GMP and cyclic AMP

Treatment	*Urinary cyclic GMP* (nmol 7 h^{-1})	*Urinary cyclic AMP* (nmol 7 h^{-1})
Vehicle	5.5±1.0	26.9±3.9
DMPPO	8.2±0.6*	30.7±3.8^{NS}

Urines were collected in conscious SHR during 7 h; cyclic nucleotide content was determined by Scintillation Proximity Assay. Values are mean±s.e.mean for n=6–7. *$P<0.05$; ^{NS}not significantly different from vehicle.

Discussion

The cyclic GMP-specific phosphodiesterase (PDE 5) is present in various tissues including blood vessels (Komas *et al.*, 1991). In rat cultured aortic smooth muscle cells, Coste & Grondin (1995) have recently shown that cyclic GMP is almost exclusively hydrolysed by PDE 5. Increases in cyclic GMP level in vascular smooth muscle cells induce relaxation (Gruetter *et al.*, 1981); hence PDE 5 may contribute to regulation of vascular tone.

DMPPO is a novel potent and highly selective PDE 5 inhibitor (Coste & Grondin, 1995). This compound acts in a reversible and competitive manner towards cyclic GMP with an apparent K_i of 3 nM.

In the present work, DMPPO (0.01 μM to 1 μM) displayed a vasorelaxant activity in phenylephrine-precontracted rat aortic rings when a functional endothelium was present but failed to relax preparations devoid of endothelium. In a similar experimental set up, zaprinast (0.1 μM to 100 μM) was reported to relax aortic rings with endothelium and very poorly those without endothelium (Harris *et al.*, 1989). In the present study, the endothelium-dependent relaxation induced by DMPPO was abolished by the soluble guanylate cyclase inhibitor, methylene blue. Therefore, it can be assumed that DMPPO potentiates the relaxant effects of cyclic GMP generated in SMC by the constitutively released endothelium-derived NO. This hypothesis is supported by the fact that in preparations without endothelium, DMPPO (30 and 100 nM) potentiated the relaxant effects of exogenously added activators of guanylate cyclase. The potentiation of ANP and SNP-mediated relaxation by DMPPO was accompanied by an increase in cyclic GMP levels in rat aortic rings. These results suggest that the vasorelaxant effects of the PDE 5 inhibitor are mediated through accumulation of cyclic GMP in VSMC and that a minimal increase in basal cyclic GMP is required for DMPPO to display relaxant properties. Indeed, cyclic AMP levels were not affected by DMPPO in rat aortic rings treated with ANP or SNP and relaxation to the adenylate cyclase activator, forskolin, was not modified by DMPPO. In contrast to our results, Silver *et al.* (1995) and Saeki *et al.* (1995) describe relaxant activities of two new PDE 5 inhibitors, WIN 58237 and E4021 respectively, at relatively high concentrations (>1 μM) in vascular preparations without endothelium. In the case of WIN 58237, the relaxant property may be accounted for by its PDE 4 (cyclic AMP-specific PDE) inhibitor activity (IC_{50}=0.3 μM). Indeed, high concentrations of rolipram and denbufylline, two PDE 4 inhibitors, can relax endothelium-denuded rat aortic rings (Komas *et al.*, 1991). For E4021, the difference might arise from the high concentration and the use of porcine coronary artery strips.

The increase in cyclic GMP levels and the extent of vasorelaxation did not seem to be simply correlated. DMPPO was devoid of a relaxant activity in aortic rings without endothelium but produced a 2.5 fold increase in intracellular cyclic GMP. This could be explained by a small increase in cyclic GMP over the whole cell. Therefore, local concentration of the cyclic nucleotide in the vicinity of cyclic GMP-dependent protein kinase would not be high enough to produce relaxation. At 30 nM, ANP increased by 3 fold the cyclic GMP content and induced 90% relaxation. In addition, SNP (30 nM) augmented cyclic GMP content by 5 fold and relaxed aortic rings by only 40%. One explanation for the lack of correlation between the intracellular cyclic GMP level and relaxation could be that SNP and ANP increase cyclic GMP content through the activation of different enzymes, i.e. cytosolic and particulate guanylate cyclase respectively. Thus, the cyclic GMP generated in different cell compartments may not be equally available for cyclic GMP-dependent protein kinase. The concept of compartmentalization of protein kinase in the cell has already been proposed by Cornwell *et al.* (1991) and Mochly-Rosen (1995). Alternatively, the kinetics of production and catabolism or egression of cyclic GMP might differ depending on whether the cyclic nucleotide is generated by soluble or particulate guanylate cyclase. These hypotheses can be ruled out since (1) at the time where we measured cyclic nucleotide levels, ANP-generated cyclic GMP could be partly metabolized whereas cyclic GMP generated by SNP could be metabolized slower. However, if this were the case, after blockade of cyclic GMP catabolism by DMPPO, the level of cyclic GMP generated by ANP should be higher than that generated by SNP and (2) for short periods of incubation with ANP, such as those used in the present study, the egression process does not substantially modify cyclic GMP levels (Coste & Grondin, 1995; Hamet *et al* 1989). These results rather support the hypothesis of different pools of intracellular cyclic GMP, as suggested for cyclic AMP in cardiomyocytes (Hayes *et al.*, 1980; Buxton & Brunton, 1983; Hohl & Li, 1991; Xiao *et al.*, 1994).

Similarly to the *in vitro* experiments described above, DMPPO potentiated the hypotensive effects of SNP *in vivo*: in DMPPO-treated animals, the magnitude of the hypotensive effect of SNP was unchanged but the fall in blood pressure persisted for a longer period of time. The latter property could be particularly interesting in a therapeutic context where it would allow reduction in dosage of NO donors and also, as shown by Silver *et al.* (1995), prevent development of tolerance reported for these drugs upon repeated administrations (Packer, 1990). In all the animal models described in this work (i.e. anaesthetized normotensive and hypertensive rats and

conscious hypertensive rats), DMPPO produced a fall in blood pressure. In the latter model, the hypotensive effect of DMPPO was accompanied by an increase in urinary excretion of cyclic GMP but not cyclic AMP. Since Moe *et al.* (1992) have shown that changes in plasma cyclic GMP closely paralleled those of urinary cyclic GMP excretion, this observation suggest that the fall in blood pressure induced by DMPPO is related to an increase in vascular cyclic GMP. The hypotensive effects of DMPPO were not accompanied by changes in heart rate. This is quite unusual since most hypotensive agents induce a reflex tachycardia (Micheli *et al.*, 1990). The lack of tachycardia could be due to a decrease in noradrenaline release at nerve endings consecutive to the increase in the cyclic GMP level, as suggested in various vascular preparations (Greenberg *et al.*, 1990a,b).

In anaesthetized rats, the magnitude of the DMPPO hypotensive effect was higher in SHR than in normotensive animals. This can partly be explained by the higher initial blood pressure and vasoconstriction level that exist in SH rats (Bohlen, 1979; Li & Joshua, 1993). Ruth *et al.* (1993) proposed that the cyclic GMP-dependent protein kinase phosphorylates either a member of the G_p protein family or an isozyme of the phospholipase C family. Therefore, activation of the kinase would interfere with the signal transduction pathway between the receptor of vasoconstractile agents such as phenylephrine or angiotensin II and inositol phosphate production. This capacity of cyclic GMP to antagonize vasopressor hormones was confirmed by the ability of DMPPO to shift to the right the dose-pressor response to phenylephrine in pithed rats (Figure 7). Moreover, it would be tempting to speculate that the activation of guanylate cyclase by endothelium-derived NO is higher in the hypertensive strain. However, conflicting results have been obtained in this field; endothelial function has been shown to be either unchanged (Sawada *et al.*, 1994) or depressed (Tominaga *et al.*, 1994) in SHR compared to WKY rats and Papapetropoulos *et al.* (1993) reported that in SHR, guanylate cyclase activity was up-regulated.

Using purified enzyme preparations, DMPPO was found to be 1000 fold more potent than zaprinast (Coste & Grondin, 1995). However, *in vivo*, DMPPO seemed only 5 fold more potent than zaprinast (compare the present work with Trapani *et al.*, 1991). This difference might be accounted for by the lack of specificity of zaprinast which possess PDE 1 inhibitory activity (Coste & Grondin, 1995).

In conclusion, we have shown that the selective PDE 5 inhibitor, DMPPO, potentiates the vasorelaxant effects of agents stimulating guanylate cyclase in vascular smooth muscle. *In vivo*, DMPPO decreases blood pressure when given i.v. or orally without modifying heart rate. As shown by the prolonged hypotensive effect of orally administered DMPPO, this compound possess good oral bioavailability and stability over time.

Therapeutically, a PDE 5 inhibitor such as DMPPO could be used for the treatment of essential hypertension. In combination with NO donors and, due to the capacity of cyclic GMP to evoke relaxation of coronary arteries (Gruetter *et al.*, 1981) and inhibit platelet aggregation (Mellion *et al.*, 1981), DMPPO should be useful for the treatment of coronary artery disease. Finally, the ability of DMPPO to potentiate ANP-dependent cyclic GMP accumulation and vasorelaxation confers on this molecule potential for treating congestive heart failure. Indeed, in the latter disease, circulating levels of ANP are increased (Burnett *et al.*, 1986) and the reponse blunted (Nakamura *et al.*, 1990; Giles *et al.*, 1991). It seems reasonable, therefore, to speculate that PDE 5 inhibitors will represent a new class of therapeutic agents useful in the treatment of cardiovascular disorders.

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