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The ruthenium-based nitric oxide scavenger, AMD6221, augments cardiovascular responsiveness to noradrenaline in rats with streptozotocin-induced diabetes

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Abstract

Excess production of nitric oxide by inducible nitric oxide synthase (iNOS) has been implicated in cardiovascular dysfunction associated with the acute phase of diabetes mellitus. We examined if the selective nitric oxide scavenger, AMD6221 (ruthenium[hydrogen(diethylenetrinitrilo) pentaacetato] chloride) improved cardiovascular function in rats with streptozotocin (60 mg/kg, i.v.)-induced diabetes. The cardiovascular effects of noradrenaline (16.5 nmol/kg/min, i.v.) were measured in thiobutabarbitone-anaesthetised diabetic and control rats before and after acute administration of AMD6221 (80 mg/kg). Rats in the acute phase of diabetes (3 weeks post injection of streptozotocin) had impaired mean arterial pressure, left ventricular systolic pressure and maximum rate of increase ($+dP/dt$) and decrease ($-dP/dt$) of left ventricular pressure responses to noradrenaline compared with control rats. AMD6221 significantly augmented noradrenaline-induced increases in left ventricular systolic pressure and $\pm dP/dt$ in the diabetic but not control rats. The results show that selective scavenging of nitric oxide by AMD6221 improved cardiac response to noradrenaline in rats with streptozotocin-induced diabetes.

Keywords: Streptozotocin-induced diabetes; Oxidative stress; Nitric oxide scavenger; Cardiovascular dysfunction

1. Introduction

In addition to the inhibition of nitric oxide synthesis, modulation at the level of the synthetic product provides an alternative strategy for the management of nitric oxide-mediated abnormalities. Both organic (nitronyl nitroxides, haemoglobin derivatives) and inorganic (transition metal complexes) scavengers of nitric oxide have been demonstrated to abrogate its physiological effects in vitro and in vivo (Maeda et al., 1994; Rioux et al., 1995; Marmion et al., 2004). Several ruthenium-based nitric oxide scavengers have been shown to attenuate the vasodilator action of *S*-nitroso-*N*-acetylpenicillamine (SNAP) in isolated, precontracted rat tail arteries (Fricker et al., 1997). Furthermore, in a rat model of bacterial lipopolysaccharide-induced septicaemic shock, ruthenium-based nitric oxide scavengers fully reversed hypotension secondary to the excessive production of nitric oxide by inducible nitric oxide synthase (iNOS) (Fricker et al., 1997). AMD6221 is a ruthenium-based nitric oxide scavenger with demonstrated activity in animal models in which excess nitric oxide production in implicated; administration of AMD6221 enhanced allograft survival in models of both acute and delayed cardiac transplant rejection (Pieper et al., 2002) and reduced the rate of tumour growth in the P22 tumour-bearing BD1X rat model (Fricker, 2004).

Previous work in this laboratory has demonstrated that selective inhibition of iNOS by 1400W ameliorates cardiovascular dysfunction associated with streptozotocin-induced diabetes (Cheng et al., 2004) adding to the existing evidence that excess nitric oxide production plays a role in the etiology of the cardiovascular dysfunction associated with diabetes mellitus. Excess nitric oxide production can result in peroxynitrite formation from the reaction of nitric oxide with superoxide (Ferdinandy and Schulz, 2003). Both peroxynitrite and nitric oxide

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are reactive nitrogen species that may attack tyrosine residues in proteins to form nitrotyrosine, an established in vivo biomarker for oxidative protein damage. Reduction in the excessive production of nitric oxide observed in streptozotocin-induced diabetes through the inhibition of iNOS has been shown to protect both cardiac and vascular function, presumably through the reduction in oxidative damage caused by reactive nitrogen species (Cheng and Pang, 2004; Smith et al., 1997).

Our hypothesis is that selective scavenging of excess nitric oxide in diabetic animals will have a beneficial effect on cardiovascular function. This study investigated if acute intravenous administration of AMD6221 increases cardiac contractility and vasoconstrictor responses in rats at the acute phase of type I diabetes through the administration of streptozotocin.

2. Materials and methods

2.1. Induction of diabetes

Male Wistar rats (300–400 g) were randomly assigned to either the control or diabetic group ($n=7$ per group). The control rats were injected intravenously via the tail vein with the vehicle (0.9% w/v NaCl), and the 'diabetic' rats were injected with streptozotocin (60 mg/kg, Sigma-Aldrich Chemicals). A small (<20 μl) blood sample was obtained from saphenous vein puncture at 48 h after injection and blood glucose was measured using Accusoft test strips read by an Accusoft Advantage blood glucose monitor (Hoffman-La Roche). Rats were considered diabetic for the purpose of this study if they had blood concentration of glucose in excess of 15 mM (McNeill, 1999).

Streptozotozin was given under light halothane anaesthesia at a standard intravenous injection volume of 1 ml/kg was used. All animals used were obtained from Charles River Canada and maintained at a temperature of 20±1 °C under a 12:12 h light/dark cycle (lights on from 07:00 to 19:00) and supplied with a standard laboratory chow diet (PMI Feeds) and water ad libitum. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.2. Surgical procedures

The rats were studied at 21 days following the injection of either streptozotocin or the vehicle. Anaesthesia was induced via intraperitoneal injection of thiobutabarbitone (100 mg/kg; Sigma-Aldrich Chemicals). Upon induction of anaesthesia, the rats were tracheotomised, and allowed to breathe spontaneously in ambient air. Body temperature was maintained at 37 °C with a rectal probe and heat lamp attached to a temperature controller (Model 71; Yellow Spring Instruments, OH, USA). Polyethylene (PE50) cannulae (Beckton Dickinson) were inserted into the right iliac artery for the withdrawal of a reference blood sample (for cardiac output measurement), into the left iliac vein for infusion of noradrenaline (Sigma-Aldrich Chemicals), and into the left iliac artery for the measurement of mean arterial pressure by a pressure transducer (P23DB, Gould Statham, CA, USA). Heart rate was derived from the left iliac artery pulse pressure using a tachograph (Model 7P4G, Grass Instruments, MA, USA). The left ventricle was also cannulated via the right common carotid artery to allow measurement of peak left ventricular systolic pressure by a pressure transducer (P23DB, Gould Statham) and intraventricular injection of radiolabelled microspheres for the measurement of cardiac output. A differentiator (Model 7P20C, Grass Instruments) was used to obtain the maximal rate of increase ($+dP/dt$) and decrease ($-dP/dt$) of left ventricular pressure during systole and diastole, respectively. All recorded variables were displayed using a polygraph (Model 7D, Grass Instruments).

2.3. Measurement of cardiac output

Radioactively labelled ([^{57}Co]) microspheres (15 μm diameter, Perkin-Elmer Canada, ON, Canada) were injected into the left ventricle of each rat during the simultaneous withdrawal of a reference blood sample at 0.35 ml/min for 45 s, as previously described (Cheng et al., 2004). Cardiac output was calculated by dividing the product of the rate of blood withdrawal and total injected radioactivity (cpm) by the cpm measured in the withdrawn blood (Rudolph and Heymann, 1967).

2.4. Experimental protocol

After 1 h equilibration, baseline measurements of cardiovascular variables mentioned above (heart rate, mean arterial pressure, cardiac output, peak left ventricular systolic pressure and $\pm dP/dt$) were made. Cardiac index was calculated as the ratio of cardiac output and body weight, whilst total peripheral resistance was calculated by dividing mean arterial pressure by cardiac output. Next, noradrenaline (16.5 nmol/kg/min) was infused intravenously for 6 min, with the above variables were measured at 5 min after the start of infusion. At 10 min following the cessation of noradrenaline infusion, AMD6221 (ruthenium[hydrogen(diethylenetrinitrilo) pentaacetato] chloride; 80 mg/kg, AnorMED Inc., BC, Canada) was injected intravenously over 90 s. The dose and duration of action of the administration of AMD6221 were derived from pilot experiments, and the administration did not produce a significant increase in mean arterial pressure in control rats. Noradrenaline infusion (16.5 nmol/kg/min) was restarted 10 min following the administration of AMD6221, and measurement of the aforementioned cardiovascular variables was performed 5 min after the start of noradrenaline infusion.

2.5. Statistical analysis

Results are presented as the mean±S.E.M. Comparison of baseline values between groups of control and diabetic rats was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-test. The effects of AMD6221 were analysed using two-way repeated-measures ANOVA with

planned comparisons of variables within the same group using the Dunn–Bonferroni post-test. All analyses were performed using InStat 3.6 software (GraphPad Software Inc., CA, USA) at $\alpha = 0.05$.

3. Results

3.1. General characteristics

The rats injected with streptozotocin had higher plasma concentration of glucose at 48 h post injection relative to the rats injected with the vehicle (24.7±1.8 and 6.1±0.2 mM, respectively; $P<0.05$). At the time of experimentation (21 days after injection of streptozotocin or vehicle), the diabetic rats had lower body weights compared with the control rats (0.36±0.01 and 0.46±0.01 kg, respectively; $P<0.05$). Baseline cardiovascular variables were generally similar between the two groups (Table 1), with the exceptions of heart rate, which was higher in the control than diabetic group, and cardiac index, which was lower in the control than diabetic group.

3.2. Effect of noradrenaline on cardiovascular function

Infusion with noradrenaline did not significantly alter cardiac output or cardiac index in either group of rats (Table 1). Noradrenaline significantly increased heart rate, total peripheral resistance, left ventricular $+dP/dt$ and $-dP/dt$ to a similar extent in both control and diabetic rats. Mean arterial pressure and peak left ventricular systolic pressure were also increased by noradrenaline in both groups, but the increases were markedly greater in control than diabetic rats.

Table 1
Cardiovascular values at baseline and the change from baseline in response to noradrenaline (NA; 16.5 nmol/kg/min) in thiobutabarbital-anaesthetised rats at 21 days following i.v. injection with either streptozotocin (60 mg/kg, diabetic) or vehicle (0.9% w/v NaCl; control)

	Control		Diabetic	
	Baseline	Change	Baseline	Change
HR (beats/min)	368±9	+47±16 [a]	319±8 [b]	+48±10 [a]
MAP (mm Hg)	105±3	+42±4 [a]	96±5	+23±2 [a, c]
CO (ml/min)	82±3	+2±7	83±4	−11±7
CI (ml/min/kg)	177±9	+5±15	232±10 [b]	−28±19
TPR (mm Hg min/ml)	1.29±0.04	+0.49±0.14 [a]	1.17±0.04	+0.68±0.22 [a]
LVSP (mm Hg)	112±4	+50±7 [a]	108±4	+19±3 [a, c]
$+dP/dt$ (mm Hg/s)	9375±675	+1875±329 [a]	9300±354	+1725±299 [a]
$-dP/dt$ (mm Hg/s)	8513±808	+2100±474 [a]	8213±929	+2465±357 [a]

Data are mean±S.E.M.; $n=7$ per group. HR, heart rate; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; CO, cardiac output; CI, cardiac index; TPR, total peripheral resistance; $+dP/dt$, maximal rate of increase of left ventricular pressure; $-dP/dt$, maximal rate of decrease of left ventricular pressure.

[a] $P<0.05$ vs. baseline (one-way repeated-measures ANOVA followed by Dunn–Bonferroni post-test).

[b] $P<0.05$ vs. control baseline.

[c] $P<0.05$ vs. control change from baseline in response to NA (one-way ANOVA followed by Tukey's post-test).

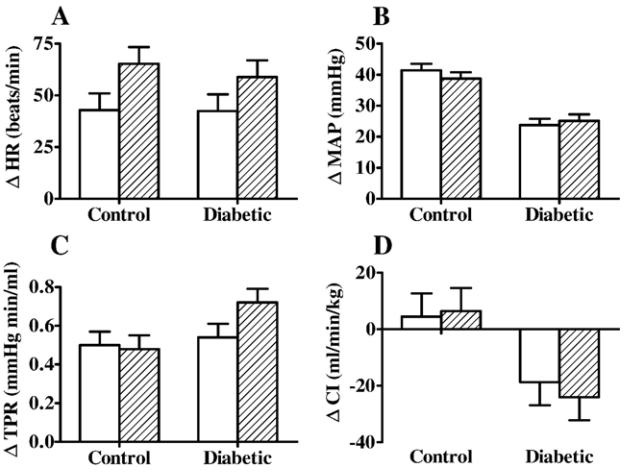

Fig. 1. Effects (mean±S.E.M.; $n=7$ per group) of noradrenaline (16.5 nmol/kg/min) on heart rate (ΔHR; A), mean arterial pressure (ΔMAP; B), total peripheral resistance (ΔTPR; C) and cardiac index (ΔCI; D) prior to (open bars), and after (solid bars), administration of AMD6221 (80 mg/kg, i.v.) in control and diabetic thiobutabarbitone-anaesthetised rats at 21 days after i.v. injection of vehicle (0.9% w/v NaCl) or streptozotocin (60 mg/kg), respectively.

3.3. Effect of AMD6221 on cardiovascular responses to noradrenaline

Administration of AMD6221 did not alter the effects of noradrenaline on heart rate, mean arterial pressure response, total peripheral resistance or cardiac index in either the control or diabetic rats (Fig. 1).

Following the administration of AMD6221, clear differences in cardiac responses to noradrenaline were observed between the two groups. AMD6221 augmented noradrenaline-induced increases in peak left ventricular systolic pressure as well as left ventricular $-dP/dt$ in the diabetic rats, but

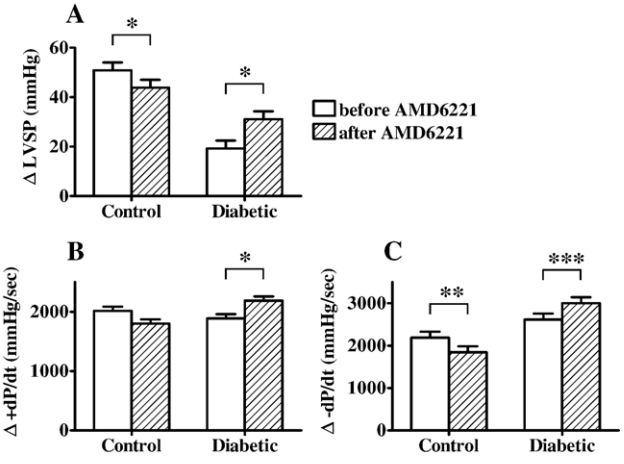

Fig. 2. Effects (mean±S.E.M.; $n=7$ per group) of noradrenaline (16.5 nmol/kg/min) on peak left ventricular (LV) systolic pressure (ΔLVSP; A), maximal rate of increase ($\Delta+dP/dt$; B) and decrease ($\Delta-dP/dt$; C) of LV pressure prior to (open bars), and after (solid bars) administration of AMD6221 (80 mg/kg, i.v.) in control and diabetic thiobutabarbitone-anaesthetised rats at 21 days after i.v. injection of vehicle (0.9% w/v NaCl) or streptozotocin (60 mg/kg), respectively. *$P<0.05$; **$P<0.01$; ***$P<0.001$ for difference in response to noradrenaline before and after administration of AMD6221, as calculated from two-way repeated measures ANOVA followed by planned Dunn–Bonferroni post-tests.

attenuated these response in control rats (Fig. 2). AMD6221 also increased left ventricular $+dP/dt$ in the diabetic group, and decrease this response insignificantly in the control rats (Fig. 2).

4. Discussion

At 21 days following injection of either streptozotocin or the vehicle, baseline haemodynamic variables did not vary between the control and diabetic rats, with the exception of lower heart rate and higher cardiac index in the diabetic group. However, upon challenge with exogenous noradrenaline, depressions of mean arterial pressure and peak left ventricular systolic pressure responses were observed in the diabetic group relative to the control group. These findings are in agreement with those from previous studies, both from this and other laboratories (Hill and Larkins, 1989; Brands et al., 2000; Cheng et al., 2004).

The underlying mechanism for cardiovascular hyporesponsiveness at the acute phase of streptozotocin-induced diabetes has yet to be fully elucidated. Whilst a multifactorial etiology is likely, there is mounting evidence to support an important role for increased nitric oxide production (and associated increase in the release of reactive nitrogen species such as peroxynitrite; $ONOO^-$). Therefore, we investigated if AMD6221, a selective nitric oxide scavenger demonstrated to reduce nitric oxide levels both in vitro and in vivo (Pieper et al., 2002; Fricker, 2004; Marmion et al., 2004), would improve cardiovascular function in diabetic animals. Acute administration of AMD6221 had no observable effects on baseline haemodynamic variables in either group, but produced marked increase of cardiac responses to noradrenaline. These changes were predominantly associated with augmentation of noradrenaline-induced increase in cardiac contractile function (peak left ventricular systolic pressure, left ventricular $+dP/dt$ and $-dP/dt$) in the diabetic group. In contrast, the same variables in control animals were decreased following the administration of AMD6221. Whereas AMD6221 did not alter pressor or cardiac index responses to noradrenaline, it slightly ($P>0.05$) increased vasoconstriction (approximately 30% increase in total peripheral resistance) to noradrenaline in the diabetic but not control rats. In a previous study, the selective iNOS inhibitor 1400W (Cheng et al., 2004) was shown to augment noradrenaline-induced increases in mean arterial pressure and total peripheral resistance. The augmentation of cardiovascular function by AMD6221 and 1400W are in accordance with what would be expected from attenuation of the excess nitric oxide production associated with streptozotocin-induced diabetes. Whilst this study did not attempt to address the possible mechanism of action of AMD6221, based on the similarity of these results with those obtained with 1400W in both the streptozotocin-induced diabetes (Cheng et al., 2004) and lipopolysaccharide-induced endotoxaemia (Cheng et al., 2003), in addition to the available in vitro data for both 1400W (Garvey et al., 1997), and AMD6221 (Mosi et al., 2002), and it is reasonable to speculate that both compounds produce their effects in vivo through reduction of nitric oxide levels and the subsequent production of reactive nitrogen species such as peroxynitrite.

Selective scavenging of nitric oxide potentially presents an alternative to inhibition of the formation of isoforms of nitric oxide synthase for conditions associated with increased nitric oxide production. Other non-ruthenium-based nitric oxide scavengers have shown activity in the streptozotocin-induced diabetic model; for example, chronic administration of the dithiocarbamate-based nitric oxide scavenger NOX-101 prevented the development of endothelial dysfunction in rats injected with streptozotocin (Pieper et al., 1998). Future studies investigating the effects of chronic administration of AMD6221 have the potential to yield similar results.

In addition to the results from this study, and from the aforementioned rat cardiac allograft and P22 tumour models, AMD6221 has shown activity across different species in several animal models of nitric oxide-mediated diseases. In a canine model of cardiac allograft rejection, animals administered with AMD6221 had reduced phenylephrine requirements together with an overall reduction of the inflammatory response, as measured by reduced neutrophil CD18 expression (Mayers et al., 2003). Also, in a rabbit model of LPS-induced uveitus, AMD6221 administration attenuated both aqueous flare and iridial hyperemia (Allen et al., 2002).

The findings from this study add not only to the growing evidence that reactive nitrogen and oxygen species are critically involved in cardiovascular dysfunction associated with diabetes, but also demonstrate that selective scavenging of nitric oxide can produce similar results as selective inhibition of iNOS in animal models of cardiovascular dysfunction associated with excess production of nitric oxide. However, rather than simply mimicking the effects of iNOS inhibition, scavenging nitric oxide may afford advantages over enzyme inhibition as the relative contribution of the various NOS isoforms in many disease states is not clear. Selective nitric oxide scavenging bypasses any disparities concerning the source of nitric oxide; assuming a second-order process, the rate of scavenging is determined by the concentration of both nitric oxide and the scavenger. This means that at appropriate dose levels, only nitric oxide in excess of normal physiological levels would be scavenged (Fricker, 2004).

In summary, our results provide further evidence that the cardiovascular dysfunction associated with streptozotocin-induced diabetes is associated with excess production of nitric oxide. Acute administration of the selective nitric oxide scavenger AMD6221 augmented cardiovascular response to noradrenaline and demonstrates that use of nitric oxide scavengers is a viable alternative to iNOS inhibitors in disease states where excess nitric oxide production is implicated.

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References

Allen, J.B., Pittman, K.A., Keng, T., Gilger, B.C., Privalle, C.T., Fricker, S.P., 2002. Nitric oxide scavenger AMD6221 suppresses endotoxin-induced uveitis (EIU) in rabbits. Proc. Assoc. Res. Vision. Ophthal., Florida. P3976.

Brands, M.W., Fitzgerald, S.M., Hewitt, W.H., Hailman, A.E., 2000. Decreased cardiac output at the onset of diabetes: renal mechanisms and peripheral vasoconstriction. Am. J. Physiol: Endocrinol. Metab. 278, E917–E924.

Cheng, X., Pang, C.C., 2004. Increased vasoconstriction to noradrenaline by 1400W, inhibitor of iNOS, in rats with streptozotocin-induced diabetes. Eur. J. Pharmacol. 484, 263–268.

Cheng, X., Leung, S.W., Lo, L.S., Pang, C.C., 2003. Selective versus non-selective suppression of nitric oxide synthase on regional hemodynamics in rats with or without LPS-induced endotoxemia. Naunyn Schmiedebergs Arch. Pharmacol. 367, 372–379.

Cheng, X., Cheng, X.S., Kuo, K.H., Pang, C.C., 2004. Inhibition of iNOS augments cardiovascular action of noradrenaline in streptozotocin-induced diabetes. Cardiovasc. Res. 64, 298–307.

Ferdinandy, P., Schulz, R., 2003. Nitric oxide, superoxide, and peroxynitrite in myocardial ischaemia–reperfusion injury and preconditioning. Br. J. Pharmacol. 138, 532–543.

Fricker, S.P., 2004. Metal complexes as therapeutic agents in nitrogen monoxide modulation. Met. Ions Biol. Syst. 41, 421–480.

Fricker, S.P., Slade, E., Powell, N.A., Vaughan, O.J., Henderson, G.R., Murrer, B.A., Megson, I.L., Bisland, S.K., Flitney, F.W., 1997. Ruthenium complexes as nitric oxide scavengers: a potential therapeutic approach to nitric oxide-mediated diseases. Br. J. Pharmacol. 122, 1441–1449.

Garvey, E.P., Oplinger, J.A., Furfine, E.S., Kiff, R.J., Laszlo, F., Whittle, B.J., Knowles, R.G., 1997. 1400W is a slow, tight binding, and highly selective inhibitor of inducible nitric-oxide synthase in vitro and in vivo. J. Biol. Chem. 272, 4959–4963.

Hill, M.A., Larkins, R.G., 1989. Alterations in distribution of cardiac output in experimental diabetes in rats. Am. J. Physiol. 257, H571–H580.

Maeda, H., Akaike, T., Yoshida, M., Suga, M., 1994. Multiple functions of nitric oxide in pathophysiology and microbiology: analysis by a new nitric oxide scavenger. J. Leukoc. Biol. 56, 588–592.

Marmion, C.J., Cameron, B., Mulcahy, C., Fricker, S.P., 2004. Ruthenium as an effective nitric oxide scavenger. Curr. Top. Med. Chem. 4, 1585–1603.

Mayers, I., Hurst, T., Radomski, A., Johnson, D., Fricker, S., Bridger, G., Cameron, B., Darkes, M., Radomski, M.W., 2003. Increased matrix metalloproteinase activity following canine cardiopulmonary bypass is suppressed by a nitric oxide scavenger. J. Thorac. Cardiovasc. Surg. 125, 661–668.

McNeill, J., 1999. Experimental Models of Diabetes. C.R.C. Press, L.L.C. Florida.

Mosi, R., Seguin, B., Cameron, B., Amankwa, L., Darkes, M.C., Fricker, S.P., 2002. Mechanistic studies on AMD6221: a ruthenium-based nitric oxide scavenger. Biochem. Biophys. Res. Commun. 292, 519–529.

Pieper, G.M., Dembny, K., Siebeneich, W., 1998. Long-term treatment in vivo with NOX-101, a scavenger of nitric oxide, prevents diabetes-induced endothelial dysfunction. Diabetologia 41, 1220–1226.

Pieper, G.M., Roza, A.M., Adams, M.B., Hilton, G., Johnson, M., Felix, C.C., Kampalath, B., Darkes, M., Wanggui, Y., Cameron, B., Fricker, S.P., 2002. A ruthenium (III) polyaminocarboxylate complex, a novel nitric oxide scavenger, enhances graft survival and decreases nitrosylated heme protein in models of acute and delayed cardiac transplant rejection. J. Cardiovasc. Pharmacol. 39, 441–448.

Rioux, F., Drapeau, G., Marceau, F., 1995. Recombinant human hemoglobin (rHb1.1) selectively inhibits vasorelaxation elicited by nitric oxide donors in rabbit isolated aortic rings. J. Cardiovasc. Pharmacol. 25, 587–594.

Rudolph, A.M., Heymann, M.A., 1967. The circulation of the fetus in utero. Methods for studying distribution of blood flow, cardiac output and organ blood flow. Circ. Res. 21, 163–184.

Smith, J.M., Paulson, D.J., Romano, F.D., 1997. Inhibition of nitric oxide synthase by L-NAME improves ventricular performance in streptozotocin-diabetic rats. J. Mol. Cell. Cardiol. 29, 2393–2402.