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Impaired arterial reactivity following cytomegalovirus infection in the immunosuppressed rat

Pedro H.A. Eerdmans, *Maike C.J. Persoons, Sjaak J.M. Debets, Harry A.J. Struijker Boudier, Jos F.M. Smits, *Catrien A. Bruggeman & [1]Jo G.R. De Mey

Departments of Pharmacology and *Medical Microbiology, Cardiovascular Research Institute Maastricht, University of Limburg, Maastricht, The Netherlands

1 Cytomegalovirus (CMV) is a major pathogen in immunocompromised individuals and may participate in the pathogenesis of atherosclerosis in the general population. We evaluated whether CMV-infection alters the function of arterial smooth muscle.

2 Blood pressure (BP) and arterial reactivity were recorded in immunosuppressed rats that had been infected with CMV (10^5 plaque forming units i.p.). Furthermore, the reactivity of isolated arteries was compared between CMV-infected rats and rats injected with bacterial endotoxin (LPS).

3 Initially resting BP and heart rate (HR) were not modified in CMV-infected rats, but baroreflex control of HR was impaired. By the eighth day post-CMV, BP dropped precipitously and could no longer be raised by phenylephrine (PHE).

4 In mesenteric resistance arteries, isolated at this stage from CMV-infected rats, contractile responses to nerve stimulation, noradrenaline, PHE and 5-hydroxytryptamine (5-HT) were virtually absent while those to high potassium and vasopressin (AVP) were not modified. In aortae of CMV-infected rats, responses to 5-HT and AVP were impaired while those to PHE or potassium were hardly affected. Reduced contractile responses could not be restored by N^G-nitro-L-arginine methyl ester (L-NAME).

5 Continuous treatment of CMV-infected rats with prazosin (0.1 mg kg^{-1} day^{-1}) prevented blood pressure lowering and resistance artery changes.

6 Observations in arteries of LPS-treated rats (5-10 mg kg^{-1}, i.p.) differed markedly from those in vessels of CMV-infected animals. The contractile reactivity of their mesenteric resistance arteries was not altered while in their aortae, responses to PHE, 5-HT and AVP were reduced. With the exception of the AVP responses, this was more pronounced in the presence of L-arginine and reversed by L-NAME.

7 These findings indicate that CMV-infection results in a reduction of resistance artery reactivity and hypotonia. This seems not to involve cytokine-mediated induction of NO synthase in the vascular wall but may be due to alterations of excitation-contraction coupling in arterial smooth muscle in response to increased sympathetic nervous input.

Keywords: Cytomegalovirus; resistance arteries; sympathetic nervous system; adrenergic responses; endotoxin; LPS; NO synthase

Introduction

Latent cytomegalovirus (CMV, a betaherpes virus) is present in a major portion of the human population. In immunologically compromised individuals, including those with acquired immunodeficiency, CMV is the cause of serious disease (Abe *et al.*, 1983; Tyms *et al.*, 1989). In the general population CMV may participate in the pathogenesis of atherosclerosis (Bruggeman & van Dam-Mieras, 1991; Hajjar, 1991; Persoons *et al.*, 1994). The virus is found more frequently in arteries of patients exhibiting grade III atherosclerosis than in those of young trauma victims (Hendrix *et al.*, 1989) and the development of atherosclerosis in heart-transplants has been observed to correlate with the presence of CMV (Grattan *et al.*, 1989; McDonald *et al.*, 1989). The mechanism of CMV's contribution to atherogenesis remains unclear. Both *in vitro* and *in vivo*, CMV increases adherence of monocytes and leucocytes to vascular endothelium (Span *et al.*, 1991b). This is mediated by interleukin 1 (Span *et al.*, 1991a). Whether CMV also affects arterial smooth muscle cells, another participant in the formation of atherosclerotic lesions and a major determinant of blood pressure, is largely unknown. In addition to potential direct effects of CMV on arterial smooth muscle cells, arterial responses to cytokines produced during CMV-infection may be considered. Cytokines such as interleukin-1 and tumour necrosis factor have been shown to induce NO synthase activity in vascular smooth muscle (Moncada *et al.*, 1989; Moncada & Higgs, 1991; Schini *et al.*, 1992). This seems to underlay hypotonic shock (Lin *et al.*, 1994) during bacterial endotoxaemia. Endotoxin would stimulate cytokine production and subsequently elevate intravascular levels of NO leading to profound vasodilatation (Julou-Schaeffer *et al.*, 1990; Moncada & Higgs, 1991; Wakabayashi *et al.*, 1991; Mombouli & Vanhoutte, 1995). This is accompanied by marked baroreflex-mediated stimulation of sympathetic nerve traffic to the heart and blood vessels (Groeneveld *et al.*, 1986). With time the reflex may adapt (Salgado & Krieger, 1978) which could result in the precipitous uncontrolled fall of blood pressure that characterizes septic shock (Groeneveld *et al.*, 1986; Julou-Schaeffer *et al.*, 1990; Wakabayashi *et al.*, 1991).

In the present study we evaluated whether CMV-infection influences arterial contractile reactivity and whether cytokine-mediated induction of NO synthase or adaptation to elevated sympathetic tone are involved therein. For this purpose we recorded blood pressure, pressor responses to phenylephrine and contractile reactivity of isolated blood vessels. Rat cytomegalovirus (Bruggeman *et al.*, 1985) was used to infect rats that had been immunosuppressed by irradiation to promote and accelerate infection. Part of the experiments were performed during continuous treatment with prazosin, in an at-

[1] Author for correspondence at: Dept. Pharmacology, University of Limburg, P.O. Box 616 6200 MD Maastricht, The Netherlands.

tempt to protect the vasculature against the effects of increased exposure to noradrenaline. Observations were compared to those in sham-infected irradiated rats and in rats injected with bacterial endotoxin as a positive control for hypotonic shock due to cytokine-induced NO synthase expression in the vasculature (Julou-Schaeffer *et al.*, 1990; Rengasamy & Johns, 1991).

Methods

Experiments were performed on 8 week old, male, specific pathogen-free, Wistar Kyoto rats (Central Animal Facilities; University of Limburg, Maastricht, The Netherlands) and were approved by the local animal welfare committee. The animals were maintained on 12 h dark – 12 h light cycles and had free access to standard lab food and tap water. Four groups of animals ($n = 10$ each) were defined: (1) untreated rats (control), (2) rats treated with bacterial endotoxin (LPS-treated), (3) immunosuppressed untreated rats (CMV-control) and (4) immunosuppressed rats that were infected with cytomegalovirus (CMV-infected). Treatment with LPS consisted of i.p. injection of 5 mg kg^{-1} endotoxin of *Escherichia coli*. Immunosuppression was achieved by 6 Gray total-body gamma irradiation. Twenty four hours later part of the animals were injected i.p. with 100,000 plaque forming units (PFU) of rat cytomegalovirus from a pad of homogenized salivary glands of acutely infected rats (Bruggeman *et al.*, 1985). CMV-controls received sterile phosphate buffered saline.

Blood pressure measurement

Ten CMV-control rats and ten CMV-infected rats were equipped with an intravenous and an intraarterial catheter for i.v. administration of agents and recording of blood pressure, respectively (Struijker Boudier *et al.*, 1982). Surgery was performed under ketamine/xylazine (5 and 1 mg kg^{-1}, i.m. respectively) anaesthesia 3 days before immunosuppression. Polyethylene tubing (PE 10) was inserted from a femoral artery into the abdominal aorta and from a femoral vein into the vena cava. The catheters were guided under the dorsal skin, exteriorized and sutured to the neck musculature. The animals were injected on three consecutive days with an antibiotic (ampicillin 0.1 g day^{-1}, s.c.).

The arterial catheter was connected to a miniature low volume displacement pressure transducer (CP-01, Century Technology Co, Inglewood Ca, U.S.A.) and the signal recorded on a polygraph (Grass model 7D; Grass Instruments, Quincy MA, U.S.A.). Heart rate was determined from the pulsatile signal by a tachograph (Grass Instruments). Mean blood pressure, heart rate and dose response curves for the vasopressor effect of i.v. infused(−)-phenylephrine, were determined in conscious freely moving animals on days 1,3, 5, 7, 8, 9, and 10 after CMV- or sham-infection. Phenylephrine infusion were started at 0.1 $\mu g\ min^{-1}$, the infusion rate was increased (0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0 $\mu g\ min^{-1}$) when a stable pressure was reached.

Similar experiments were performed in rats that were continuously treated with 0.1 mg kg^{-1} day^{-1} prazosin. During placement of the catheters, these rats were also implanted s.c. with an osmotic minipump (Alzet 2ML2; Alza Co, Palo Alto, Ca, U.S.A.) filled with prazosin dissolved in 50% dimethylsulphoxide (DMSO) in H_2O. In a previous study (van Kleef *et al.*, 1992) this dose of prazosin was observed to produce an 80 fold shift of the dose-response curve for the pressor action of phenylephrine, indicating significant α_1-adrenoceptor antagonism. Up to 14 days of treatment with the solvent did not affect blood pressure, pressor responses to phenylephrine or the reactivity of isolated blood vessels. Three days after initiation of the prazosin-treament the rats were irradiated. Twenty four hours later some of them were infected with CMV ($n = 10$) and the rest were sham-infected ($n = 10$).

Contractile reactivity of isolated blood vessels

Rats were killed by abdominal aorta puncture under ether anaesthesia 48 to 72 h after LPS treatment, 10 days after sham-infection or 7 – 10 days after CMV-infection. The decision to kill CMV-infected rats was based on their blood pressure and overall appearance. Furthermore, 8 untreated control animals were used. From each rat the thoracic aorta and the mesentery were isolated. From the latter a fourth order resistance-sized side branch of the superior mesenteric artery (diameter approximately 200 μm) was dissected. Aortic segments and mesenteric small artery segments (length 2 mm) were mounted horizontally in an organ chamber between a isometric force transducer (Statham UC3 and Kistler Morse DSC6 for large and small vessels, respectively) and a displacement device (Boonen & De Mey, 1990a; 1991; Eerdmans *et al.*, 1991). The organ chamber was filled with Krebs-Ringer bicarbonate solution (KRB, 25 and 10 ml for large and small vessels, respectively) that was maintained at 37°C and aerated with 95% O_2 and 5% CO_2. Prior to experimentation the internal circumference of the thoracic aorta segments was set at 4.25 mm, which was observed in preliminary experiments to yield strong and reproducible contractile responses. Mesenteric resistance arteries were stretched to their individual optimal internal circumference for isometric force development (Boonen & De Mey, 1990a, b; 1991; Eerdmans *et al.*, 1991). Their circumference was increased by 60 μm every 5 min with intermittent exposure to high potassium solution (125 mM K^+; KRB in which all NaCl had been replaced by an equimolar amount of KCl). This length-tension protocol was continued until maximal contractile responses to the depolarizing stimulus were obtained. Further experimentation was performed at this optimal internal circumference.

Contractile responses were induced with potassium, noradrenaline, phenylephrine, 5-hydroxytryptamine or Arg-vasopressin and relaxing responses to isoprenaline or acetylcholine were induced after the preparations had been made to contract with 30 nM Arg-vasopressin. These agents were tested in the same vessels but the order of testing was randomized to exclude the possibility that responses to a given agonist were influenced by pretreatment with another agent. Part of the experiments were repeated in the presence of 1 mM L-arginine or of 0.1 mM N^G-nitro-L-arginine methyl ester (L-NAME), a substrate and an inhibitor of NO synthase, respectively (Moncada *et al.*, 1989). Preparations were preincubated with these agents for at least 30 min before exposure to contractile or relaxing agonists; also the order of exposure to L-arginine and L-NAME was randomized. This did not affect the results. To stimulate sympathetic nerves in mesenteric resistance arteries, 2 platinum electrodes connected to a constant current source (amplitude 85 mA) were placed longitudinally across the mounted vessel segments and frequency response curves (1 to 32 Hz, pulse duration 2 ms) were constructed (Nilsson, 1985; Eerdmans *et al.*, 1991). The experiments were performed in parallel in isolated aortae and resistance artery segments and took approximately 6 h. It has previously been demonstrated that long term incubation of blood vessels *in vitro* may be accompanied by the induction of additional NO synthesis (Moncada *et al.*, 1989; Moncada & Higgs, 1991). However, in the present experiments the sensitivity to vasocontrictor agonists and the effects of L-arginine and L-NAME on contractile responses to agonists did not differ when evaluated after 1 and 6 h of incubation of preparations of control rats ($n = 6$). Therefore, the interpretation of the present findings is not likely to be complicated by the induction of NO synthase during the *in vitro* experimentation.

Drugs and solutions

KRB had the following millimolar composition: NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4.7H_20$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0,

glucose 11.1. L-Arginine hydrochloride, N^G-nitro-L-arginine methyl ester, (−)-isoprenaline hydrochloride, LPS from *Escherichia coli*, (−)-noradrenaline hydrochloride, (−)-phenylephrine hydrochloride, (−)-prazosin hydrochloride, and (±)-propranolol hydrochloride were obtained from Sigma Chemical Co (St Louis, Mo U.S.A.). Acetylcholine hydrochloride and 5-hydroxytryptamine creatinine sulphate monohydrate were purchased from Janssen Chimica (Beerse, Belgium) and Arg-vasopressin from Sandoz (Basel, Switzerland).

Data analysis

Contractile responses of isolated blood vessels to agonists were expressed as increases in wall tension (increases in isometric force divided by twice the segment length) and relative to the amplitude of the contraction induced by 125 mM potassium in the same preparation. Sensitivity for the pressor action of phenylephrine *in vivo* and for the contractile effect of agonists *in vitro* was determined by interpolation on a least square sigmoidal curve fit of the concentration-response curves (Inplot, GraphPad, San Diego, Ca, U.S.A.). Data are shown as mean ± s.e.mean. Statistical significance of effects and of differences between treatment groups was evaluated by Student's *t* test for paired observations, Student's *t* test for unpaired observations or analysis of variance followed by Bonferroni's *t* test or Student's *t* test where appropriate (Wallenstein *et al.*, 1980) using commercially available software (Crunch, Software Corp., San Francisco, Ca, U.S.A.).

Results

Blood pressure and heart rate

In irradiated rats that had been sham-infected, basal mean arterial pressure (MAP) and heart rate (HR), the sensitivity and maximal pressor response to i.v. infused phenylephrine

Table 1 Effect of phenylephrine on blood pressure in sham-infected and CMV-infected rats[a]

	Sham-infected		*CMV-infected*	
	ED_{50} ($\mu g\,min^{-1}$)	E_{max} (mmHg)	ED_{50} ($\mu g\,min^{-1}$)	E_{max} (mmHg)
Day 1	0.16 ± 0.03	51 ± 7	0.22 ± 0.03	46 ± 8
Day 3	0.20 ± 0.02	64 ± 5	0.21 ± 0.03	68 ± 4
Day 5	0.22 ± 0.03	64 ± 3	0.22 ± 0.07	58 ± 4
Day 7	0.14 ± 0.01	61 ± 2	0.55 ± 0.17	47 ± 8
Day 8	0.22 ± 0.02	69 ± 4	> 2	9 ± 4*

[a]Irradiated rats were injected i.p. with 10^5 PFU of CMV or sham-infected on Day 0. Cumulative concentration-response curves for the pressor effect of phenylephrine (0.1 − 2 $\mu g\,min^{-1}$) were constructed by i.v. infusion of the agonist in conscious unrestrained animals. Data are expressed as the dose required to induce a half maximal response (ED_{50}) and as the maximal increase in mean arterial blood pressure (E_{max}), and are shown as mean ± s.e.mean ($n = 7-10$). *Statistically significant difference from sham-infected animals ($P < 0.05$).

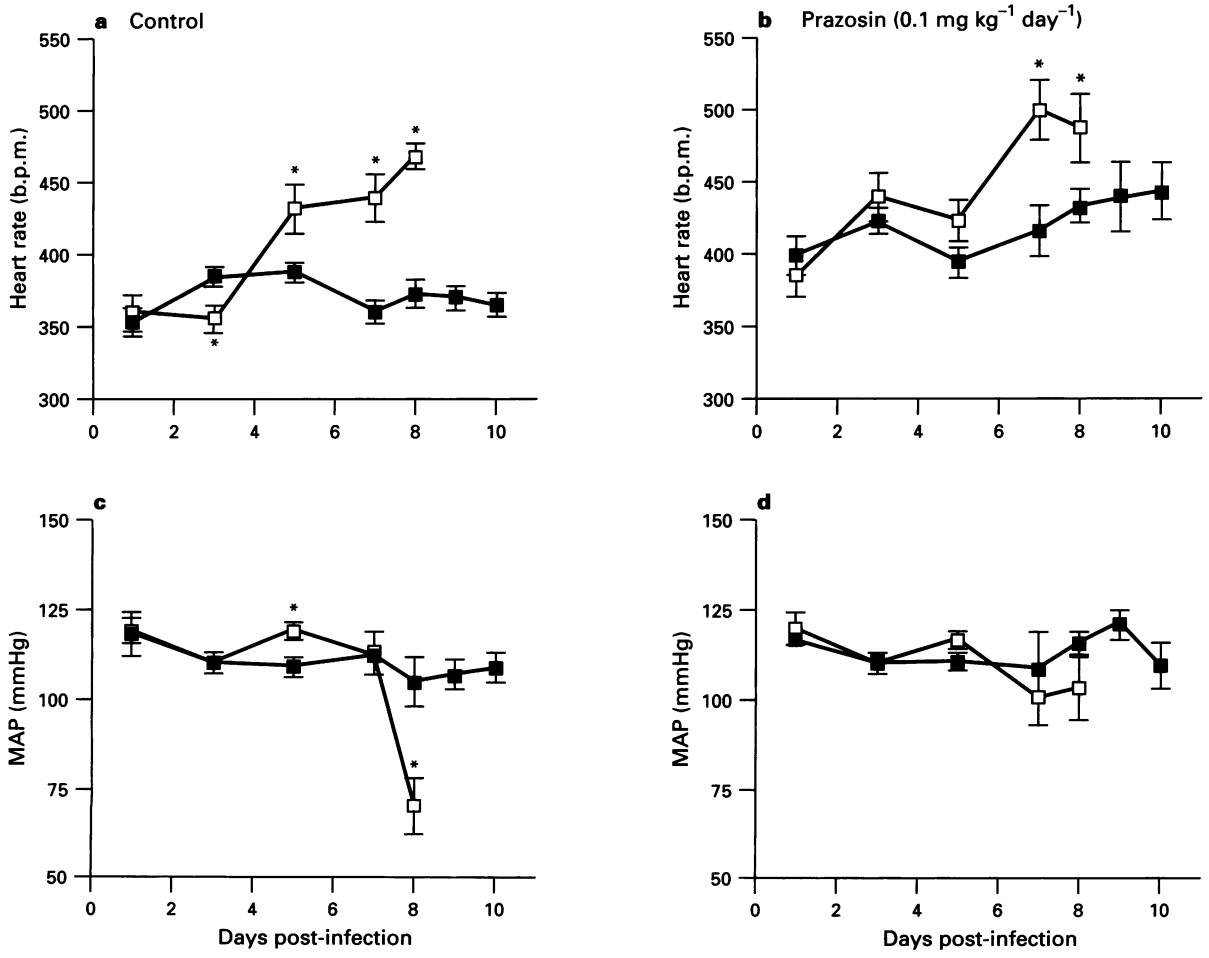

Figure 1 Basal heart rate (HR, a,b) and mean arterial pressure (MAP, c,d) in conscious unrestrained irradiated rats that had been i.p. injected on day 0 with 10^5 PFU rat cytomegalovirus (CMV, □) or sham-infected (■) without(a,c) and with (b,d) continuous treatment with prazosin 0.1 mg kg^{-1} day^{-1}, initiated at day −3. Data are shown as mean ± s.e.mean ($n = 7-10$). *Statistically significant difference from sham-infected animals ($P < 0.05$).

(PHE) and the bradycardia accompanying the elicited pressure elevation, did not change significantly during the study period (Table 1, Figures 1 and 2).

One day after CMV-infection the recorded variables did not differ from those in sham-infected rats. Three days following CMV-infection, MAP was not altered by HR was significantly reduced (Figure 1). The sensitivity and maximal pressor response to PHE were not modified at this stage (Table 1, Figure 2). The baroreflex, as reflected by heart rate changes during pharmacologically induced pressure elevation was, however, impaired (Figure 2). The relationship between changes in heartperiod and changes in MAP was less pronounced in CMV-infected rats (correlation coefficient, r^2 0.271) than in shams (r^2 0.508) and was more shallow (slope 0.712 ± 0.179 ms mmHg^{-1} versus 1.653 ± 0.217 ms mmHg^{-1} in shams). By day 5, both MAP and HR were significantly elevated (Figure 1). The sensitivity and maximal pressor response to PHE were not altered (Table 1). The baroreflex was restored but seemed to be operating around a higher heart rate (Figure 2). At the 7th day after CMV-infection, basal HR remained elevated while MAP was comparable to that in shams. The animals had become less sensitive to the pressor action of PHE (Table 1), but still displayed bradycardia in response to pressure elevation (Figure 2). Although the range of pressure and heart rate changes was narrower, the relationship between MAP and HR was shifted as observed on day 5 post-CMV. On day 8, CMV-infected rats displayed very low MAP and high HR (Figures 1 and 2). At this stage even high doses of PHE had minimal effects on blood pressure.

Blood pressure and heart rate were recorded in additional animals that were infected with CMV or sham-infected during continuous treatment with prazosin, 0.1 mg kg^{-1} day^{-1}. The long-term treatment with the α_1-adrenoceptor antagonist did not modify MAP but tended to increase HR (Figure 1). In prazosin-treated animals the development of tachycardia during CMV-infection was delayed and no precipitous fall of blood pressure was noted on the 8th day (Figure 1). In a separate experiment it was observed that 6 out of 10 prazosin-treated rats survived the CMV-infection for at least 10 days, while this was the case for only 3 out of 10 CMV-infected animals that were not treated with prazosin.

Isolated blood vessels

Thoracic aortae Eight days following CMV-infection, contractile responses of thoracic aorta segments to 125 mM potassium were not significantly modified. They averaged 3.8 ± 0.5 mN/mm^{-1} compared to 4.8 ± 0.2 mN mm^{-1} in aortae of sham-infected rats. The sensitivity of the aorta to PHE was decreased and contractile responses to 1–10 μM 5-HT and those to 30 nM Arg-vasopressin (AVP) were markedly reduced (Figure 3). These differences between the aortae of CMV-infected and sham-infected rats were not accentuated in the presence of 1 mM L-arginine and were not abolished by 0.1 mM L-NAME (Figure 3).

Mesenteric resistance arteries Eight days after CMV-infection the size of the mesenteric resistance arteries was not modified and the amplitude of their responses to 125 mM potassium and 30 nM AVP was not altered (Table 2). Mesenteric small arteries obtained from CMV-infected rats failed, however, to contract to adrenergic stimulation (Figure 4). Virtually no contractile responses were obtained in response to nerve stimulation or exogenous noradrenaline. The β-adrenoceptor

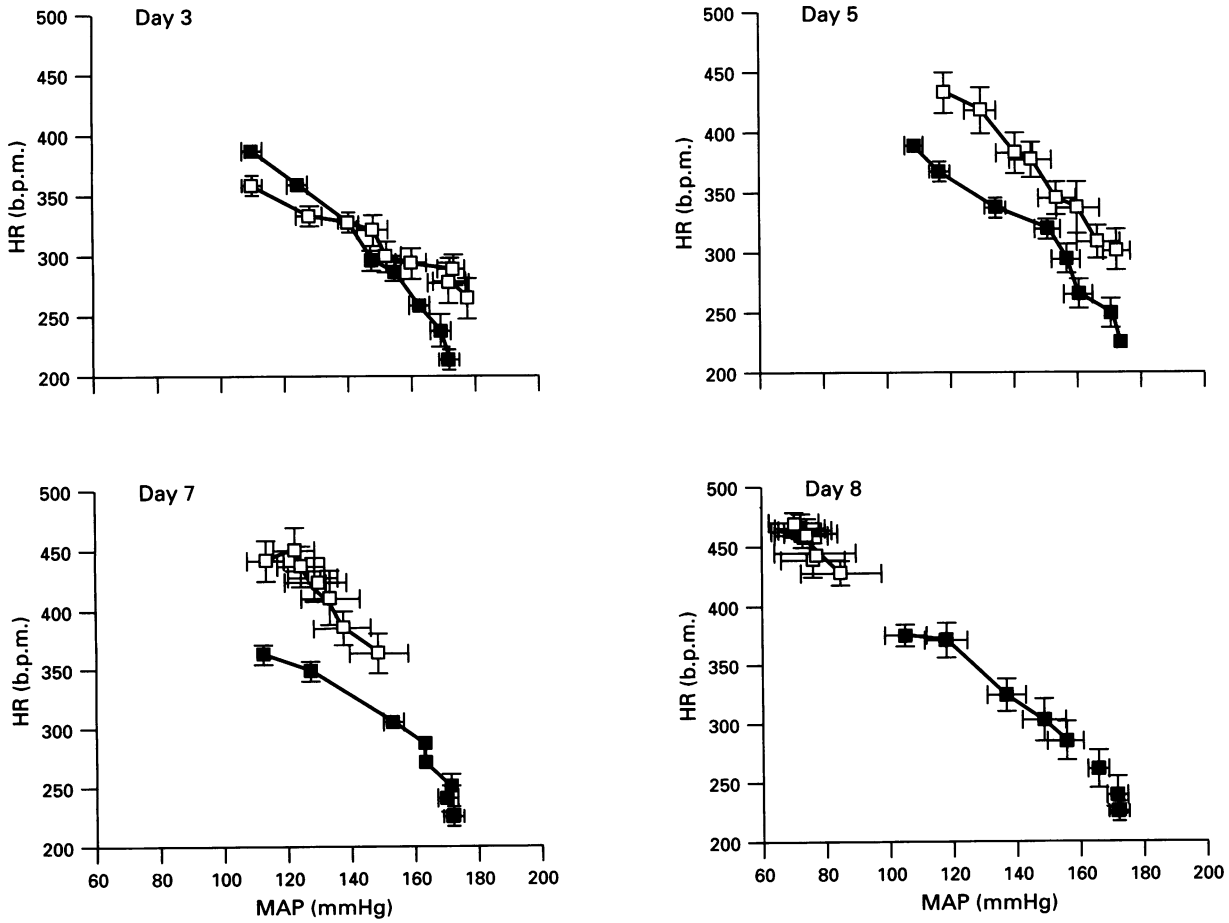

Figure 2 Relationship between heart rate (HR) and mean arterial pressure (MAP) before and during i.v. infusion of phenylephrine (0.1 to 2.0 μg.min^{-1}) in conscious unrestrained irradiated rats that had been infected with CMV (□) or sham-infected (■) on day 0. For sensitivity and maximal pressor responses to the agonist see Table 1. Data are shown as mean ± s.e.mean ($n = 7-10$).

blocking agent, propranolol (1 μM) did not unmask responses to these interventions (data not shown, $n=8$) and also the selective α_1-adrenoceptor agonist, PHE, failed to induce contraction (Figure 4). Furthermore, responses to 5-HT were significantly smaller in mesenteric resistance arteries of CMV-infected than in those of sham-infected rats (Figure 4). The sensitivity (pD_2: 6.7 ± 0.4 versus 7.2 ± 0.4) and maximal relaxing responses ($96\pm4\%$ versus $88\pm8\%$) to isoprenaline, ad-

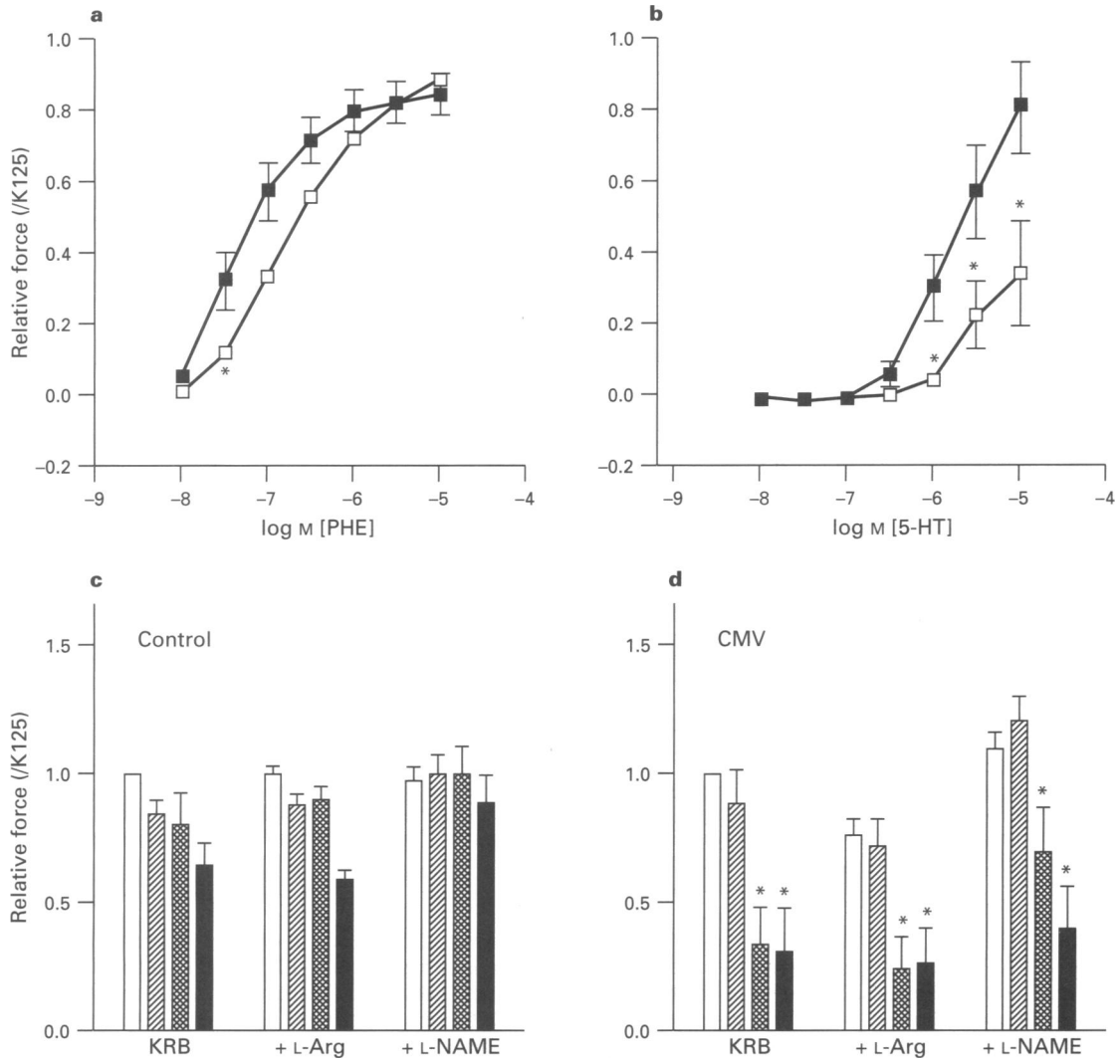

Figure 3 Contractile reactivity of aortic segments isolated 8 days after CMV- or sham-infection. Shown are concentration-response curves for phenylephrine (PHE, a) and 5-hydroxytryptamine (5-HT, b; sham, ■; CMV, □) along with (c,d) the amplitude of contractile responses to 125 mM potassium (open columns), 10 μM phenylephrine (hatched columns), 10 μM 5-HT (cross-hatched columns) and 30 nM Arg-vasopressin (solid columns) in the absence (KRB) or presence of 1 mM L-arginine (L-Arg) or 0.1 mM L-NAME. The data are expressed as fraction of the contractile response to 125 mM potassium in drug-free solution (4.8 ± 0.2 and 3.8 ± 0.5 mN mm^{-1} for control and CMV-rats, respectively) and are shown as mean$\pm$s.e.mean ($n=8$). *Statistically significant difference from sham-infected rats ($P<0.05$).

Table 2 Maximal contractile responses to agonists in mesenteric resistance-sized arteries of rats infected with cytomegalovirus (CMV)[a]

	OID (μm)	*K^+* (mN mm^{-1})	*AVP* (mN mm^{-1})	*NA* (mN mm^{-1})	*5-HT* (mN mm^{-1})
Untreated					
Sham-infected	201 ± 9	2.6 ± 0.4	2.6 ± 0.3	3.0 ± 0.4	2.6 ± 0.2
CMV-infected	193 ± 12	2.8 ± 0.3	2.4 ± 0.3	0.1 ± 0.5*	0.6 ± 0.6*
Prazosin (0.1 mg kg^{-1} day^{-1})					
Sham-infected	200 ± 11	3.3 ± 0.4	2.6 ± 0.5	3.3 ± 0.3	2.1 ± 0.3
CMV-infected	242 ± 28	2.6 ± 0.3	3.2 ± 0.3	1.3 ± 0.9*	2.7 ± 0.7

[a]Small arteries were isolated from the mesentery of irradiated rats 8 days after sham- or CMV-infection with and without continuous treatment with prazosin. The vessels were stretched to their individual optimal internal diameter (OID) for isometric force development. The amplitude of contractile responses to 125 mM potassium (K^+), 30 nM Arg-vasopressin (AVP), 10 μM noradrenaline (NA) and 10 μM 5-hydroxytryptamine (5-HT) were expressed as increases in wall tension and are shown as mean$\pm$s.e.mean ($n=7-10$). *Statistically significant difference sham-infected animals ($P<0.05$).

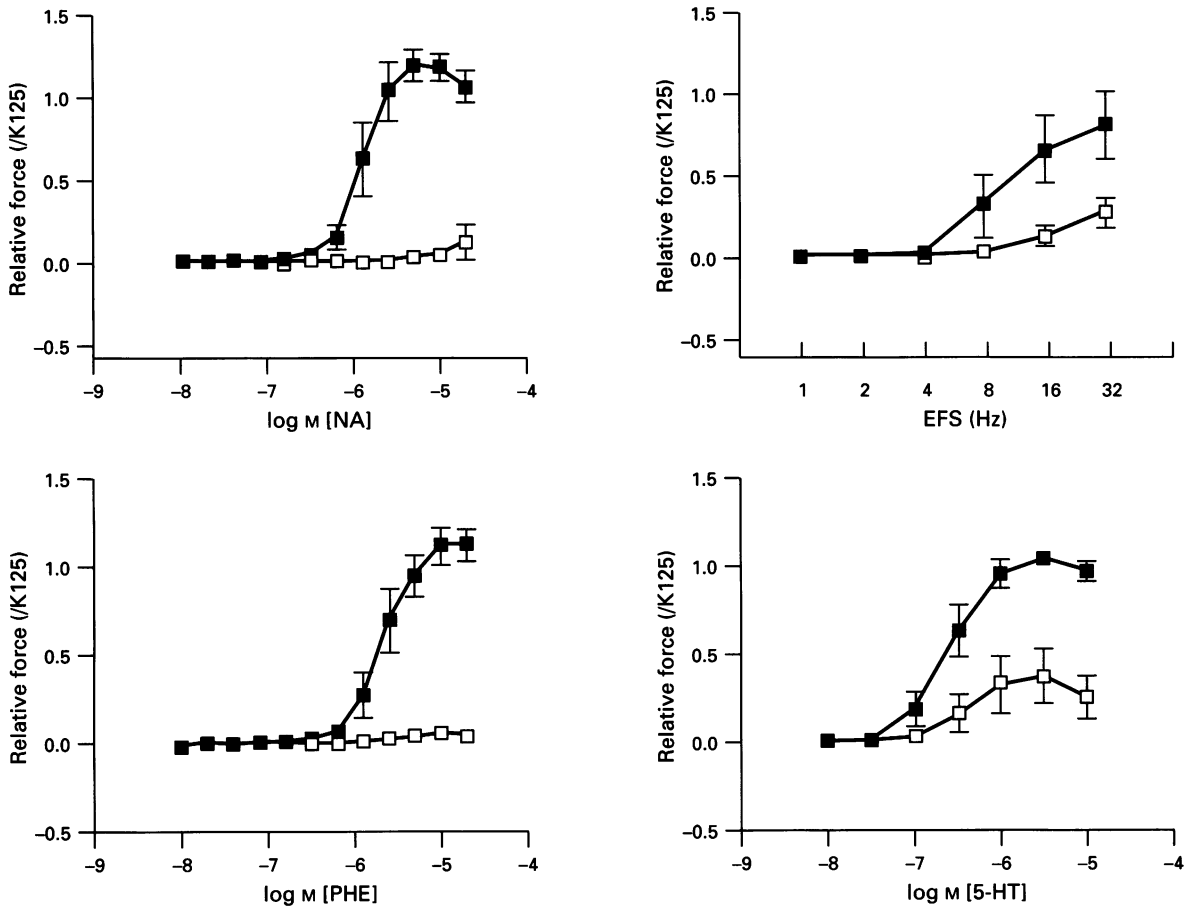

Figure 4 Contractile responses in mesenteric small artery segments isolated 8 days after CMV- (□) or sham-infection (■). Concentration-response curves for noradrenaline (NA), phenylephrine (PHE) and 5-hydroxytryptamine (5-HT) are shown along with frequency-response curves for electrical field stimulation of perivascular nerves (EFS). The data are expressed relative to the amplitude of contractile responses to 125 mM potassium (for absolute values see Table 2) and are shown as mean ± s.e.mean ($n = 7-10$). The differences between both groups are statistically significant.

ministered during AVP-induced contraction, were not altered in small arteries of CMV-infected rats (Figure 5). In resistance arteries of rats that had been exposed to the virus, the maximal relaxing response to acetylcholine (54 ± 17% versus 90 ± 3%) was significantly reduced but the sensitivity to the endothelium-dependent agonist was not modified (pD_2: 7.1 ± 0.2 versus 6.9 ± 0.3) (Figure 5).

Pretreatment with L-NAME, or administration of the inhibitor during exposure to agonists, did not unmask contractile responses to noradrenaline or PHE and did not enhance contractile responses to 5-HT in mesenteric resistance arteries of CMV-infected rats (not shown).

Figure 6 illustrates responses to vasoconstrictors in mesenteric resistance arteries obtained from rats that had been sham- or CMV-infected during continuous treatment with prazosin, 0.1 mg kg^{-1} day^{-1}. As was observed for the untreated animals, the optimal diameter and the amplitude of responses to 125 mM potassium and 30 nM AVP did not differ between vessels of the sham- and the CMV-infected rats (Table 2). But unlike untreated rats, the responsiveness to 5-HT in mesenteric small arteries isolated from prazosin-treated CMV-infected rats was comparable to that in small vessels of prazosin-treated sham-infected animals (Figure 6). Furthermore, sensitivity but not maximal responses to PHE were reduced in mesenteric resistance arteries of rats infected with CMV during continuous treatment with prazosin (Figure 6, Table 2).

Vessels of LPS-treated rats

Compared to controls and sham-infected immunosuppressed rats, the contractile reactivity of thoracic aorta segments of LPS-treated rats was markedly altered. The amplitude of contractile responses to PHE, 5-HT and AVP was significantly reduced (Figure 7). Furthermore, in aortae of LPS treated rats, 1 mM L-arginine reduced contractile responses and 0.1 mM L-NAME increased contractile responses to PHE and 5-HT (Figure 7). In the aortae of control rats, the effects of L-arginine and L-NAME were not statistically significant.

In contrast to the thoracic aorta, the contractile reactivity of mesenteric resistance arteries obtained from the same LPS-treated rats, did not differ from that of small arteries isolated from control animals (Figure 7). The amplitude of maximal contractile responses to potassium, PHE, 5-HT and AVP were not altered following administration of LPS. Furthermore, the presence of 1 mM L-arginine or 0.1 mM L-NAME did not unmask differences between resistance arteries of LPS-treated and control rats (Figure 7).

Discussion

In immunosuppressed rats, CMV infection was found to alter heart rate, blood pressure and arterial reactivity. The arterial changes were more marked in small arteries than in the aortae and differed from those observed following LPS. They could not be reversed by L-NAME but were prevented by prazosin treatment. This suggests that CMV resulted in vascular failure independently of NO synthase, but through activation of the sympathetic nervous system.

Herpes viruses, including cytomegalovirus are widespread in the general population and it has been suggested that they are involved in the pathogenesis of atherosclerosis (Bruggeman

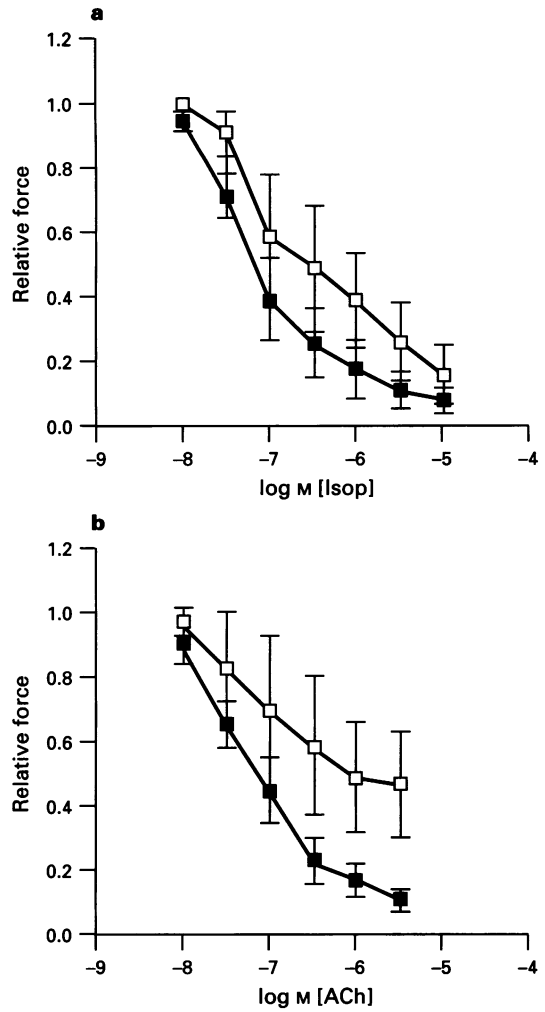

Figure 5 Relaxant responses in mesenteric small artery segments isolated 8 days after CMV- (□) or sham-infection (■). Concentration-response curves for (a) isoprenaline (Isop) and (b) acetylcholine (ACh) were constructed during contraction induced by 30 nM Arg-vasopressin. The data are expressed as a fraction of the tone observed before addition of the relaxing agent and are shown as mean ± s.e.mean ($n = 7-10$).

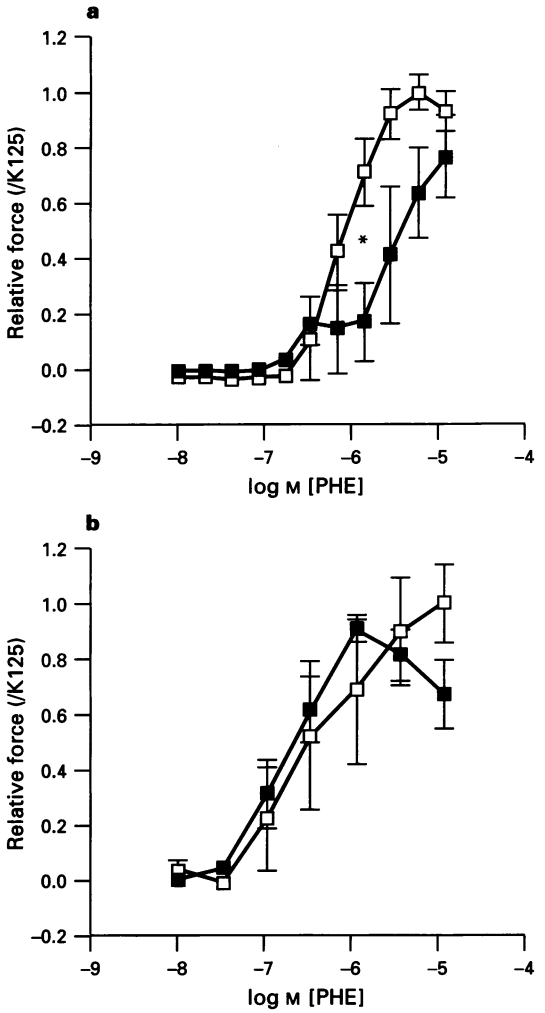

Figure 6 Contractile responses in mesenteric small artery segments isolated 8 days after CMV- (□) or sham-infection (■) during continuous treatment *in vivo* with prazosin 0.1 mg kg^{-1} day^{-1}. Concentration-response curves for phenylephrine (PHE) and 5-hydroxytryptamine (5-HT) are shown. The data are expressed relative to the amplitude of contractile responses to 125 mM potassium (for absolute values see Table 2) and are shown as mean ± s.e.mean ($n = 7-10$). *Statistically significant difference between CMV- and sham-infected rats.

& van Dam-Mieras, 1991; Hajjar, 1991; Persoons *et al.*, 1994). CMV was observed to promote integrin production and adhesion of monocytes and leucocytes to endothelium (Span *et al.*, 1991a,b). This is mediated by cytokines such as interleukin-1 (Span *et al.*, 1991a). The goal of the present study was to evaluate whether CMV-infection *in vivo* also influences the function of arterial smooth muscle. The experiments were performed following immunosuppression by irradiation because like human CMV, rat CMV is pathogenic almost exclusively under immunocompromised conditions (Bruggeman *et al.*, 1985). Observations with respect to blood pressure and arterial reactivity in immunosuppressed sham-infected rats were comparable to those in control age-matched rats (Struijker Boudier *et al.*, 1982; Boonen & De Mey, 1990a; Eerdmans *et al.*, 1991).

CMV-infected rats ultimately displayed tachycardia, low blood pressure and impaired pressor responses to PHE, reminiscent of hypotonic shock during bacterial sepsis (Groeneveld *et al.*, 1986; Julou-Schaeffer *et al.*, 1990). Observations in mesenteric small arteries indicate that this was due to extensive peripheral vasodilatation. These resistance-sized arteries were markedly hyporesponsive to sympathetic nerve stimulation and α_1-adrenoceptor stimulation by exogenous noradrenaline or PHE. Also during bacterial sepsis, extensive peripheral vasodilatation underlies the precipitous fall in blood pressure (Groeneveld *et al.*, 1986; Julou-Schaeffer *et al.*, 1990). However, regional- and agonist-selectivity of the changes suggest that the mechanisms differ from those engaged following administration of LPS. Selective alterations were found in small mesenteric arteries. They consisted of drastically impaired responses to α_1-adrenoceptor and 5-HT receptor stimulation, despite maintenance of responses to potassium and vasopressin. This cannot be attributed to induction of NO synthase because (i) small artery responses to phenylephrine, 5-HT and vasopressin are equally sensitive to NO (Eerdmans & De Mey, unpublished observations), (ii) the NO synthase inhibitor L-NAME did not restore responses and because (iii) LPS-treatment, which has been shown to result in cytokine-mediated induction of NO synthase in the vascular wall (Moncada *et al.*, 1989; Julou-Schaeffer *et al.*, 1990; Moncada & Higgs, 1991), altered the reactivity of the aortae but not that of small resistance vessels. It is more likely that a selective alteration of signal-transduction developed in small artery smooth muscle during the course of the CMV-infection. We previously suggested that small artery responses to α_1-adrenoceptor stimulation and 5-HT are mediated by pertussis toxin-sensitive G-proteins, affecting voltage-operated calcium channels (Boonen & De Mey, 1990a,b; 1991). Depolarization

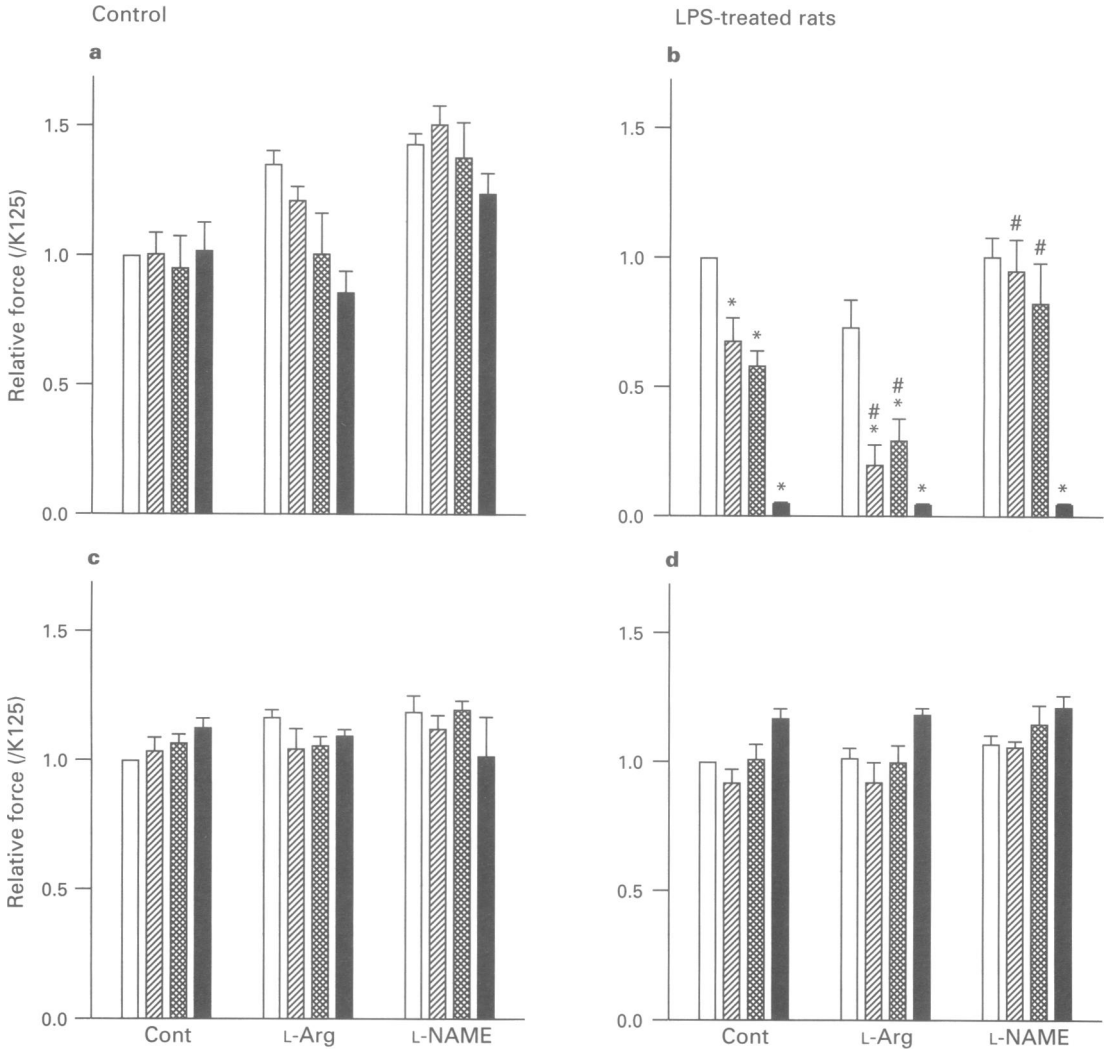

Figure 7 Effects of L-arginine (L-Arg) and N^G-nitro-L-arginine methyl ester (L-NAME) on contractile responses of thoracic aortae segments (a,b) and mesenteric resistance artery segments (c,d) of rats that had been injected 48–72 h earlier with 5–10 mg/kg^{-1} LPS (b,d) and untreated controls (a,c). Contractions were induced with 125 mM potassium (open columns), 10 μM phenylephrine (hatched columns), 10 μM 5-HT (cross-hatched columns) and 30 nM Arg-vasopressin (solid columns) in drug-free solution (KRB) at 45 and 15 min interval in aortae and small artery preparations, respectively. They were then repeated in the continuous presence of 1 mM L-arginine and thereafter in the presence of 100 μM L-NAME. Contractions were expressed as fraction of the response to K^+ in KRB (3.5±0.6 and 2.9±0.5 mN mm^{-1} for control and LPS-treated aortae, respectively, and 2.9±0.3 and 3.2±0.3 mN mm^{-1} for control and LPS-treated mesenteric small artery, respectively). The data are shown as mean±s.e.mean (n=8). *Statistically significant difference from control rats; #the effect of L-Arg or L-NAME is statistically significant.

opens these channels directly while vasopressin most likely induces contraction through phospholipase C (Boonen & De Mey, 1990a,b; 1991). Furthermore, contractile responses of the rat aortae to PHE are rather insensitive to pertussis toxin and calcium channel blockers (Nichols *et al.*, 1989; Oriowo & Ruffolo, 1992). Thus, the observed regional- and agonist selectivity suggest changes at the level of pertussis toxin sensitive G-proteins, but this remains to be firmly established.

That mechanisms other than induction of NO synthase would be involved in vascular changes following CMV-infection is suggested by their resistance to arginine and arginine-analogues and by their regional distribution which differs from that observed following LPS. We observed that 2 days after bolus administration of LPS, contractile reactivity was reduced in aortae but not resistance vessels and that exogenous arginine caused relaxation in aortae but not resistance vessels. This large artery selectivity contrasts with observations of reduced coronary resistance and relaxation of resistance arteries induced by arginine in rabbit hearts and rat mesenteries 4–6 h after being injected i.p. with 20 mg kg^{-1} LPS (Smith *et al.*, 1991). This discrepancy may be due to differences in concentration- and time-dependency of responses to LPS and cytokines in large and small arteries. In the present study we deliberately used a low concentration of LPS and a long 'incubation' time in an attempt to mimic the progressive nature of the changes following CMV-infection.

Prior to changes in vasopressor responsiveness to PHE, CMV-infected rats displayed changes in basal heart rate and in heart rate regulation. Upon elevation of blood pressure with PHE, heart rate is normally reduced through a decrease of sympathetic and an increase of parasympathetic outflow. Three days after CMV-infection the capacity to reduce heart rate in response to pressure elevation was impaired. Although the reflex recovered, it was apparently shifted to a higher set-point. Thereafter basal heart rate increased even further. It is of interest that even at 8 days post-infection, when high heart rate and low blood pressure were observed, administration of the β-adrenoceptor antagonist, propranolol (1 mg kg^{-1}, i.v.) reduced heart rate (unpublished observations). Combined, these data suggest that increased sympathetic output was maintained for several days after CMV-infection.

Several days were needed before arterial reactivity changed following CMV-infection. This may be indicative of an indirect mechanism. Our findings with prazosin suggest a possible

contribution of sympathetic nervous input to the vascular alterations. Because CMV-infection ultimately resulted in hypotonic shock preceded by tachycardia, we anticipated that prazosin-treatment would accelerate the development of the precipitous fall of blood pressure. Surprisingly it rather delayed the changes in heart rate and blood pressure. Furthermore, it protected small arteries against the selective reactivity changes. From this we suggest that excessive α_1-adrenergic stimulation of arterial smooth muscle contributes to the selective alteration of excitation-contraction coupling. Through a more or less classical down-regulation process it would primarily affect the action of postjunctional α_1-adrenoceptors and that of other agonists, such as 5-HT, that share the same signalling mechanisms in the vasculature (Boonen & De Mey, 1990a,b; 1991). It would not affect vascular β-adrenergic responses, because we have previously shown that in mesenteric small arteries postjunctional β-adrenoceptors are situated beyond the reach of neuronally released noradrenaline (Eerdmans *et al.*, 1991). Furthermore, α_1-adrenergic responses of the aortae could escape from this process because, in the adult rat, this vessel is not innervated.

In summary, we observed that CMV-infection of irradiated rats resulted in altered heart rate regulation, tachycardia, changes in arterial reactivity and marked reduction of blood pressure. The changes in vascular function were agonist- and regionally selective. They differed from those observed following administration of endotoxin. Alterations of excitation-contraction coupling in blood vessels are more likely to be involved than cytokine-mediated induction of NO synthase. α_1-Adrenoceptors may participate in the initiation of these changes.

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