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Cardiovascular effects of noradrenaline in hypovolemic haemorrhage: role of inducible nitric oxide synthase

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Abstract

Hypovolemia has been associated with the induction of nitric oxide synthase which is believed to result in an over-production of nitric oxide. In the present study, we have examined the effects of noradrenaline following haemorrhage on cardiac output, blood pressure, mean circulatory filling pressure and vascular resistance in anaesthetized rats after pre-treatment with nitric oxide synthase inhibitor, L-N^6-(1-iminoethyl)lysine or dexamethasone. Hypovolemic haemorrhage resulted in induction of nitric oxide synthase, as measured in lungs, and both dexamethasone and L-N^6-(1-iminoethyl)lysine inhibited the activity of the inducible form of nitric oxide synthase. An infusion of noradrenaline significantly increased cardiac output, blood pressure and mean circulatory filling pressure in animals pre-treated with L-N^6-(1-iminoethyl)lysine and dexamethasone when compared with saline pre-treatment. In addition, the administration of noradrenaline significantly reduced venous resistance in animals pre-treated with L-N^6-(1-iminoethyl)lysine when compared with saline pre-treatment. The results of this investigation indicated that the impact of noradrenaline on cardiac output, blood pressure and mean circulatory filling pressure was greater in hypovolemic rats treated with L-N^6-(1-iminoethyl)lysine or dexamethasone. In addition, we found that in the hypovolemic state, the greater increase in cardiac output during the infusion of noradrenaline after inhibition of nitric oxide synthase was predominantly due to reduced resistance to venous return.

Keywords: Cardiac output; Hemorrhage; Venous resistance; Noradrenaline; Nitric oxide (NO) synthase; Blood pressure

1. Introduction

Nitric oxide (NO) is an important mediator involved in the regulation of vascular tone, and, therefore, blood flow and blood pressure (Huang et al., 1995; Takahashi et al., 1995; Kassab et al., 1998). However, an over-production of NO seems to result in the demise of the cardiovascular system in pathophysiological conditions such as endotoxic and haemorrhagic shock (for review see Szabó and Thiemermann, 1994). In general, NO serves as a mediator in physiological processes such as host defense, cardiovascular regulation and neuronal communication.

Nitric oxide synthase is responsible for conversion of L-arginine into NO and citrulline (for review see Moncada et al., 1989). At present, at least three forms of nitric oxide synthase have been identified, cloned, sequenced and expressed. These are isoform I, found in neuronal cells, isoform II, present in the '*activated*' macrophages and also in many other organs, tissues and cells, and isoform III, found in endothelial cells (for review see Moncada et al., 1991). Induction of nitric oxide synthase, which results in an increased level of NO, has been implicated in a number of pathophysiological conditions, such as circulatory shock (Thiemermann et al., 1993a), cancer (Langrehr et al., 1993), diabetes (Corbett et al., 1992), and chronic inflammation (Vane et al., 1994).

Haemorrhage has also been implicated as a process that results in the induction of nitric oxide synthase, and, therefore, an increased level of NO (Thiemermann, 1994). An increase in the level of NO has been suggested to be a key factor responsible for reduced responsiveness of blood vessels towards vasoconstrictors. The hypoactivity of blood vessels towards vasoconstrictors may account for reduced blood pressure following haemorrhage (Thiemermann et al., 1993a). Moreover, diminished responsiveness of blood vessels towards vasoconstrictor agents can, in part, account for reduced cardiac output. However, it is apparent from the current literature that the impact of exogenously infused vasoconstrictor agents on cardiac output, arterial

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resistance and venous resistance in the state of oligemia after inhibition of inducible nitric oxide synthase remains unknown and needs to be characterized. Therefore, in the present investigation, we have examined the effects of a selective inhibitor of inducible nitric oxide synthase, L-N^6-(1-iminoethyl)lysine (Moore et al., 1994), on the cardiovascular (cardiac output, blood pressure, venous tone, arterial and venous resistances) effects of noradrenaline after hypovolemic haemorrhage. In addition, we have compared the influence of L-N^6-(1-iminoethyl)lysine to that of dexamethasone, which is also known to selectively inhibit inducible nitric oxide synthase.

2. Methods

2.1. Surgical preparation of animals

Male Long–Evans rats (340–370 g) were anaesthetized with thiobutabarbital (100 mg kg^{-1}) i.p.. Catheters (polyethylene tubing; i.d. 0.58 mm, o.d. 0.965 mm) were inserted into the left and right iliac arteries and veins. The left venous catheter was advanced into the inferior vena cava and used for the measurement of central venous pressure. The left arterial and right venous catheters were used for the measurement of blood pressure, and drug/vehicle administration, respectively, while the right arterial catheter was used for blood withdrawal of radiolabeled microspheres. A catheter (polyethylene tubing; i.d. 0.28 mm, o.d. 0.61 mm) was inserted into a branch of the left jugular vein for infusion of chemicals. An additional catheter was inserted into the left ventricle via the right carotid artery for measurement of the left ventricular end-diastolic pressure and injection of radiolabeled microspheres. A saline-filled balloon-tipped catheter was placed in the right atrium via the right external jugular vein for the purpose of transient circulatory stop as necessary for the measurement of mean circulatory filling pressure (Tabrizchi, 1997). The animals were tracheotomized and allowed to stabilize for a period of 1 h while arterial pressure, central venous pressure and heart rate were monitored continuously.

All catheters were filled with heparinized saline (25 IU ml^{-1}). Body temperature was maintained at 37°C using a heating lamp and monitored using a rectal thermometer. Arterial blood pressure and central venous pressures were recorded with a pressure transducer (Gould Statham, USA; Model PD23B) connected to an amplifier (DA 100A) which was connected to a universal interface module (UIM 100) which interfaced with an acquisition unit (MP 100). The data was collected using AcqKnowledge III (BIOPAC System USA) and stored on an IBM compatible microcomputer. Heart rate was calculated from the blood pressure signal using the AcqKnowledge III system. Cardiac output was measured using the reference sample microsphere method, and mean circulatory filling pressure was measured after circulation was transiently stopped by inflating the balloon in the right atrium. Final arterial pressure and venous plateau pressure were recorded at 5–7 s after the circulatory stop (Pang and Tabrizchi, 1986).

2.2. Measurement of cardiac output

This technique has been described in detail elsewhere (Pang, 1983). Briefly, suspensions of microspheres (Mandel Canada; 15 μm diameter) labeled with ^{57}CO (20,000–22,000 in 150 μl) were injected into the left ventricle over a period of 10 s. Blood was withdrawn from the right femoral artery at the rate of 0.35 ml min^{-1} starting 15 s. before microsphere injection using an infusion/withdrawal pump (Kd Scientific USA; Model 120) for 1 min. The blood sample and syringes used for injection of microspheres or withdrawal of blood were counted for radioactivity at 80–160 keV using a dual channel automatic gamma counter (LKB Wallac, Clinic Gamma Counter, Canada; Model 1272). The withdrawn blood sample was slowly injected back into the animals immediately after counting of radioactivity.

2.3. Experimental protocol

Animals were randomly assigned to four groups ($n = 6$): saline-treated (0.54 ml kg^{-1} bolus and 0.54 ml kg^{-1} h^{-1} infusion; Group I), dexamethasone-treated (5 mg kg^{-1}; Group II) and L-N^6-(1-iminoethyl)lysine-treated (100 μg kg^{-1} bolus and 100 μg kg^{-1} h^{-1} infusion; Group III and 300 μg kg^{-1} bolus and 300 μg kg^{-1} h^{-1} infusion; Group IV). After the completion of surgery, blood pressure and heart rate were continuously monitored for 60 min, after which each animal received either saline or drugs. 30 min after the administration of saline or drugs, the first measurement of haematocrit, cardiac output and mean circulatory filling pressure were made. Animals were haemorrhaged (0.90 ml kg^{-1} for 15 min) and, subsequently, blood pressure and heart rate were continuously monitored for a period of 3.5 h. The second haemocrit was taken and each rat was then treated with propranolol (4 mg kg^{-1}). Five min after treatment with propranolol, the second measurements of cardiac output and mean circulatory filling pressure were taken. After the second measurements of cardiac output and mean circulatory filling pressure, a dose of noradrenaline (0.1, 0.3 and 1.0 μg kg^{-1} min^{-1}) was infused and cardiac output and mean circulatory filling pressure were measured 12–14 min after the start of infusion. In each animal, repeated cardiac output and mean circulatory filling pressure measurements were made during the infusion of each dose of noradrenaline. The time allowed between each dose of noradrenaline was 15–16 min. At the end of each experiment, the lungs were quickly excised, placed in liquid nitrogen and stored at −80°C.

Table 1
Basal cardiac output (CO; ml min^{-1}), blood pressure (BP; mm Hg); mean circulatory filling pressure (P_{mcf}; mm Hg), heart rate (HR; beats min^{-1}), arterial resistance (A_R; mm Hg min ml^{-1}) and venous resistance (V_R; mm Hg min ml^{-1}) in various groups of animals treated with saline (0.54 ml kg^{-1} and 0.54 ml kg^{-1} h^{-1}), dexamethasone (DEX; 5 mg kg^{-1}), and *l*-N^6-(1-iminoethyl)lysine (IEL; 100 μg kg^{-1} and 100 μg kg^{-1} h^{-1} and 300 μg kg^{-1} and 300 μg kg^{-1} h^{-1}) before haemorrhage and 3.5 h post-haemorrhage after treatment with propranolol (+Prop; 4 mg kg^{-1}).

Groups	CO	BP	P_{mcf}	HR	A_R	V_R
Saline	105 ± 4	108 ± 5	5.9 ± 0.2	360 ± 8	1.00 ± 0.04	0.040 ± 0.001
DEX	109 ± 5	122 ± 3	5.6 ± 0.17	371 ± 10	1.12 ± 0.08	0.035 ± 0.001
IEL (100)	112 ± 5	110 ± 5	5.74 ± 0.20	360 ± 8	0.92 ± 0.04	0.035 ± 0.004
IEL (300)	108 ± 5	115 ± 6	5.39 ± 0.28	373 ± 4	1.05 ± 0.02	0.035 ± 0.001
+Prop						
Saline	73 ± 2*	72 ± 4*	4.2 ± 0.3*	325 ± 12*	0.96 ± 0.06	0.038 ± 0.003
DEX	84 ± 1*	77 ± 3*	4.76 ± 0.17*	322 ± 6*	0.95 ± 0.05	0.036 ± 0.002
IEL (100)	82 ± 5*	79 ± 3*	4.47 ± 0.17*	341 ± 4*	0.99 ± 0.08	0.033 ± 0.003
IEL (300)	70 ± 3*	85 ± 5*	4.74 ± 0.13*	330 ± 8*	1.09 ± 0.07	0.044 ± 0.002

Each value represent means of six experiments ± S.E.M.
* Significantly different from pre-haemorrhage; $P < 0.05$.

2.4. Nitric oxide synthase assay in lungs

Nitric oxide synthase was assessed by measuring the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline as described by Thiemermann et al. (1993b), with slight modifications. Frozen lungs were homogenized on ice in buffer composed of (in mM): Tris–HCl, 50; EDTA, 0.1; EGTA, 0.1; 2-mercaptoethanol, 12; and phenylmethylsulfonyl fluoride, 1 (pH 7.4). 50 μl of homogenates were incubated in the presence of [^{3}H]L-arginine/L-arginine (10 μM), NADPH (1.0 mM), calmodulin (10 μg ml^{-1}), tetrahydrobiopterin (5.0 μM) and Ca^{2+} (2.0 mM) (total volume of 200 μl) at 37°C for 30 min. The reaction was stopped using stop buffer (1.0 ml) of the following composition (in mM): HEPES, 20; EDTA, 2.0; and EGTA, 2.0 (pH 5.5). Each sample was applied to a 2-ml column of Dowex 50W-X8 (sodium form) (Bio-Rad Laboratory, Canada) and eluted four times with 1.0 ml of stop buffer. Radioactivity in each sample was measured using a scintillation counter (Beckman, USA; Model LS 3801). Assays were performed in duplicate in the presence of NADPH to determine constitutive nitric oxide synthase activity, the absence of NADPH to determine the extent of [^{3}H]L-citrulline formation independent of nitric oxide synthase, and in a Ca^{2+}-free buffer containing NADPH and EGTA (5 mM) to determine Ca^{2+}-independent (induced) nitric oxide synthase activity. Protein concentration was measured using Bradford's method (Bradford, 1976).

2.5. Chemicals

L-N^6-(1-Iminoethyl)lysine, propranolol, thiobutabarbital and L-arginine were purchased from Research Biochemical International (Natick, MA, USA). All other fine chemicals were purchased from Sigma (Ontario, Canada).

2.6. Calculations and statistical analysis

Cardiac output (ml min^{-1}) was calculated as the rate of withdrawal of blood multiplied by total injected c.p.m. divided by c.p.m. in withdrawn blood. Arterial resistance (mm Hg min ml^{-1}) was obtained by dividing blood pressure by cardiac output, and venous resistance (mm Hg min ml^{-1}) was calculated as the difference of mean circulatory filling pressure and central venous pressure divided by cardiac output (Wang et al., 1995). Percent changes in plasma volume were estimated using the formula $(100/100 - H_i) \times 100\,(H_i - H_f)/H_f$. H_i and H_f were the initial and final haemocrit readings, respectively (Davies et al., 1976).

The data were analyzed by One-way Analysis of Variance with repeated measures for comparison. Duncan's multiple range test was used for comparison between means. A difference of $P < 0.05$ was considered to be significant.

3. Results

There were no differences between basal haemodynamics measurements in the various groups of animals (Table 1). The percent changes in plasma volume following haemorrhage were between 24–28% in the various groups of animals (Table 2). Cardiac output, blood pressure, mean

Table 2
Values of percent change in plasma volume (% ΔPV) after haemorrhage and enzymatic activity of inducible nitric oxide synthase (iNOS) and constitutive (cNOS) forms of nitric oxide synthase (pmol mg^{-1} protein min^{-1}) of lungs in various groups of animals treated with saline (0.54 ml kg^{-1} and 0.54 ml kg^{-1} h^{-1}), dexamethasone (DEX; 5 mg kg^{-1}), and *l*-N^6-(1-iminoethyl)lysine (IEL; 100 μg kg^{-1} and 100 μg kg^{-1} h^{-1} and 300 μg kg^{-1} and 300 μg kg^{-1} h^{-1}). Each value represents mean of six experiments ± s.e.m.

Groups	Saline	DEX	IEL (100)	IEL (300)
% ΔPV	26 ± 2	28 ± 2	25 ± 2	24 ± 2
iNOS	2.1 ± 0.5	0.13 ± 0.04*	0.75 ± 0.25*	0.18 ± 0.05*
cNOS	0.48 ± 0.18	0.23 ± 0.08	0.37 ± 0.10	0.24 ± 0.09

* Significantly different from pre-treatment with saline; $P < 0.05$.

circulatory filling pressure and heart rate were significantly reduced in animals 3.5 h post-haemorrhage (Table 1). However, no significant differences were found in arterial and venous resistances after haemorrhage (Table 1). There were no significant differences in percent change in plasma volume after haemorrhage in the different groups of animals. Both dexamethasone and L-N^6-(1-iminoethyl)lysine inhibited inducible nitric oxide synthase activity without affecting constitutive nitric oxide synthase activity (Table 2).

An infusion of noradrenaline at 0.1 and 0.3 μg kg^{-1} min^{-1} resulted in a significant increase in cardiac output in animals pre-treated with L-N^6-(1-iminoethyl)lysine (100 μg kg^{-1} bolus and 100 μg kg^{-1} h^{-1}; 300 μg kg^{-1} bolus and 300 μg kg^{-1} h^{-1}) when compared with saline pre-treatment. In addition, the infusion of noradrenaline at 1.0 μg kg^{-1} min^{-1} significantly increased cardiac output in animals treated with the higher dose of L-N^6-(1-iminoethyl)lysine when compared to saline treatment (Fig. 1A,B,C). In contrast, the administration of noradrenaline only at 0.1 μg kg^{-1} min^{-1} significantly increased cardiac output in dexamethasone (5 mg kg^{-1}) pre-treated animals when compared with saline pre-treatment (Fig. 1A,B,C). Furthermore, the infusion of noradrenaline did not affect heart rate at any dose level in animals pre-treated with dexamethasone (ranging between 335–339 beats min^{-1})

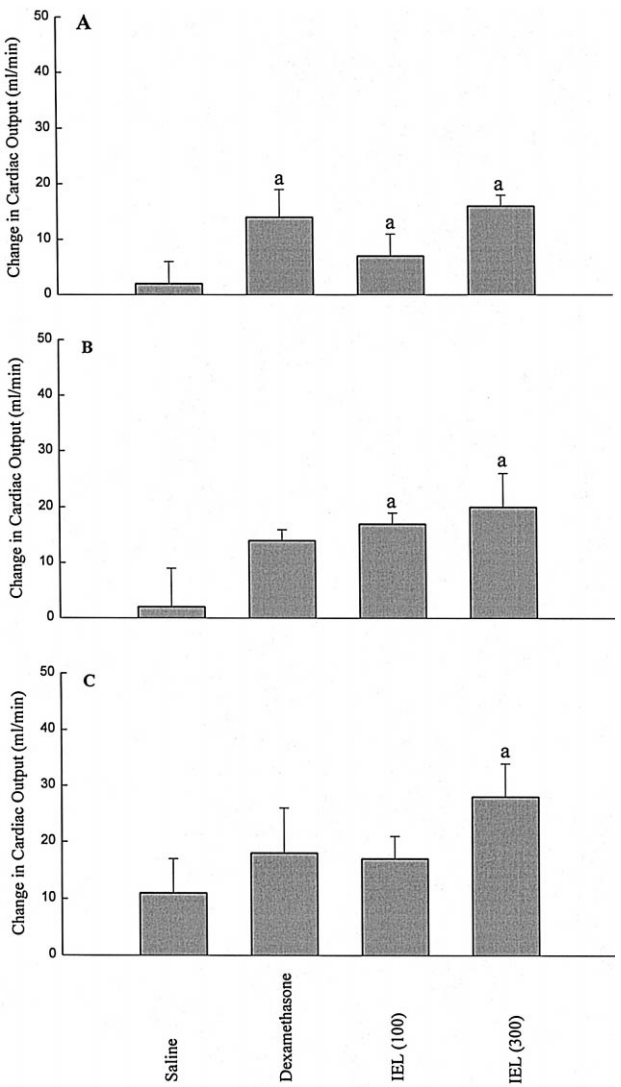

Fig. 1. Changes in cardiac output post-haemorrhage during infusion of noradrenaline (A) (0.1 μg kg^{-1} min^{-1}), (B) (0.3 μg kg^{-1} min^{-1}) and (C) (1.0 μg kg^{-1} min^{-1}) in animals pretreated with saline (0.54 ml kg^{-1} (bolus) and 0.54 ml kg^{-1} h^{-1} (infusion)), dexamethasone (DEX; 5 mg kg^{-1}), and *l*-N^6-(1-iminoethyl)lysine (IEL; 100 μg kg^{-1} (bolus) and 100 μg kg^{-1} h^{-1} (infusion) and 300 μg kg^{-1} (bolus) and 300 μg kg^{-1} h^{-1} (infusion)). [a]Significantly different from saline group, $P < 0.05$.

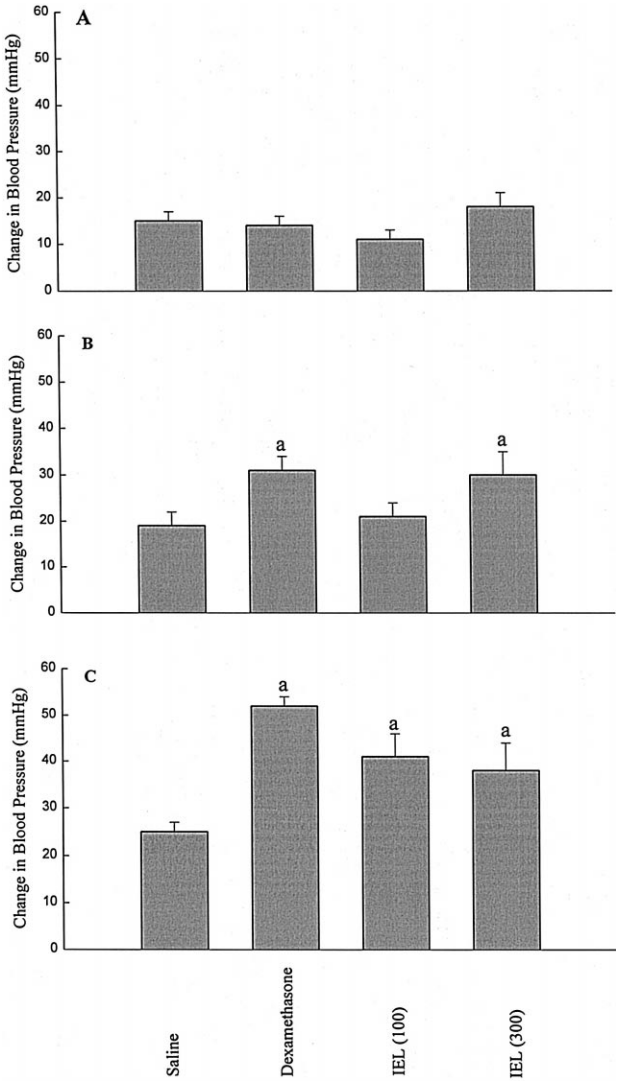

Fig. 2. Changes in blood pressure post-haemorrhage during infusion of noradrenaline (A) (0.1 μg kg^{-1} min^{-1}), (B) (0.3 μg kg^{-1} min^{-1}) and (C) (1.0 μg kg^{-1} min^{-1}) in animals pretreated with saline (0.54 ml kg^{-1} (bolus) and 0.54 ml kg^{-1} h^{-1} (infusion)), dexamethasone (DEX; 5 mg kg^{-1}), and *l*-N^6-(1-iminoethyl)lysine (IEL; 100 μg kg^{-1} (bolus) and 100 μg kg^{-1} h^{-1} (infusion) and 300 μg kg^{-1} (bolus) and 300 μg kg^{-1} h^{-1} (infusion)). [a]Significantly different from saline group, $P < 0.05$.

and L-N^6-(1-iminoethyl)lysine (ranging 339–347 beats min^{-1}) when compared to saline (ranging between 339–343 beats min^{-1}) treatment. Administration of noradrenaline at 0.1 μg kg^{-1} min^{-1} also failed to significantly increase blood pressure in dexamethasone- and L-N^6-(1-iminoethyl)lysine-treated animals when compared to saline treatment (Fig. 2A). However, administration of noradrenaline both at 0.3 and 1.0 μg kg^{-1} min^{-1} resulted in a significant increase in blood pressure in animals pre-treated with either dexamethasone or the higher dose of L-N^6-(1-iminoethyl)lysine in comparison to saline treatment (Fig. 2BC). An infusion of noradrenaline at the lowest dose

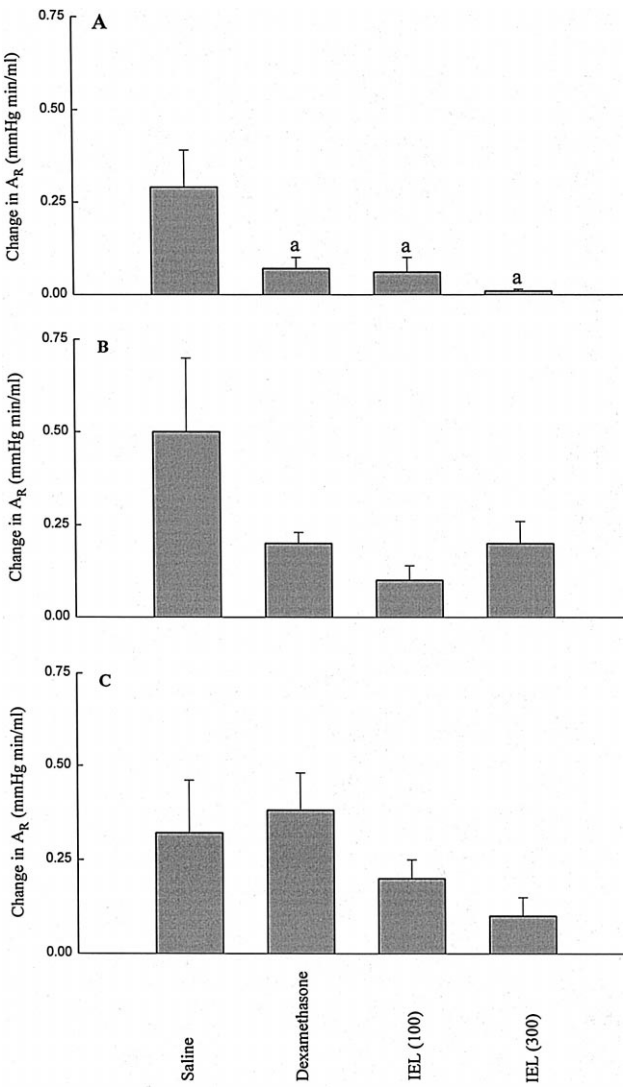

Fig. 3. Changes in arterial resistance (A_R) post-haemorrhage during infusion of noradrenaline (A) (0.1 μg kg^{-1} min^{-1}), (B) (0.3 μg kg^{-1} min^{-1}) and (C) (1.0 μg kg^{-1} min^{-1}) in animals pretreated with saline (0.54 ml kg^{-1} (bolus) and 0.54 ml kg^{-1} h^{-1} (infusion)), dexamethasone (DEX; 5 mg kg^{-1}), and *l*-N^6-(1-iminoethyl)lysine (IEL; 100 μg kg^{-1} (bolus) and 100 μg kg^{-1} h^{-1} (infusion) and 300 μg kg^{-1} (bolus) and 300 μg kg^{-1} h^{-1} (infusion)). [a]Significantly different from saline group, $P < 0.05$.

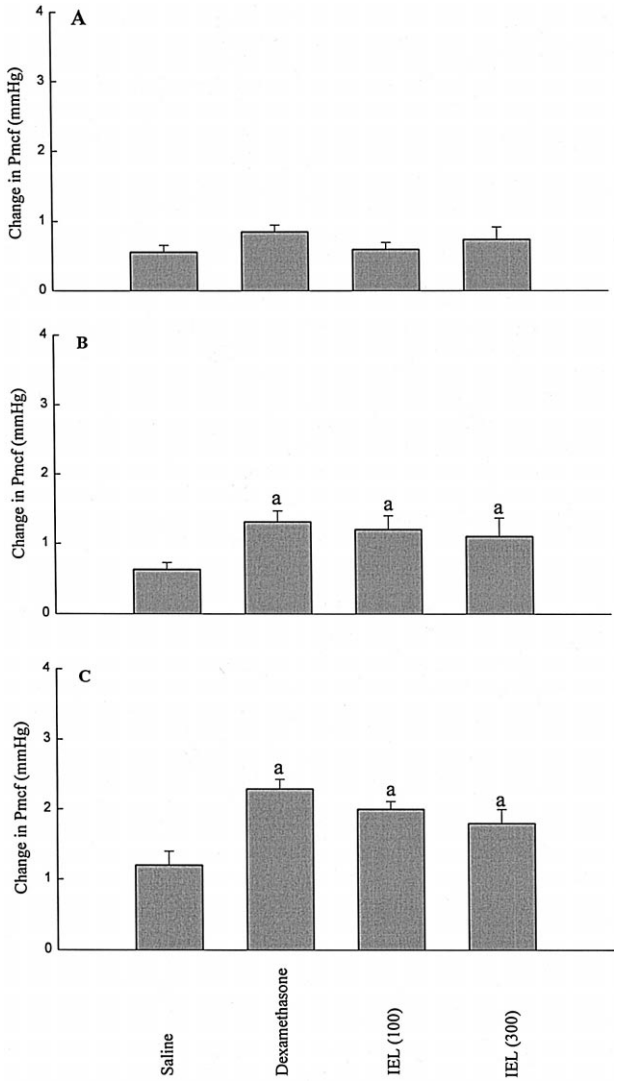

Fig. 4. Changes in mean circulatory filling pressure (P_{mcf}) post-haemorrhage during infusion of noradrenaline (A) (0.1 μg kg^{-1} min^{-1}), (B) (0.3 μg kg^{-1} min^{-1}) and (C) (1.0 μg kg^{-1} min^{-1}) in animals pretreated with saline (0.54 ml kg^{-1} (bolus) and 0.54 ml kg^{-1} h^{-1} (infusion)), dexamethasone (DEX; 5 mg kg^{-1}), and *l*-N^6-(1-iminoethyl)lysine (IEL; 100 μg kg^{-1} (bolus) and 100 μg kg^{-1} h^{-1} (infusion) and 300 μg kg^{-1} (bolus) and 300 μg kg^{-1} h^{-1} (infusion)). [a]Significantly different from saline group, $P < 0.05$.

significantly decreased arterial resistance in dexamethasone- and L-N^6-(1-iminoethyl)lysine-treated rats when compared to saline treatment (Fig. 3A).

Administration of the lowest dose of noradrenaline failed to significantly influence mean circulatory filling pressure in dexamethasone and L-N^6-(1-iminoethyl)lysine pre-treated rats when compared with saline pre-treatment (Fig. 4A). However, an infusion of noradrenaline at 0.3 and 1.0 μg kg^{-1} min^{-1} significantly increased mean circulatory filling pressure in animals pre-treated with dexamethasone or L-N^6-(1-iminoethyl)lysine when compared with saline pre-treatment (Fig. 4B,C). Administra-

tion of noradrenaline at 0.1 and 0.3 μg kg^{-1} min^{-1} significantly reduced venous resistance in animals pre-treated with L-N^6-(1-iminoethyl)lysine when compared with saline pre-treatment (Fig. 5A,B). An infusion of noradrenaline at 1.0 μg kg^{-1} min^{-1} did not significantly influence venous resistance in L-N^6-(1-iminoethyl)lysine pre-treated animals when compared with saline pre-treatment (Fig. 5C). In dexamethasone pre-treated animals, only the infusion of the lowest dose of noradrenaline significantly reduced venous resistance in comparison to saline pre-treatment (Fig. 5A).

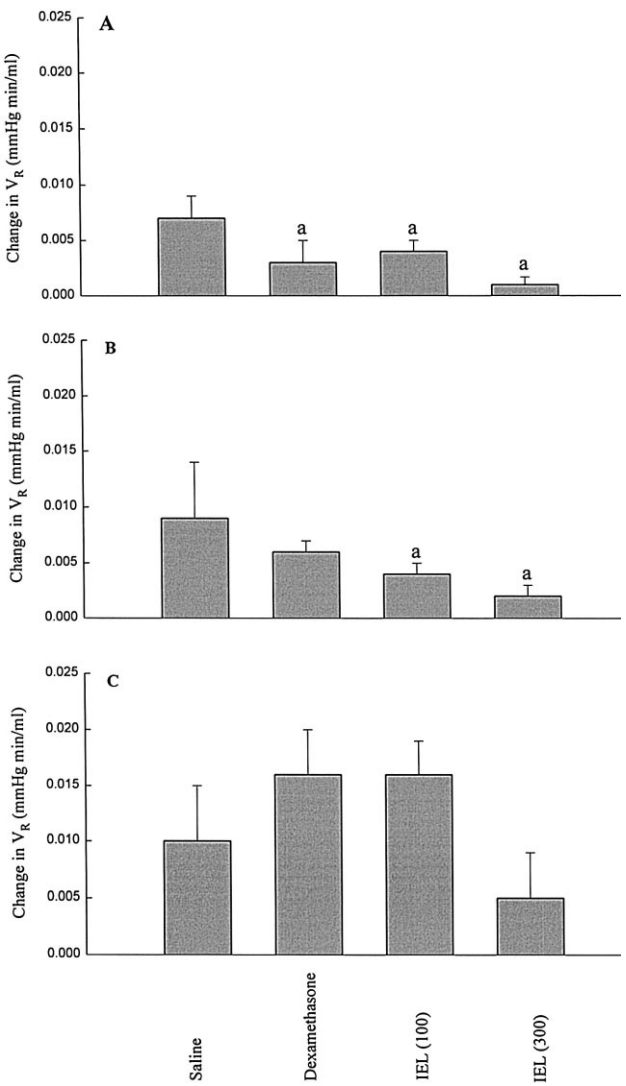

Fig. 5. Changes in venous resistance (V_R) post-haemorrhage during infusion of noradrenaline (A) (0.1 μg kg^{-1} min^{-1}), (B) (0.3 μg kg^{-1} min^{-1}) and (C) (1.0 μg kg^{-1} min^{-1}) in animals pretreated with saline (0.54 ml kg^{-1} (bolus) and 0.54 ml kg^{-1} h^{-1} (infusion)), dexamethasone (DEX; 5 mg kg^{-1}), and *l*-N^6-(1-iminoethyl)lysine (IEL; 100 μg kg^{-1} (bolus) and 100 μg kg^{-1} h^{-1} (infusion) and 300 μg kg^{-1} (bolus) and 300 μg kg^{-1} h^{-1} (infusion)). [a]Significantly different from saline group, $P < 0.05$.

4. Discussion

It is recognized that vascular responses are reduced during the refractory period after haemorrhage. Thiemermann et al. (1993a) had demonstrated that vascular hyporeactivity to the pressor actions of noradrenaline occurs following haemorrhagic shock. In addition, it was also reported that responses to noradrenaline and potassium in rat isolated aortic rings, obtained from animals subjected to haemorrhage, were reduced in comparison to controls. Moreover, it was demonstrated that vascular decompensation, resulting from prolonged periods of haemorrhage, was associated with induction of nitric oxide synthase (Thiemermann et al., 1993a). It has been speculated that an increase in plasma levels of tumor necrosis factor-α is the trigger responsible for the induction of inducible nitric oxide synthase. In short, it seems that the loss of vascular tone following haemorrhage is, in part, due to an increase in the levels of NO and that this increase in NO is attributed to an induction of nitric oxide synthase in the system (for review see Thiemermann, 1994). In the present investigation, it is apparent that responses to noradrenaline were augmented in the inducible nitric oxide synthase-inhibited hypovolemic state. The inhibition of inducible nitric oxide synthase in hypovolemic animals results in a greater effect by noradrenaline on cardiac output, blood pressure and venous tone. It is evident that the increase in cardiac output, following infusion of noradrenaline in L-N^6-(1-iminoethyl)lysine pre-treated animals, is the result of enhanced venous return. This suggests that an over-production of NO has an impact on venous circulation. This is not surprising since previous evidence in the literature had indicated that a certain degree of loss in venomotor function occurs in a state of haemorrhage (Alexander, 1955; Green, 1961). Our current findings support the view that reduced responsiveness of the venous circulation to noradrenaline during the hypovolemic state is, in part, due to induction of nitric oxide synthase resulting in an over-production of NO. Moreover, an enhanced reduction in resistance to venous return appears to be responsible for increased cardiac output during noradrenaline infusion following inducible nitric oxide synthase inhibition.

Certainly, the influence of nitric oxide on venous resistance is quite complex. Although more than one factor may account for the relaxant actions of endothelium-derived relaxing factor (Furchgott and Zawadzki, 1980), there is strong evidence that nitric oxide accounts for its main biological actions (Palmer et al., 1987). Current evidence in the literature tends to support the view that endothelium-dependent relaxation is greater in arteries than veins (De Mey and Vanhoutte, 1982; Vanhoutte and Miller, 1985; Seidel and LaRochelle, 1987). In normovolemic animals, N^G-monomethyl-L-arginine, an inhibitor of nitric oxide synthase, has been reported to increase venous resistance, thereby suggesting a role for spontaneously released NO (Ekelund and Mellander, 1990). In addition, N^G-

monomethyl-L-arginine has been reported to cause a small increase in mean circulatory filling pressure in rats (Glick et al., 1993). Administration of another nitric oxide synthase inhibitor, N^G-nitro-L-arginine, was reported to significantly increase central venous pressure, thus suggesting that endothelium derived relaxing factor contributed substantially to the control of larger veins (Schwarzacher et al., 1992). Furthermore, inhibition of nitric oxide synthase by N^G-nitro-L-arginine, in normovolemic animals, has been found to increase resistance to venous return and decrease cardiac output without changing mean circulatory filling pressure (Wang et al., 1995). It would appear that a reduction in cardiac output, after the administration of nitric oxide synthase inhibitor in normovolemic animals, is a result of an increase in vascular impedance (Bower and Law, 1993; Wang et al., 1995). Taken together, available evidence in the current literature indicates that spontaneously released NO in normovolemic animals plays a relatively modest role in the maintenance of venous tone.

The influence of vasoactive agents on the cardiovascular system in the hypovolemic state is complex. The venous circulation contains approximately 75% of blood volume (for review see Pang, 1994) and it is well recognized that venous circulation plays a critical role in the regulation of cardiac output (for review see Greenway, 1982; Rothe, 1993). Therefore, it is not surprising that a reduction in vascular capacitance after haemorrhage results in a reduction in cardiac output and, thus, blood pressure. However, it is quite evident that the loss of blood volume is not the only factor contributing to a reduction in cardiac output over time. Previous evidence in the literature has indicated that the deleterious effects of prolonged haemorrhage on the pre-capillary resistance vessels are quite severe. Moreover, the segment of blood vessels which are least sensitive to the effects of haemorrhage were reported to be the post-capillary resistance vessels (Mellander and Lewis, 1963). In a state of haemorrhage, there is more venoconstriction than arteriole constriction. This results in an increase in mean capillary hydrostatic pressure and net outward movement of intravascular fluid (Mellander and Lewis, 1963; Nickerson and Sutter, 1964). Therefore, in a state of oligemia, infusion of vasoconstrictor agents may result in either no change or a reduction in cardiac output due to loss of intravascular fluid. In the present study, we believe that a balance between the actions of noradrenaline on arterial and venous blood vessels was responsible for the observed increase in cardiac output after the inhibition of inducible nitric oxide synthase. Alternatively, the greater effect of noradrenaline on veins versus arteries in the systemic circulation of hypovolemic animals may be explained by the fact that NO may have a limited role in reducing venous tone. However, it is also possible that infusion of noradrenaline following haemorrhage in saline-treated animals resulted in loss of intravascular fluid leading to reduction in cardiac output. Indeed, it has been reported that plasma leakage occurs after administration of lipopolysaccharide and subsequent to induction of nitric oxide synthase (Bernareggi et al., 1997; Filep et al., 1997).

The infusion of noradrenaline in L-N^6-(1-iminoethyl)lysine treated animals produced a dose-dependent increase in cardiac output. This was not the case in animals that were pre-treated with dexamethasone. The differential effect of dexamethasone versus L-N^6-(1-iminoethyl)lysine on cardiac output is most likely the result of additional cardiovascular effects of dexamethasone. Glucocorticoids are known to be important in the maintenance of vascular tone and cardiac function (Baxter and Rousseau, 1979). For example, reduced pressor responses to catecholamines have been reported in adrenalectomized dogs (Leffer and Sutfin, 1964), and the 'permissive' actions of glucocorticoids in response to noradrenaline have been described (Granner, 1979). Here, in the present study, a lack of increase in cardiac output, following the highest administered dose of noradrenaline in dexamethasone treated rats, is best explained by the greater increase in vascular impedance (afterload) by the infusion of catecholamine. We have previously reported that cardiac output can be impaired in anaesthetized rats by an increase in vascular impedance following continuous infusion with phenylephrine (Nekooeian and Tabrizchi, 1996).

In summary, the present investigation indicates that hypovolemic haemorrhage results in induction of nitric oxide synthase and that L-N^6-(1-iminoethyl)lysine- and dexamethasone-treatment results in selective inhibition of inducible nitric oxide synthase. In the state of hypovolemia, infusion of noradrenaline produces a greater increase in cardiac output, blood pressure and venous tone following inhibition of inducible nitric oxide synthase.

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