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Short communication

N-Nitro-L-arginine methyl ester blocks the decompensatory phase of acute hypovolaemia in conscious rabbits by a brainstem mechanism

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Abstract

Graded caval occlusion in conscious rabbits caused a biphasic response. Phase I was characterized by a fall in conductance so that arterial pressure was maintained. When cardiac output had fallen to 71 ± 4% of its baseline level, phase II supervened. During phase II, conductance rose abruptly and arterial pressure fell to a life threatening level (< 40 mm Hg). When administered into the fourth ventricle, the nitric oxide synthase inhibitor *N*-nitro-L-arginine methyl ester prevented the onset of phase II. The mean threshold dose for this effect was 4 μmol (range: 0.4–11). When administered intravenously, a dose of 275 μmol *N*-nitro-L-arginine methyl ester prevented the onset of phase II in only one out of six rabbits. It is concluded that a central brainstem nitrergic mechanism is involved in the onset of the decompensatory phase II of the haemodynamic response to hypovolaemia.

Keywords: Hypovolemia; Hemorrhagic shock; Nitric oxide (NO); *N*-Nitro-L-arginine methyl ester; (Rabbit, conscious)

1. Introduction

The cardiovascular response to acute haemorrhage in unanaesthetized rabbits consists of two phases. Initially, systemic vascular conductance falls as blood volume and cardiac output fall, so that arterial pressure is well maintained (Schadt et al., 1984; Ludbrook and Rutter, 1988). This compensatory vasoconstriction is attributable to the action of the arterial baroreceptor reflex (Ludbrook and Graham, 1984; Schadt and Gaddis, 1986). If acute blood loss exceeds 30% of blood volume the compensatory vasoconstriction fails and blood pressure falls abruptly (Schadt et al., 1984; Ludbrook and Rutter, 1988). A similar, biphasic response occurs when central hypovolaemia is produced by graded inflation of a cuff on the inferior vena cava (Ludbrook et al., 1988; Evans et al., 1989).

Nitric oxide production has been proposed to play an important role in the pathophysiology of haemorrhagic shock (Zingarelli et al., 1992; Thiemermann et al., 1993). This was based on the effects of *N*-nitro-L-arginine methyl ester, a selective inhibitor of nitric oxide production from L-arginine, injected intravenously into rats subjected to experimental haemorrhagic shock. *N*-Nitro-L-arginine methyl ester increased survival rate and time and improved blood pressure. This reversal of haemorrhagic shock could be prevented if the dose of *N*-nitro-L-arginine methyl ester was followed by administration of a bolus dose of L-arginine (Zingarelli et al., 1992). L-Arginine, given on its own, enhanced the effects caused by haemorrhage. Central production of nitric oxide may also play a role in haemorrhagic shock since nitric oxide production has been shown to increase in the brain of rats following hypotensive haemorrhage, and this effect can be inhibited by intravenous administration of *N*-nitro-L-arginine methyl ester (Sato et al., 1993).

The experiments described here were aimed at investigating whether nitric oxide is involved in the mediation of the decompensatory phase of acute central hypovolaemia in conscious rabbits.

2. Materials and methods

Eight New Zealand White rabbits were used, weighing 2.25–2.75 (mean 2.52) kg. The experiments were

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done in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990), and were approved in advance by the Animal Ethics Committee of the Royal Melbourne Hospital.

2.1. Surgical procedures

Major procedures

These were performed under halothane anaesthesia after induction with i.v. thiopentone sodium (25 mg/kg) and endotracheal intubation. At a first operation, an inflatable cuff was placed around the thoracic inferior vena cava (caval cuff). Two weeks later, an ultrasonic (transit time) flow probe (Transonic Systems, Ithaca, NY, USA; Type 6S) was placed extrapericardially around the ascending aorta. Two weeks later, a 0.3 mm o.d. polyvinyl chloride tube (Dural Plastics SV10) was introduced through the atlanto-occipital membrane so that its tip lay in the fourth ventricle. Its dead space of 18 μl was filled with 154 mM NaCl.

Minor procedures on study days

These were done under local anaesthesia with 0.5% lignocaine HCl. The rabbit was placed in a $15 \times 17 \times 40$ cm box fitted with a wire mesh lid, 180 min before the beginning of the study. The tubes leading to the caval cuff and fourth ventricular catheter (when used) and the connecting plug for the flow probe were retrieved from their subcutaneous positions. A catheter was inserted into a central ear artery and advanced to the route of the ear, for measuring arterial pressure. For intravenous drug administration a catheter was inserted into a marginal ear vein.

2.2. Haemodynamic variables

Arterial pressure was measured by connecting the ear artery catheter to a Stratham P23Dc transducer which was placed at heart level, 50 mm above the floor of the rabbit's box. The flow probe was connected to a flowmeter (Transonic Systems, Ithaca, NY, USA; Model T206) to measure ascending aortic flow (cardiac output). Heart rate was measured by a tachometer that was actuated by the flow pulse.

The signals were amplified and recorded on a Grass Model 7 Polygraph, and sent to an Olivetti M24 computer equipped with an A-D converter which provided 10 s mean values for arterial pressure (mm Hg), heart rate (beats/min) and cardiac output (ml/min). The computer also calculated 10 s means for cardiac index (cardiac output/body weight in kg) and systemic vascular conductance index (100 × cardiac index/mean arterial pressure).

2.3. Graded caval occlusion

Central hypovolaemia was produced by using a micrometer-driven syringe to gradually inflate the caval cuff so that cardiac index fell at a constant rate of $8.5 \pm 0.3\%$ of its baseline level per minute. This corresponds approximately to blood loss at a rate of 7% of blood volume per minute (Ludbrook et al., 1988). The caval cuff was deflated when mean arterial pressure had fallen to ≤ 40 mm Hg, or after 8 min of cuff inflation when cardiac index had fallen to $\sim 34\%$ of its baseline level, whichever occurred first.

2.4. Drugs

The drug used was the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester hydrochloride (Research Biochemicals). *N*-Nitro-L-arginine methyl ester was dissolved and diluted to the required concentrations in sterile 154 mM NaCl.

2.5. Experimental protocol

The aim was to determine the critical (threshold) dose of *N*-nitro-L-arginine methyl ester which prevented circulatory decompensation during graded caval occlusion, when administered either centrally or intravenously. Loading doses of *N*-nitro-L-arginine methyl ester were injected into the fourth ventricle in a volume of 15 μl over 1 min, 10 min before the commencement of the caval cuff inflation. This was followed by a slow infusion at 0.75 μl/min until the caval cuff was deflated. Intravenous *N*-nitro-L-arginine methyl ester was given as an initial loading dose in 0.2–0.3 ml over 1 min followed by a slow infusion at 5% of the initial rate. *N*-Nitro-L-arginine methyl ester was given either intravenously or into the fourth ventricle on different days.

The first graded caval occlusion in each study was performed after either fourth ventricular or intravenous administration of saline. Graded caval occlusions were then repeated at 90 min intervals after doses of *N*-nitro-L-arginine methyl ester that ascended in half-logarithmic units until a threshold (critical) dose was reached. No more than six caval cuff inflations were performed on any one experimental day.

2.6. Analysis of results

The levels of the haemodynamic variables were compared by analysis of variance (ANOVA). The Dunn-Šidák correction (Ludbrook, 1991) was applied when multiple contrasts were made. Levels of haemodynamic variables are expressed as between-rabbit mean $\pm$ 1 S.E.M.

The critical doses were logarithmically transformed to calculate between-rabbit geometric means, on the assumption that the dose-response relationship is distributed lognormally.

3. Results

Prior to the injection of saline vehicle across all study days the baseline mean arterial pressure was 77 ± 2 mm Hg; heart rate was 240 ± 7 beats/min and cardiac index was 162 ± 9 ml/min. These were within the normal range for our laboratory (Evans et al., 1989). Injection of saline by either route did not affect the level of any of the haemodynamic variables ($P \geq 0.77$). Intravenous infusion of *N*-nitro-L-arginine methyl ester (100 μmol/kg) produced a consistent rise in mean arterial pressure and falls in heart rate, cardiac index and systemic vascular conductance (Table 1). Fourth ventricular injection of critical doses of *N*-nitro-L-arginine methyl ester produced a consistent rise in mean arterial pressure (see Table 1) and inconsistent falls in heart rate, cardiac index and systemic vascular conductance ($P \geq 0.069$, see Table 1).

After treatment with saline vehicle, the haemodynamic response to graded caval occlusion was biphasic. During the initial compensatory phase I, systemic vascular conductance fell at 4.7 units/min, heart rate rose at 7.2 beats/min and mean arterial pressure fell at 1.4 mm Hg/min. The decompensatory phase II began when cardiac index had fallen to 71 ± 4% of its pre-treatment level, at which point systemic vascular conductance index rose abruptly and mean arterial pressure fell precipitately. The caval cuff was deflated when mean arterial pressure had fallen to 33 ± 1 mm Hg, at which point cardiac index was 64 ± 5% of its baseline value.

Table 1
Haemodynamic variables after centrally and intravenously administered critical doses of *N*-nitro-L-arginine methyl ester (L-NAME) or saline

	Intravenous		Fourth ventricular	
	Saline	L-NAME	Saline	L-NAME
Baseline				
MAP [a]	77 ± 2	92 ± 3 [gg]	77 ± 3	88 ± 5 [g]
HR [b]	244 ± 13	187 ± 4 [gg]	231 ± 5	187 ± 9
CI [c]	159 ± 9	112 ± 11 [gg]	136 ± 9	120 ± 11
SVCI [d]	209 ± 13	121 ± 14 [gg]	178 ± 11	141 ± 19
Phase II				
ABSCI [e]	113 ± 10	70 ± 11 [gg]	99 ± 5	40 ± 4 [gg]
PERCI [f]	71 ± 5	64 ± 9	71 ± 6	36 ± 5 [gg]
Critical dose (μmol)				
Mean	–	> 275	–	3.6
Range	–	–	–	(0.4–11.1)

[a] MAP = mean arterial pressure (mm Hg), [b] HR = heart rate (beats/min), [c] CI = cardiac index (ml/min/kg), [d] SVCI = systemic vascular conductance index (100 × CI/MAP), [e] ABSCI = cardiac index at the time of onset of the decompensatory phase II of caval cuff inflation, [f] PERCI = percentage of baseline cardiac index at the time of onset of the decompensatory phase II of caval cuff inflation. Pairwise contrasts between L-NAME and the saline vehicle were made by ANOVA applying the Dunn-Šidák correction for multiple contrasts. [g] $P \leq 0.05$, [gg] $P \leq 0.01$. Values for i.v. administration are the means ± S.E.M. from six experiments. Values for fourth ventricular administration are the means ± S.E.M. from five experiments.

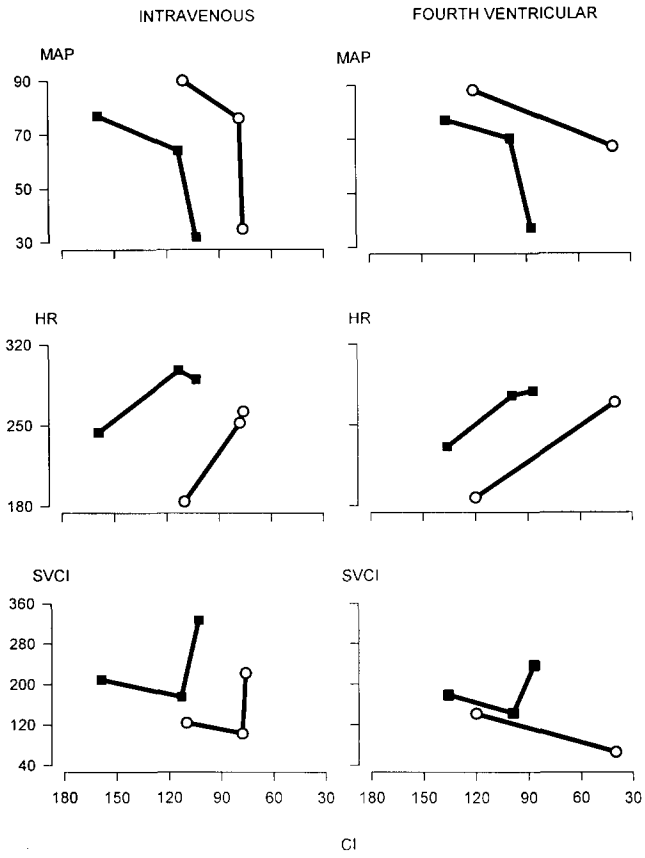

Fig. 1. Haemodynamic changes during graded caval occlusion after intravenous or fourth ventricular administration of saline or *N*-nitro-L-arginine methyl ester. CI = cardiac index (ml/min/kg), MAP = mean arterial pressure (mm Hg), HR = heart rate (beats/min) and SVCI = systemic vascular conductance (100 × cardiac index/mean arterial pressure). Lines join average coordinates for five to six rabbits. The treatments were: saline (■), fourth ventricular critical dose (mean: 3.6 μmol, range: 0.4–11.1) of *N*-nitro-L-arginine methyl ester (○), intravenous administration of *N*-nitro-L-arginine methyl ester (100 μmol/kg) (○). Values for the solitary rabbit in which phase II did not occur following intravenous administration of *N*-nitro-L-arginine methyl ester (100 μmol/kg) are not shown.

Despite the marked effects of intravenous *N*-nitro-L-arginine methyl ester on the baseline haemodynamic variables, the rates of change mean arterial pressure, heart rate and systemic vascular conductance index during phase I of the haemodynamic response to graded caval occlusion were unaffected by intravenous administration of *N*-nitro-L-arginine methyl ester (100 μmol/kg) ($P \geq 0.95$) (see Fig. 1). Phase II of the

haemodynamic response to graded caval occlusion occurred in five out of six rabbits. Although the absolute cardiac index at the time of onset of phase II was consistently lower than following intravenous saline infusion, when expressed as a percentage of its baseline level the cardiac indices at the time of onset of phase II were not different (see Table 1). In one rabbit, intravenous infusion of *N*-nitro-L-arginine methyl ester (100 μmol/kg) prevented phase II of the haemodynamic response to graded caval occlusion, so that throughout graded caval occlusion, there was a steady fall of systemic vascular conductance index and rise in heart rate, with only a small fall of mean arterial pressure (Fig. 1).

Following fourth ventricular administration of *N*-nitro-L-arginine methyl ester the rates of change of the haemodynamic variables were not different from those during phase I of graded caval occlusion following fourth ventricular injection of saline ($P \geq 0.99$ in all cases) (see Fig. 1). However, phase II of the haemodynamic response to graded caval occlusion was abolished in all rabbits, so that throughout graded caval occlusion there was a steady fall of systemic vascular conductance index and rise of heart rate, with only a small fall of mean arterial pressure (Fig. 1). When the caval cuff was deflated, mean arterial pressure was 67 ± 9 mm Hg, at which point cardiac index averaged $33 \pm 3\%$ of its baseline level. The mean critical dose for this phase II blocking effect was 3.6 μmol (range: 0.4–11).

4. Discussion

Others have shown that, in conscious rabbits, blockade of nitric oxide formation by intravenous *N*-nitro-L-arginine lowers hindlimb vascular conductance and heart rate and elevates arterial pressure (Du et al., 1991; Ward and Angus, 1993). Our results show that intravenous *N*-nitro-L-arginine methyl ester's vasoconstrictor action is widespread since it markedly lowered overall vascular conductance (Table 1). It has also been shown in conscious rabbits that intravenous *N*-nitro-L-arginine methyl ester has the added effect of lowering renal sympathetic nerve activity and heart rate while raising arterial pressure (Hasser et al., 1994). This implies that intravenous blockade of nitric oxide production has a direct vasoconstrictor effect, and the rise in arterial pressure causes a baroreflex fall in sympathetic nerve activity and heart rate.

Our more important finding was that *N*-nitro-L-arginine methyl ester is at least 75 times more potent at inhibiting the decompensatory phase II of the haemodynamic response to graded caval occlusion in conscious rabbits when administered into the fourth ventricle than when it is infused intravenously. This suggests that central, rather than peripheral, production of nitric oxide is involved in the onset of the decompensatory phase II of acute hypovolaemia. Indeed the maximum intravenous dose of 100 μmol/kg prevented phase II in only one of six rabbits (Fig. 1). Although the observed fourth ventricular critical doses of *N*-nitro-L-arginine methyl ester covered a wide range, the highest fourth ventricular critical dose obtained (11.1 μmol) was still approximately 25 times less than the highest dose given intravenously, which of course failed to block phase II of the haemodynamic response to caval cuff inflation.

Fourth ventricular *N*-nitro-L-arginine methyl ester may have exerted its effect on phase II in one of two ways. Microinjection of the nitric oxide inhibitor *N*-monomethyl-L-arginine into the nucleus tractus solitarius of the anaesthetized rabbit has been shown to increase tonic sympathetic drive (Harada et al., 1993). This would render an incoming sympathoinhibitory signal less effective. The pressor effect of fourth ventricular *N*-nitro-L-arginine methyl ester that we observed (Table 1) is consistent with this explanation. Alternatively, *N*-nitro-L-arginine methyl ester may have acted directly to block the phasic sympathoinhibitory mechanism that results in the sudden haemodynamic events of phase II.

Drugs which prevent or reverse the decompensatory phase of haemorrhage might be useful in clinical practice. However *N*-nitro-L-arginine methyl ester would appear to have little therapeutic value since its fourth ventricular effects could not be reproduced by intravenous administration. Moreover the peripheral vasoconstrictor action of intravenous *N*-nitro-L-arginine methyl ester might in fact prove harmful in the treatment of haemorrhagic shock, given that it decreases tissue perfusion. Adrenocorticotropic hormone-(1–24) offers a more attractive alternative, as it prevents the occurrence of phase II during hypovolaemia by a brainstem action, as well as increasing vascular conductance and cardiac output, thereby increasing tissue perfusion, by a peripheral action (Ludbrook and Ventura, 1994).

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