ELSEVIER

European Journal of Pharmacology 294 (1995) 369–375

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L-Arginine reverses the abolition of hypovolaemic decompensation by *N*-nitro-L-arginine methyl ester and naloxone in conscious rabbits

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Received 1 June 1995; revised 22 August 1995; accepted 29 August 1995

Abstract

Graded caval occlusion in conscious rabbits caused a biphasic haemodynamic response. Phase I was characterized by a fall in systemic vascular conductance so that arterial pressure was maintained. When cardiac output had fallen to $65 \pm 2\%$ 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). Fourth ventricular administration of either *N*-nitro-L-arginine methyl ester or naloxone prevented the occurrence of phase II. Fourth ventricular administration of L-arginine had no effect on the response to graded caval occlusion but was able to reverse the phase II blocking action of *N*-nitro-L-arginine methyl ester and naloxone. It is concluded that central nitrergic and opioid mechanisms interact to cause the vasodilatation characteristic of the decompensatory phase II of the cardiovascular response to acute hypovolaemia.

Keywords: Hypovolemia; *N*-Nitro-L-arginine methyl ester; L-Arginine; Naloxone; Hemorrhagic shock; Nitric oxide (NO); (Conscious rabbit)

1. Introduction

The cardiovascular response to acute haemorrhage in conscious rabbits, as in all mammals, consists of two phases. In phase I, systemic vascular conductance falls pari passu with blood volume and cardiac output, so that arterial pressure is well maintained (Schadt et al., 1984; Ludbrook and Rutter, 1988). This compensatory vasoconstriction is chiefly attributable to the action of the arterial baroreceptor reflex (Ludbrook and Graham, 1984; Schadt and Gaddis, 1986). If acute blood loss exceeds about 30% of blood volume phase II commences abruptly. In phase II the compensatory vasoconstriction is superseded by decompensatory vasodilatation and blood pressure falls steeply (Schadt et al., 1984; Ludbrook and Rutter, 1988). A similar, biphasic response occurs when acute central hypovolaemia is produced by graded inflation of a cuff on the inferior vena cava (Ludbrook et al., 1988; Evans et al., 1989). In rabbits, phase II is associated with a dramatic fall in sympathetic vasoconstrictor drive (Burke and Dorward, 1988), and is dependent on a brainstem δ_1-opioid receptor mechanism (Ludbrook and Ventura, 1994).

We demonstrated recently that a central brainstem nitrergic mechanism is involved in the onset of the decompensatory phase II of the haemodynamic response to hypovolaemia (Ventura and Ludbrook, 1995). This was based on the effects of central administration of the selective inhibitor of nitric oxide production, *N*-nitro-L-arginine methyl ester, which prevented the occurrence of the decompensatory phase II of the haemodynamic response to acute hypovolaemia in conscious rabbits.

Recent research into the mechanisms and modes of action of central nitrergic mechanisms has revealed that central nitrergic and opioid mechanisms can interact to cause potentiating effects. For instance, the nitric oxide precursor L-arginine has been shown to increase morphine-induced changes in locomotion and food intake in mice (Calignano et al., 1993). L-Arginine can also accelerate tolerance to opioid analgesia in the mouse, and this accelerating effect of L-arginine can be

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SSDI 0014-2999(95)00555-2

prevented by the nitric oxide synthase inhibitor *N*-nitro-L-arginine (Babey et al., 1994). Similarly, β-endorphin induced antinociception in the mouse can be attenuated by nitric oxide synthase inhibitors, while activation of the nitric oxide system also potentiates the antinociception induced by β-endorphin (Yu Xu and Tseng, 1994). The opioid receptor agonist morphine has also been shown to decrease nitric oxide synthase activity in rat cerebral cortex homogenates (Barjavel and Bhargava, 1994).

The experiments described in this paper were primarily designed to investigate further the role of nitric oxide in the onset of the decompensatory phase II of acute hypovolaemia, by examining whether the nitric oxide precursor L-arginine is able to reverse the prevention of phase II induced by central administration of the nitric oxide synthase inhibitor *N*-nitro-L-arginine methyl ester. A secondary aim of these experiments was to determine whether there is any interaction between central nitrergic and opioid mechanisms during the haemodynamic response to acute hypovolaemia in the conscious rabbit.

2. Materials and methods

Seven New Zealand White rabbits were used, weighing 2.22–3.13 kg (mean 2.65 kg). The experiments were 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 intravenous 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. A 0.3 mm o.d. polyvinylchloride tube (Dural Plastics SV10) was introduced 2 weeks later through the atlanto-occipital ligament 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 × 17 × 40 cm box filled with a wire mesh lid, 180 min before the beginning of the study. The tubes leading to the caval cuff and fourth ventricular catheter 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 root of the ear for measuring arterial pressure.

2.2. Haemodynamic variables

Arterial pressure was measured by connecting the ear artery catheter to a Viggo-Spectramed P23XL transducer which was placed at the level of the heart, 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 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 central hypovolaemia

The caval cuff was gradually inflated by a micrometer-driven syringe so that cardiac index fell at a constant rate of 8.2 ± 0.1% 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 when cardiac index had fallen to ~ 34% of its baseline level, whichever occurred first.

2.4. Drugs

The drugs used were: the nitric oxide synthase inhibitor *N*-nitro-L-arginine methyl ester hydrochloride (Research Biochemicals), the nitric oxide precursor L-arginine (Sigma), the δ_1-opioid receptor agonist [D-Pen^2,D-Pen^5]enkephalin (DPDPE) (Sigma) and the non-selective opioid receptor antagonist naloxone hydrochloride (Sigma). The drugs were dissolved and diluted to the required concentrations in sterile 154 mM NaCl.

2.5. Experimental protocol

Six different experimental protocols were performed. Only one protocol was performed on one rabbit on any given day, and rabbits were studied at intervals of 2–7 days. During each study caval cuff

inflation was repeated up to 5 times at intervals of 90 min. Loading doses of drugs were injected into the fourth ventricle in a volume of 15 μl over 1 min, 10 min before the commencement of caval cuff inflation. This was followed by a slow infusion of 0.75 μl/min until the caval cuff was deflated. In our extensive experience of fourth ventricular administration of drugs we have never observed any effect of administering vehicle at the same rate and volume as in this regimen.

Critical doses of N-nitro-L-arginine methyl ester and naloxone

The aim was to determine the critical (threshold) doses of *N*-nitro-L-arginine methyl ester (protocol 1, seven rabbits) or naloxone (protocol 2, three rabbits) which prevented circulatory decompensation during caval cuff inflation, when administered into the fourth ventricle. The first caval cuff inflation on each day was performed after fourth ventricular administration of saline. Caval cuff inflations were then repeated at 90 min intervals after doses of *N*-nitro-L-arginine methyl ester (0.1–11 μmol) or naloxone (1–100 nmol) ascending in half-logarithmic units until a critical dose was reached. The critical dose was taken as the dose at which phase II of the cardiovascular response to caval cuff inflation did not occur.

Effects of L-arginine

In these studies the effect of fourth ventricular saline administration on the response to caval cuff inflation was first tested, then 90 min later the effect of fourth ventricular administration of L-arginine (2 μmol) (protocol 3, four rabbits) on the response to caval cuff inflation was tested.

Interactive effects of L-arginine and DPDPE

In these studies the effect of fourth ventricular saline administration on the response to caval cuff inflation was first tested, then 90 min later the effect of fourth ventricular administration of either: L-arginine (2 μmol) in combination with the critical dose of *N*-nitro-L-arginine methyl ester (protocol 4, four rabbits), L-arginine (2 μmol) in combination with the critical dose of naloxone (protocol 5, three rabbits); or DPDPE (50 nmol) in combination with the critical dose of *N*-nitro-L-arginine methyl ester (protocol 6, four rabbits) was tested on the response to caval cuff inflation. We have previously shown that this dose of DPDPE (50 nmol) is capable of reversing the anti-shock actions of fourth ventricular administration of opioid antagonists (Evans et al., 1989).

2.6. Analysis of results

Haemodynamic variables before and during caval cuff inflation, and during saline infusion were compared with the corresponding variables during infusion of the various drug treatments by two-way analysis of variance (ANOVA), the two factors being drug treatment and rabbits. Levels of haemodynamic variables are tabulated as between-rabbit means ± 1 S.E.

The critical doses of the drugs were logarithmically transformed to calculate between rabbit geometric means with ranges in parentheses, on the assumption that the dose-response relationship is distributed log-normally.

Table 1
Haemodynamic variables after fourth ventricular administration of drugs or saline

	Saline [a]	L-Arginine 2 μmol	L-NAME [b] mean = 4.6 μmol range = 4.0–11	Naloxone mean = 45 nmol range = 30–100	L-NAME [b] mean = 4.6 μmol range = 4.0–11 + L-arginine 2 μmol	Naloxone mean = 45 nmol range = 30–100 + L-arginine 2 μmol	L-NAME [b] mean = 4.6 μmol range = 4.0–11 + DPDPE 50 nmol
		$n = 4$	$n = 7$	$n = 3$	$n = 4$	$n = 3$	$n = 4$
Baseline							
MAP [c]	79 ± 2	91 ± 2 [i]	88 ± 2 [i]	76 ± 3	73 ± 5	86 ± 5	109 ± 6 [i]
HR [d]	229 ± 4	232 ± 7	190 ± 10 [j]	205 ± 11	218 ± 12	237 ± 11	275 ± 8 [i]
CI [e]	176 ± 5	170 ± 17	156 ± 13 [i]	183 ± 22	181 ± 21	189 ± 14	169 ± 21
SVCI [f]	228 ± 10	187 ± 18	180 ± 17 [i]	241 ± 29	253 ± 38	224 ± 31	161 ± 28
Phase II							
ABSCI [g]	114 ± 5	98 ± 4	62 ± 6 [j]	76 ± 18 [i]	99 ± 13	116 ± 15	103 ± 24
PERCI [h]	65 ± 2	59 ± 4	40 ± 3 [j]	41 ± 9 [i]	55 ± 1	61 ± 4	59 ± 8

[a] Values for saline are the means ± S.E.M. for all 25 experiments in the seven rabbits. [b] L-NAME = *N*-nitro-L-arginine methyl ester, [c] MAP = mean arterial pressure (mm Hg), [d] HR = heart rate (beats/min), [e] CI = cardiac index (ml/min/kg), [f] SVCI = systemic vascular conductance index (100 × CI/MAP), [g] ABSCI = cardiac index at the time of onset of the decompensatory phase II of caval cuff inflation, [h] PERCI = percentage of baseline cardiac index at the time of onset of the decompensatory phase II of caval cuff inflation. Pairwise contrasts between drugs and the saline vehicle were made within two-way ANOVA. [i] $P \leq 0.05$, [j] $P \leq 0.005$.

3. Results

3.1. Effects of fourth ventricular saline and L-arginine

Resting haemodynamic variables

Before and after fourth ventricular administration of saline, the levels of the haemodynamic variables in all seven rabbits were within the normal range for our laboratory on all days on which studies were performed (Table 1) (Evans et al., 1989; Ludbrook and Ventura, 1994, 1995). Fourth ventricular administration of L-arginine (2 μmol) raised mean arterial pressure and inconsistently lowered systemic vascular conductance index. Heart rate and cardiac index were not affected.

Haemodynamic responses to caval cuff inflation

After saline injection the haemodynamic response to caval cuff inflation was biphasic (Fig. 1). During phase I, systemic vascular conductance fell steadily so that mean arterial pressure fell only slightly. Heart rate rose steadily during phase I. Phase II began when cardiac index had fallen to 65 ± 2% of its resting level, at which point systemic vascular conductance rose abruptly and mean arterial pressure fell precipitately (Fig. 1). The haemodynamic response to caval cuff inflation was not affected by fourth ventricular administration of L-arginine (2 μmol) (Fig. 1, Table 1).

3.2. Effects of N-nitro-L-arginine methyl ester and naloxone

Resting haemodynamic variables

As we have previously described (Ventura and Ludbrook, 1995) fourth ventricular administration of *N*-nitro-L-arginine methyl ester raised mean arterial pressure, while lowering heart rate, cardiac index and systemic vascular conductance index (Fig. 2, Table 1). Fourth ventricular administration of naloxone had no consistent effect on the resting haemodynamic variables (Fig. 2, Table 1).

Haemodynamic responses to caval cuff inflation

Fourth ventricular administration of *N*-nitro-L-arginine methyl ester and naloxone abolished the decompensatory phase II of the response to caval cuff inflation. That is, throughout the caval cuff inflation there was a steady fall in systemic vascular conductance and rise in heart rate, with only a small fall in mean arterial pressure (Fig. 2). The critical doses of fourth ventricular *N*-nitro-L-arginine methyl ester and naloxone needed to abolish phase II were 4.6 μmol (range: 4.0–11) and 45 nmol (range: 30–100) respectively.

3.3. Interactive effects of L-arginine with N-nitro-L-arginine methyl ester and naloxone

Resting haemodynamic variables

Fourth ventricular administration of L-arginine in combination with *N*-nitro-L-arginine methyl ester was able to abolish the effects of *N*-nitro-L-arginine methyl ester on the resting haemodynamic variables. Similarly, the acute effects of L-arginine were not seen when a combination of these drugs was used. That is, the

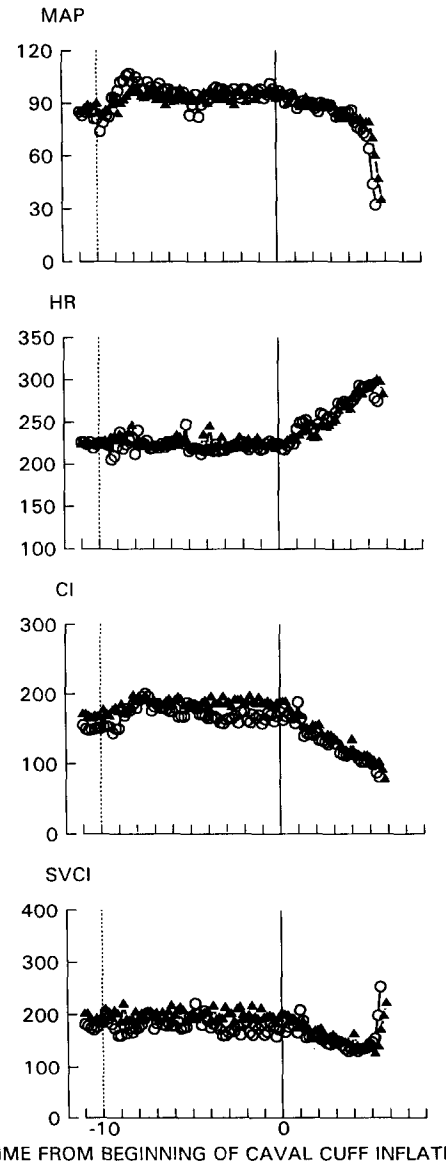

Fig. 1. Haemodynamic changes during graded caval occlusion after fourth ventricular administration of saline vehicle (○) or L-arginine (2 μmol) (▲) in one rabbit. MAP = mean arterial pressure (mm Hg), HR = heart rate (beats/min), CI = cardiac index (ml/min/kg) and SVCI = systemic vascular conductance index (100 × CI/MAP). Symbols represent the mean estimate over 10 s. Bolus infusions were made 10 min before the beginning of caval cuff inflation which was commenced at time = 0.

haemodynamic variables after fourth ventricular administration of a combination of these drugs were not different from those following fourth ventricular saline administration (Table 1). Similarly, fourth ventricular administration of L-arginine in combination with the critical dose of naloxone did not alter the resting haemodynamic variables (Table 1).

Haemodynamic responses to caval cuff inflation

Fourth ventricular administration of L-arginine (2 μmol) in combination with the critical dose of either *N*-nitro-L-arginine methyl ester or naloxone reversed the abolition of phase II caused by these drugs (Table 1, Fig. 2). When L-arginine was administered into the fourth ventricle together with either *N*-nitro-L-arginine methyl ester or naloxone, the haemodynamic response to caval cuff inflation was again biphasic. As cardiac index fell during phase I, systemic vascular conductance fell steadily until phase II began, at which point systemic vascular conductance rose abruptly and mean arterial pressure fell precipitately (Fig. 2).

3.4. Interactive effects of DPDPE

Resting haemodynamic variables

Fourth ventricular administration of DPDPE (50 nmol) in combination with the critical dose of *N*-nitro-

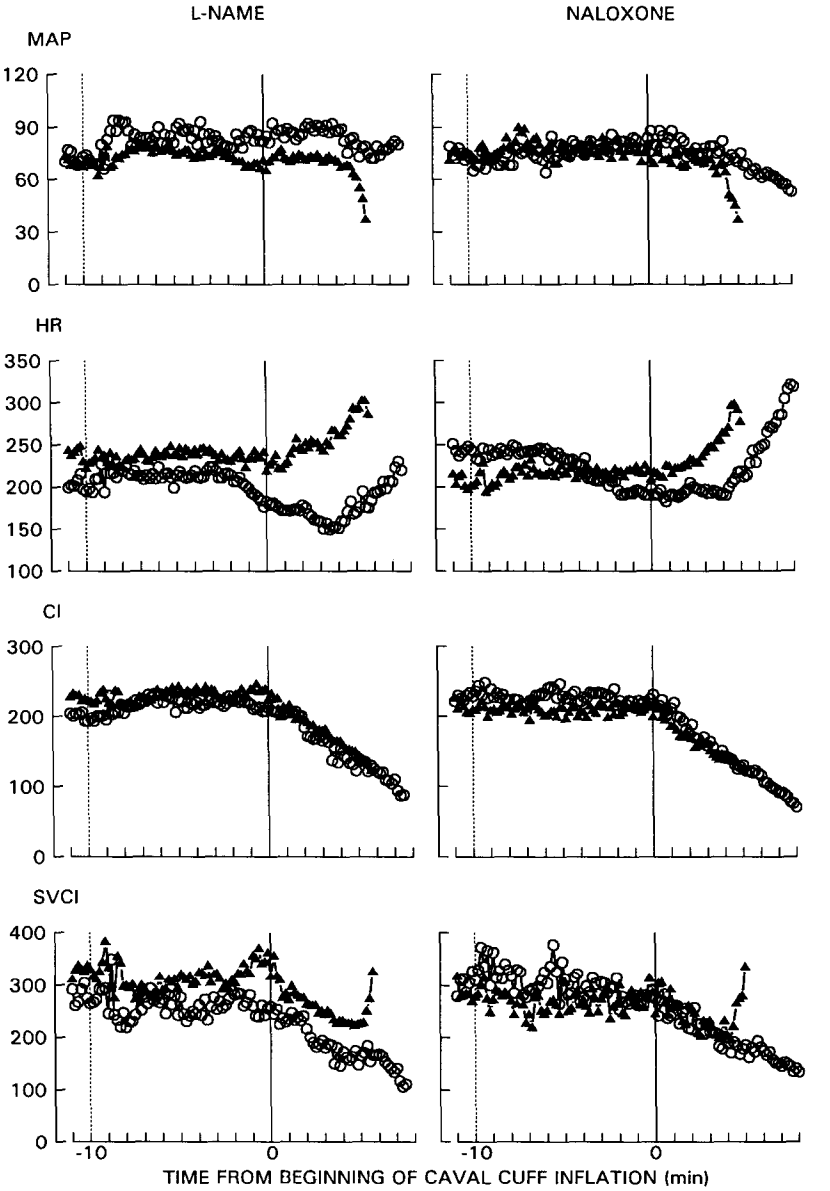

Fig. 2. Haemodynamic changes during graded caval occlusion after fourth ventricular administration of the critical dose of *N*-nitro-L-arginine methyl ester or naloxone (○) or the critical dose of *N*-nitro-L-arginine methyl ester or naloxone in combination with L-arginine (2 μmol) (▲) in one rabbit. In all rabbits the mean critical dose of fourth ventricular *N*-nitro-L-arginine methyl ester was 4.6 μmol (range: 4–11) and for naloxone was 45 nmol (range: 30–100). Symbols represent the mean estimate over 10 s. Bolus infusions were made 10 min before the beginning of caval cuff inflation which was commenced at time = 0. Abbreviated haemodynamic variables are the same as in Fig. 1.

L-arginine methyl ester caused large increases in mean arterial pressure and heart rate, and inconsistent falls in systemic vascular conductance. Cardiac index was unaffected (Table 1).

Haemodynamic responses to caval cuff inflation

During caval cuff inflation following fourth ventricular administration of both DPDPE (50 nmol) and the critical dose of *N*-nitro-L-arginine methyl ester, phase II of the biphasic response to caval cuff inflation occurred in two out of four rabbits. In the other two rabbits phase II did not occur, so that systemic vascular conductance fell steadily throughout the caval cuff inflation and mean arterial pressure was well maintained. The values shown in Table 1 for this experiment combine the results obtained from all four rabbits that underwent this protocol.

4. Discussion

Our experiments have resulted in two main findings. The first is that the nitric oxide precursor L-arginine given into the fourth ventricle is able to reverse the central anti-shock activity of the nitric oxide synthase inhibitor *N*-nitro-L-arginine methyl ester. The second is that the central nitrergic and opioid mechanisms involved in the onset of the decompensatory phase II of the haemodynamic response to acute hypovolaemia appear to interact with each other. A third finding of less importance was that not only did a combination of both L-arginine and *N*-nitro-L-arginine methyl ester abolish the fourth ventricular effects of *N*-nitro-L-arginine methyl ester but also the acute fourth ventricular effects of L-arginine. It is interesting that both L-arginine and *N*-nitro-L-arginine methyl ester when given centrally increase arterial pressure and decrease systemic vascular conductance and yet when administered together have no effect on haemodynamics. This suggests that perhaps L-arginine may have further actions than merely to act as a substrate for nitric oxide synthase and reverse the effects of *N*-nitro-L-arginine methyl ester.

We have confirmed our earlier observations that a central nitrergic pathway is involved in the onset of the decompensatory phase II of the response to hypovolaemia (Ventura and Ludbrook, 1995), by showing that the abolition of phase II by fourth ventricular administration of the nitric oxide synthase inhibitor *N*-nitro-L-arginine methyl ester can be overcome by simultaneous fourth ventricular administration of the nitric oxide precursor, L-arginine. The acute cardiovascular effects of central administration of *N*-nitro-L-arginine methyl ester on the resting haemodynamic variables were also seen to be abolished by L-arginine.

The observation that fourth ventricular administration of L-arginine could reverse the abolition of phase II caused by naloxone suggests that they are involved in the same pathway. Central opioid and nitrergic mechanisms have been observed to interact in rodents to affect a variety of systems (Calignano et al., 1993; Babey et al., 1994; Barjavel and Bhargava, 1994; Yu Xu and Tseng, 1994). In all of these experiments the central nitrergic and opioid systems under investigation produced potentiating effects. The central pathway which initiates the decompensatory phase II of the response to acute hypovolaemia may involve a similar system, by which nitric oxide causes the release of opioids in the brain or vice versa.

It would appear that nitric oxide released during the compensatory phase I of acute hypovolaemia may cause the release of opioids, since L-arginine was able to reverse the central anti-shock effects of naloxone. Injection of *N*-nitro-L-arginine methyl ester into the brain would stop the release of nitric oxide and therefore also the subsequent release of opioids. This could be reversed by injection of L-arginine which would replenish the supply of released nitric oxide. It seems less likely that opioids are releasing nitric oxide, since DPDPE could reverse the anti-shock activity of *N*-nitro-L-arginine methyl ester in only two out of four cases and we have previously shown that this dose of DPDPE is able to reverse the anti-shock effect of naloxone (Evans et al., 1989). In the two rabbits in which phase II was not abolished following administration of both DPDPE and *N*-nitro-L-arginine methyl ester, a small amount of nitric oxide may still have been released, which in turn released enough opioid to bring the opioid concentration to the level needed to set off phase II.

An alternate mode of action of nitric oxide in this pathway may be that it inhibits the release of adrenocorticotrophic hormone (ACTH). Fourth ventricular ACTH-(1–24) has also been shown to abolish the decompensatory phase II of acute hypovolaemia by a brainstem mechanism which also involves opioids (Ludbrook and Ventura, 1995). The nitric oxide donor sodium nitroprusside has been shown to inhibit corticotropin releasing hormone release and therefore also the release of ACTH in human placental syncytiotrophoblasts (Sun et al., 1994). It is, therefore, conceivable that nitric oxide may set off phase II by inhibiting the release of ACTH.

Although it is of great physiological significance that central nitrergic, opioid and perhaps ACTH mechanisms interact to cause decompensation during acute hypovolaemia, it seems unlikely that nitric oxide synthase inhibitors such as *N*-nitro-L-arginine methyl ester will be of therapeutic value in the treatment of haemorrhagic shock. A therapeutically advantageous drug would have to be able to be administered peripherally to be of any value. The fact that intravenously adminis-

tered *N*-nitro-L-arginine methyl ester has little or no effect on the time of onset of phase II of the response to acute hypovolaemia (Ventura and Ludbrook, 1995; Koch et al., 1995) makes this class of drugs poor candidates for use in the first-aid treatment of haemorrhagic shock.

Acknowledgements

The authors thank Giannina Joshua and Linda Cornthwaite for their excellent technical assistance. This work was supported by a grant from the National Health and Medical Research Council of Australia.

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