

## The effects of primary thoracic blast injury and morphine on the response to haemorrhage in the anaesthetised rat

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**Primary thoracic blast injury causes a triad of bradycardia, hypotension and apnoea mediated in part via a vagal reflex. Blast casualties may also suffer blood loss, and the response to progressive simple haemorrhage is biphasic: an initial tachycardia followed by a vagally mediated reflex bradycardia which can be attenuated by  $\mu$  opioid agonists. The aims of this study were to determine the effects of thoracic blast injury on the response to subsequent haemorrhage, and the effects of morphine, administered after blast, on the response to blood loss. Male Wistar rats, terminally anaesthetised with alphadolone–alphaxolone (19–21 mg kg<sup>-1</sup> h<sup>-1</sup> i.v.), were allocated randomly to one of three groups: Group I, sham blast; Group II, thoracic blast; Group III, thoracic blast plus morphine (0.5 mg kg<sup>-1</sup> i.v. given 5 min after blast). Blast (Groups II and III) resulted in significant ( $P < 0.05$ , ANOVA) bradycardia, hypotension and apnoea. Sham blast (Group I) had no effect. Ten minutes later, haemorrhage (40 % of the estimated total blood volume (BV)) in Group I produced a biphasic response comprising a tachycardia followed by a peak bradycardia after the loss of 33 % BV. Arterial blood pressure did not fall significantly until the loss of 13.3 % BV. In Group II the haemorrhage-induced tachycardia was absent and the bradycardia was augmented: peak bradycardia was seen after the loss of 23 % BV. Mean arterial blood pressure (MBP) began to fall as soon as haemorrhage commenced and was significant after the loss of 10 % BV. Morphine (Group III) prevented the haemorrhage-induced bradycardia and delayed the significant fall in MBP until the loss of 30 % BV. It is concluded that the response to thoracic blast injury augments the depressor response to haemorrhage while morphine attenuates this response. *Experimental Physiology* (2002) 87.6, 683–689.**

Blast weapons are a threat to both military and civilian populations (Cooper *et al.* 1983). Blast produces a spectrum of injuries ranging from the direct effects of the blast wave (primary blast injury), occurring predominantly at gas-containing organs, to the effects of debris colliding with the body and gross displacement of the victim causing injury to many body systems (secondary and tertiary blast injuries, respectively, Cooper *et al.* 1983).

Primary blast injury to the thorax produces bradycardia, hypotension and apnoea (Krohn *et al.* 1942; Guy *et al.* 1998). This is a reflex response involving the vagus nerve (Irwin *et al.* 1999; Ohnishi *et al.* 2001). The bradycardia can be abolished by vagotomy and reduced by 94 % by pretreatment with atropine (Ohnishi *et al.* 2001). The apnoea is also entirely a vagal reflex as it is abolished by vagotomy (Ohnishi *et al.* 2001). The hypotension is partly a vagal reflex as the fall in blood pressure is reduced by 42 % in vagotomised animals (Ohnishi *et al.* 2001).

However, impaired myocardial performance (Wikoff *et al.* 1999) and reduced peripheral vascular resistance (E. Kirkman & M. Sawdon, unpublished observations) seen during the hypotensive phase after blast injury are likely to contribute to the fall in blood pressure. Although the reflex mediating the response to thoracic blast has not been fully characterised, the response has many similarities to that induced by activation of the pulmonary afferent C-fibres (Daly & Kirkman, 1988). In addition, the persistence of hypotension and bradycardia following blast injury suggests that there may also be a modulation of the arterial baroreceptor reflex (Ohnishi *et al.* 2001).

Blast-injured casualties will often sustain haemorrhage as a consequence of their injuries (Cooper *et al.* 1983). The pattern of physiological responses to progressive simple haemorrhage (blood loss in the absence of tissue damage and nociception) is biphasic (Barcroft *et al.* 1944), with an initial tachycardia (due to sympathoexcitation and vagal

inhibition) and maintenance of blood pressure via the arterial baroreceptor reflex (Secher & Bie, 1985; Little *et al.* 1989). As haemorrhage progresses, and blood loss exceeds 20–30% of total blood volume, a depressor phase becomes apparent. This involves a vagally mediated bradycardia (inhibited by atropine, Little *et al.* 1989), a reduction in peripheral vascular resistance (Barcroft *et al.* 1944; Evans & Ludbrook, 1991) and a marked fall in arterial blood pressure. This second phase is not due to a failure of the baroreflex, since the latter's sensitivity is increased at this stage (Little *et al.* 1984), nor is it a preterminal event (Hoffman, 1972; Sander-Jensen *et al.* 1986), but rather it is due to the activation of additional reflex(es). The identity of the afferent limbs of these reflexes is currently uncertain (Scherrer *et al.* 1990; Shen *et al.* 1990; Kirkman *et al.* 1994), although the cardiac afferent C-fibres may be involved (see Evans *et al.* 2001).

It is recognised that concomitant injury may attenuate the depressor response to haemorrhage. The response to musculo-skeletal injury reduces or abolishes the bradycardia and delays the onset of hypotension (Little *et al.* 1989). However, the interaction, if any, of blast injury with haemorrhage is unknown. Since a blast casualty may also suffer from haemorrhage, it was important to determine whether the physiological response to thoracic blast could modify that to subsequent haemorrhage.

Blast casualties may also receive morphine as part of their clinical treatment. Morphine modifies several vagally mediated reflexes including the bradycardia induced by severe haemorrhage (Ohnishi *et al.* 1997). By contrast, morphine has no effect on the vagally mediated bradycardia induced by primary thoracic blast, but augments the apnoea and delays the recovery of blood pressure (Ohnishi *et al.* 1999). The effect of morphine when administered following primary thoracic blast is unknown.

The aims of this study were to determine the effect of thoracic blast injury on the response to subsequent haemorrhage and assess the effects of morphine on the combined response to blast and haemorrhage.

## METHODS

Male Wistar rats (Harlan Olac, Bicester, UK; body weight range, 265–337 g) were used. They were maintained on a 12 h–12 h light–dark cycle, fed on Beekay standard rat and mouse diet (B & K Universal Ltd, UK) and allowed access to food and water *ad libitum*.

### Surgical preparation and physiological measurements

Anaesthesia was induced with isoflurane by inhalation (Abbott Laboratories Ltd, UK; 3.5% in O<sub>2</sub>–N<sub>2</sub>O; inspired O<sub>2</sub> fraction ( $F_{i,O_2}$ ) 0.5) in an anaesthetic chamber and subsequently maintained by delivery of isoflurane (2.5–3.5%) via a co-axial anaesthetic system and face cone (Fluovac, International Market Supplies, UK). Once surgical anaesthesia had been attained, a cannula (2FG, Portex Ltd, UK) was inserted into the ventral tail artery and advanced until its tip lay in the abdominal aorta. Arterial blood pressure was monitored via this cannula using a strain gauge

manometer (Sensoron 840, SensoNor a.s., Norway). Both lateral tail veins were cannulated (2FG, Portex Ltd) for drug administration. All cannulae were initially filled with heparinised saline (20 i.u. ml<sup>-1</sup> heparin, Monoparin, CP Pharmaceuticals, UK, in 0.9% saline). The electrocardiogram was recorded using needle electrodes placed in the skin of the ventrum, and heart period was measured from the electrocardiogram. All physiological variables were amplified and recorded using a computerised data acquisition system (MacLab 8s, ADInstruments, UK). Body temperature was monitored using a rectal thermistor (Medical Precision Thermometer; Ellab Copenhagen DM 852) and maintained at 38.0 ± 0.1 °C (mean ± s.d.) throughout the study using a thermally insulated operating mat and a heating lamp.

### Blast wave generator

The blast wave generator has previously been described (Jaffin *et al.* 1987). Briefly, compressed air at a pressure of approximately 1500 p.s.i. was directed by a solenoid to a 0.55 mm thick aluminium bursting disc mounted in a nozzle. The blast wave left the device through a 20 mm (internal diameter) nozzle, directed at the animal lying below. Animals were positioned supine under the blast apparatus, with the blast nozzle 3.5 cm above the ventral surface of the thorax. Those subjected to sham blast were positioned 10 cm from the blast apparatus, outside of the zone of the blast wave.

### Experimental protocol

Following the surgical preparation, the administration of isoflurane was discontinued and anaesthesia was maintained with alphadolone–alphaxalone (Saffan, Pitman-Moore, UK, 19–21 mg kg<sup>-1</sup> h<sup>-1</sup> i.v.) using an infusion pump (Harvard 22, Harvard Apparatus Ltd, UK) while the animals breathed air. Rats were allowed to stabilise for 60 min before baseline measurements of heart period and blood pressure were made. Animals were then allocated randomly to one of three groups: Group I ( $n = 8$ ), sham blast, saline and haemorrhage; Group II ( $n = 8$ ), thoracic blast, saline and haemorrhage; Group III ( $n = 5$ ), thoracic blast, morphine and haemorrhage.

Cardiovascular measurements were made continuously for 5 min commencing immediately prior to blast (or sham blast), whilst respiratory movements were determined visually. Five minutes after blast (or sham blast) animals received either 0.9% saline (1 ml kg<sup>-1</sup>; Groups I and II) or morphine (0.5 mg kg<sup>-1</sup> in 1 ml kg<sup>-1</sup> 0.9% saline; Group III) intravenously. After a further 5 min cardiovascular measurements were repeated in all groups. Arterial blood was then withdrawn anaerobically into heparinised syringes from the ventral tail artery. Blood was withdrawn in 12 equal aliquots, each over a 100 s cycle, giving an overall haemorrhage rate of 2% total estimated blood volume (TBV = 6.06 ml (100 g body weight)<sup>-1</sup>; Elebute & Little, 1978) per minute, which resulted in a loss of 40% BV. Cardiovascular measurements were repeated after the withdrawal of each aliquot of blood, and each blood sample was analysed using a blood gas analyser (ABL5, Radiometer, Denmark).

At the end of the experiment the animals were killed with an overdose of sodium pentobarbitone administered intravenously. Post-mortem examination showed that blast had not caused any obvious skin damage. The chest was then opened and the extent of intra-thoracic damage assessed visually. There were areas of contusion on the lungs of all rats exposed to blast, the right mid and inferior lobes being affected the greatest. There were no consistent differences between groups of animals exposed to blast. There were no contusions on the lungs of rats exposed to sham blast. There was no evidence of intra-thoracic or intra-abdominal haemorrhage in any of the groups.

### Statistical analysis

Data are presented as mean  $\pm$  S.E.M., unless indicated otherwise. Statistical analyses were conducted using two-way analysis of variance for repeated measures (time) (SPSSPC + v4.01) followed, where appropriate, by Tukey's *post hoc* test unless indicated otherwise. A value of  $P < 0.05$  was considered statistically significant.

This study was conducted in accordance with the Animals (Scientific Procedures) Act, 1986. Some of these data have previously been presented (Kirkman *et al.* 2000a,b).

## RESULTS

There were no significant differences in the baseline (pre-blast or pre-sham blast) heart period or mean arterial blood pressure between any of the groups (Table 1).

### Effects of thoracic blast

Sham blast (Group I) produced no significant changes in heart period or mean arterial blood pressure (Fig. 1). Thoracic blast (Group II) produced a significant increase in heart period of  $317 \pm 28$  ms with a latency of  $6.0 \pm 0.7$  s from a pre-blast control of  $149 \pm 5$  ms, and a significant fall in mean arterial blood pressure of  $71.8 \pm 7.5$  mmHg with a latency of  $3.2 \pm 0.4$  s from a pre-blast level of  $105.4 \pm 5.1$  mmHg (Fig. 1). Thereafter, there was a partial recovery of heart period and mean arterial blood pressure although the animals remained bradycardic and hypotensive for the subsequent 10 min. In Group II heart period was significantly greater, and mean arterial blood pressure significantly less, than the corresponding levels recorded in

**Table 1. Initial cardiovascular and physiological data recorded before blast or sham blast**

	Group I <i>n</i> = 8	Group II <i>n</i> = 8	Group III <i>n</i> = 5
Body weight (g)	306.9 $\pm$ 3.5	314.3 $\pm$ 7.6	319.2 $\pm$ 12.3
HP (ms)	139.1 $\pm$ 4.7	149.1 $\pm$ 5.1	155.2 $\pm$ 8.4
MBP (mmHg)	112.0 $\pm$ 4.5	105.4 $\pm$ 5.1	108.5 $\pm$ 8.4
Hct (%)	39.9 $\pm$ 1.0	39.4 $\pm$ 0.6	40.9 $\pm$ 0.4
Temp ( $^{\circ}$ C)	37.9 $\pm$ 0.1	37.6 $\pm$ 0.1	37.7 $\pm$ 0.2

Values are means  $\pm$  S.E.M. *n*, number of rats; HP, heart period; MBP, mean arterial blood pressure; Hct, haematocrit; Temp, body temperature.

Group I at 10 min after blast. Blast in Group III produced effects on heart period and mean arterial blood pressure similar to those recorded in Group II and there were no significant differences between these two groups during the 10 min after blast (Fig. 1).

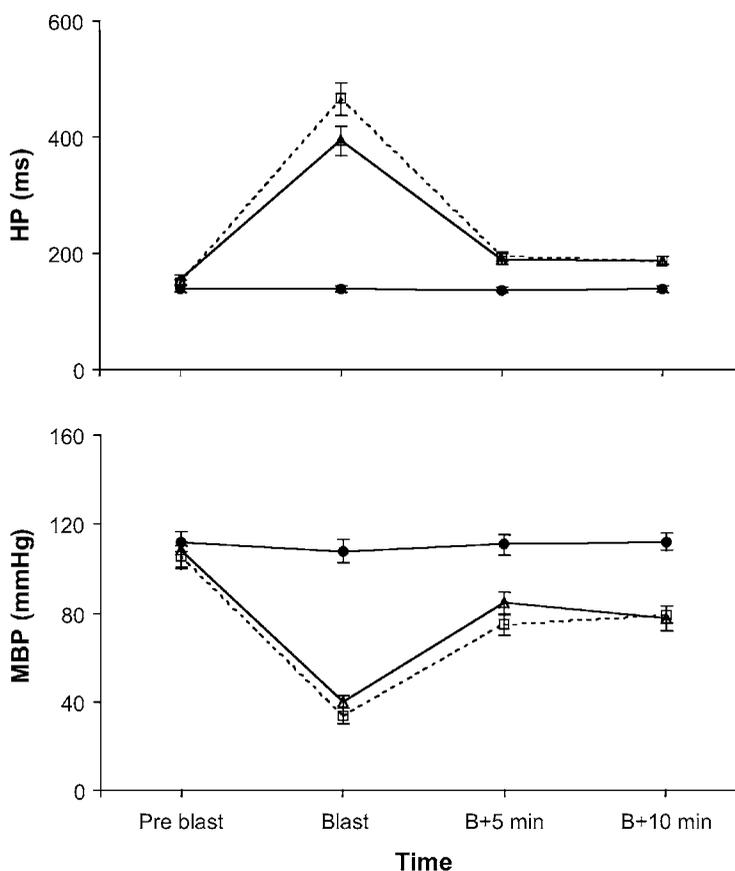
Thoracic blast produced apnoea of duration  $19.0 \pm 2.0$  s and  $16.5 \pm 1.7$  s in Groups II and III, respectively; there was no significant difference (Student's independent *t* test) in the duration of apnoea between these two groups. Sham blast did not produce apnoea.

### Effects of progressive haemorrhage

In Group I progressive haemorrhage produced a biphasic response (Fig. 2). There was an initial tachycardia, with heart period initially decreasing in all animals from  $137 \pm 5$

**Figure 1**

Effects of blast on heart period (HP) and mean arterial blood pressure (MBP) in anaesthetised rats. Data recorded immediately before (Pre blast) and after (Blast) blast or sham blast and then at 5 and 10 min after blast (B + 5 min and B + 10 min). Saline (Groups I and II) or morphine (Group III) was administered immediately after B + 5 min. ●, Group I, sham blast, saline; □, Group II, thoracic blast, saline; △, Group III, thoracic blast, morphine. Data are mean  $\pm$  S.E.M.



to  $132 \pm 4$  ms after the loss of 6.7% BV. This difference was not statistically significant. Thereafter heart period increased significantly above pre-haemorrhage levels, attaining its maximal value after the loss of 33% BV. Mean arterial blood pressure was initially maintained at a pre-haemorrhage level of  $110.8 \pm 3.3$  mmHg before falling progressively, the hypotension attaining statistical significance after the loss of 13.3% BV.

In Group II pre-haemorrhage heart period was significantly higher, and mean arterial blood pressure significantly lower, than the corresponding values in Group I. The pattern of response to haemorrhage was significantly different in Group II compared to Group I. The initial compensatory phase of the response to blood loss was absent in Group II. There was no tachycardia and heart period increased significantly attaining its maximum after the loss of 23% (Fig. 2). Furthermore, in Group II mean arterial blood pressure was not maintained during haemorrhage and started to fall after the first aliquot of blood had been removed (Fig. 2), the hypotension achieving statistical significance (compared to pre-haemorrhage control) after the loss of 10% BV.

#### Effects of morphine and progressive haemorrhage

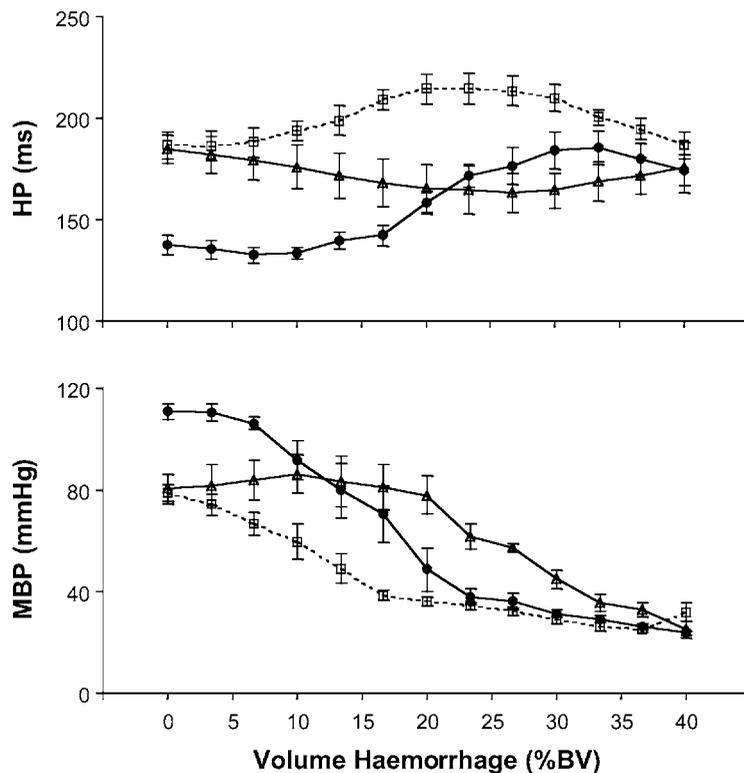
There were no significant differences in pre-haemorrhage heart period or mean arterial blood pressure in the morphine-treated animals (Group III) when compared to Group II. However, the pattern of response to haemorrhage was significantly different in Group III, and animals exhibited a tachycardia with the heart period reaching a nadir after the loss of 27% BV, while there was no

significant bradycardia in this group. In addition mean arterial blood pressure was maintained until the loss of 20% BV, after which it fell and was significantly less than pre-haemorrhage levels after the loss of 30% BV.

#### Effects of haemorrhage on arterial blood gases

Progressive haemorrhage led to a significant increase in  $P_{a,O_2}$  and a fall in  $P_{a,CO_2}$  and arterial pH. There was no significant difference in the pattern of response between the groups. However, there was a significant difference in the absolute levels between groups, with Group I displaying the highest  $P_{a,O_2}$  and arterial pH and the lowest  $P_{a,CO_2}$ , while Group III displayed the lowest  $P_{a,O_2}$  and arterial pH and the highest  $P_{a,CO_2}$  (Fig. 3).

In summary, progressive haemorrhage in the absence of thoracic blast produced a biphasic response with tachycardia followed by bradycardia, and with mean arterial blood pressure being maintained initially before falling. Following thoracic blast the initial compensatory phase of the response to haemorrhage was abolished, the tachycardia was absent while mean arterial blood pressure fell as soon as haemorrhage commenced. Although it is impossible to compare the absolute values between Groups I and II due to the different pre-haemorrhage baselines, the bradycardic, hypotensive response to haemorrhage was recorded after a significantly smaller blood loss in Group II compared with Group I. Administration of morphine after thoracic blast, but before haemorrhage, abolished the bradycardia induced by haemorrhage and led to maintenance of mean arterial blood pressure until greater volumes of blood loss were incurred.



**Figure 2**

Effects of a progressive haemorrhage of 40% total estimated blood volume (BV) at a rate of  $2\% \text{ BV min}^{-1}$  on heart period (HP) and mean arterial blood pressure (MBP) in anaesthetised rats. ●, Group I, sham blast, saline; □, Group II, thoracic blast, saline; △, Group III, thoracic blast, morphine. Data are mean  $\pm$  S.E.M.

## DISCUSSION

Results from this study demonstrate that blast injury augmented the bradycardic, hypotensive response to haemorrhage, and that morphine administered following blast injury could attenuate this effect. Administration of morphine after blast did not affect either the blast-induced bradycardia (which might be predicted from earlier studies; Ohnishi *et al.* 1999) or hypotension (which could not be predicted since pretreatment with morphine delayed the recovery of blood pressure after thoracic blast; Ohnishi *et al.* 1999).

The results indicate that exposure to thoracic blast augmented the hypotensive, bradycardic second phase of the response to blood loss. Peak bradycardia occurred after significantly less blood loss compared with the response recorded in the absence of blast. Enhanced blood loss can be discounted as a mechanism for the blast-induced augmentation of the second phase of the response to haemorrhage since there was no post-mortem evidence of additional blood loss into the body cavities. Furthermore, although the possibility that the blast injury may have affected intra-abdominal organs cannot be discounted, other studies have shown that abdominal blast has relatively little cardiovascular effect (Guy *et al.* 1998). In the present study the blast was focused on the thorax and the response to thoracic blast raises the possibility that the blast-induced augmentation of the second phase of the response to haemorrhage might be a consequence of a blast-induced modulation of the baroreflex.

Prolonged hypotension induced by thoracic blast *per se* is accompanied by a bradycardia rather than a tachycardia (Ohnishi *et al.* 2001), as might be expected if the reflex was functioning normally. To date, there are no reported studies of the effects of thoracic blast on the function of the baroreflex. However, the effects of morphine reported here would not support this modulation of the baroreflex as the cause of the early onset of the depressor response. Morphine modified the response to haemorrhage after blast so that the compensatory phase was apparent, with an initial maintenance of arterial blood pressure and tachycardia, while the bradycardia associated with severe haemorrhage was abolished. It is unlikely that morphine achieved this effect by increasing the sensitivity of the baroreflex since it is known that  $\mu$  opioid receptor agonists, in the absence of blast, reduce baroreflex sensitivity (Gordon, 1990; Hamra *et al.* 1999).

The alternative, and more likely, explanation is that blast augmented the depressor reflex(es) initiated by severe haemorrhage, and is consistent with the effects of morphine described in this study. Morphine modified the cardiovascular response to haemorrhage after blast, resulting in an initial maintenance of arterial pressure and a tachycardia, with abolition of the bradycardia normally associated with haemorrhage. It has previously been shown that morphine and other  $\mu$  opioid receptor agonists attenuate the depressor reflex associated with severe

haemorrhage (Evans *et al.* 1989; Evans & Ludbrook, 1990, 1991; Ohnishi *et al.* 1997). Therefore, the most likely explanation is that the response to blast augmented the depressor reflex associated with severe haemorrhage, which overrode the baroreflex, leading to an early fall in blood pressure and bradycardia. When this depressor reflex was attenuated by morphine, the baroreflex-mediated compensatory phase was again apparent.

It is impossible to determine the site of action of morphine in attenuating the depressor response to severe haemorrhage. A number of central nervous loci are known to show increased activity during severe haemorrhage. These areas include the ventrolateral periaqueductal grey and the rostral ventrolateral medulla (see Evans *et al.* 2001). It is known that the endogenous opioid system participates in the depressor response to severe haemorrhage; in the rat this is predominantly via activation of  $\delta_1$  receptors in the periaqueductal grey (Cavun *et al.* 2001) and  $\delta_1$  and  $\mu$  receptors in the spinal cord (Ang *et al.* 1999). The picture is complex because other studies have shown that activation of  $\mu$  opioid receptors can also attenuate the

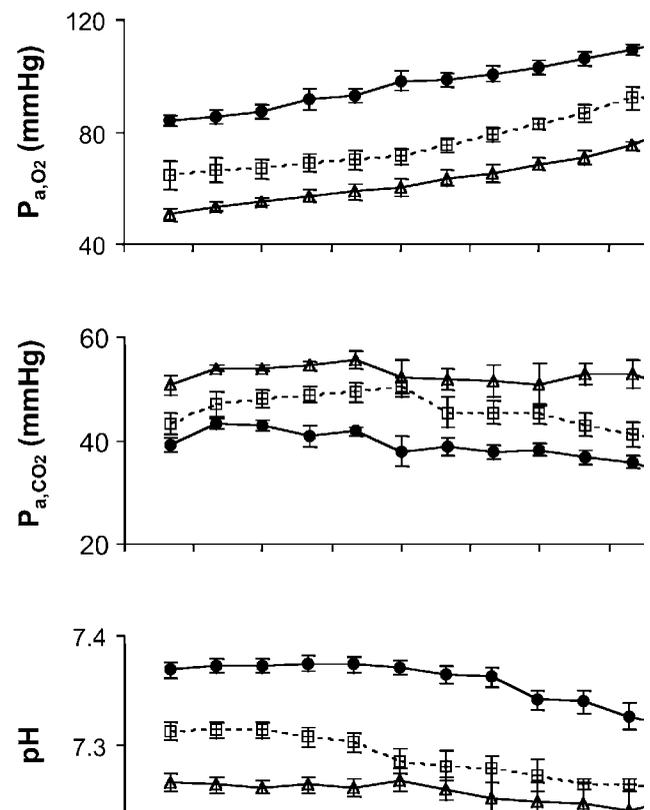


Figure 3

Effects of a progressive haemorrhage of 40% total estimated blood volume (BV) at a rate of  $2\% \text{ BV min}^{-1}$  on arterial oxygen ( $P_{a,O_2}$ ) and carbon dioxide ( $P_{a,CO_2}$ ) tensions and pH in anaesthetised rats. ●, Group I, sham blast, saline; □, Group II, thoracic blast, saline; △, Group III, thoracic blast, morphine. Data are mean  $\pm$  S.E.M.

depressor response to haemorrhage (Evans *et al.* 1989; Evans & Ludbrook, 1990, 1991; Ohnishi *et al.* 1997). However, it is unlikely that morphine is acting within the spinal cord to block the depressor response to severe haemorrhage since it is blockade, rather than activation, of  $\mu$  receptors at this site that attenuates the depressor response to blood loss (Ang *et al.* 1999). Potential sites of action for morphine include the nucleus tractus solitarius, an afferent nucleus for a number of cardiovascular reflexes, the rostral ventrolateral medulla and the nucleus ambiguus (Evans *et al.* 1989; Evans & Ludbrook, 1990, 1991). Since morphine attenuated both the bradycardia and hypotension during severe haemorrhage it is possible that it is acting early in the reflex pathway, before the sympathetic and vagal limbs diverge.

The change in arterial blood gases after blast and subsequent haemorrhage are consistent with previous studies (Ohnishi *et al.* 2001). Animals subjected to thoracic blast displayed a lower  $P_{a,O_2}$  and arterial pH and higher  $P_{a,CO_2}$  when compared to those subjected to sham blast. This alteration in blood gas is unlikely to be because of respiratory depression at this time since previous studies have shown that after a brief blast-induced apnoea respiratory activity returns to normal levels within 5 min (Ohnishi *et al.* 2001). However, the hypoxia and hypercarbia could be consistent with impaired pulmonary gas transport after blast (Damon *et al.* 1971). The fall in arterial pH is consistent with the development of a metabolic acidosis, possibly due to a failure of oxygen delivery to metabolically active tissues.

Although the combination of hypoxia, hypercarbia and acidosis are likely to activate the arterial chemoreceptors, this is unlikely to be the cause of the blast-induced augmentation of the bradycardic, hypotensive response to blood loss since any chemoreceptor-induced increase in ventilation is likely to attenuate or leave unmodified rather than augment a vagally mediated bradycardia (Daly *et al.* 1988; Daly & Kirkman, 1989). In addition, the changes in blood gases were augmented by morphine while the haemorrhage-induced bradycardia and hypotension were attenuated.

The further reduction in  $P_{a,O_2}$  and elevation in  $P_{a,CO_2}$  in morphine-treated animals may be explained by the respiratory depressant effect of morphine. Subsequent elevation of  $P_{a,O_2}$  and reduction in  $P_{a,CO_2}$  during haemorrhage in each of the groups would have been due to haemorrhage-induced increase in ventilation following activation of arterial chemoreceptors as a consequence of reduced blood flow to these structures (Daly *et al.* 1954; Acker & O'Regan, 1981; Potter & McCloskey, 1987). However, these changes in respiratory activity and arterial blood gases are unlikely to account for the effects of morphine on the cardiovascular response to severe haemorrhage. Indeed, any respiratory depression would be predicted to enhance, rather than reverse, the bradycardia associated with severe haemorrhage (Daly & Kirkman 1988, 1989; Daly *et al.* 1988; Blake *et al.* 1994).

In conclusion, results from this study indicate that thoracic blast injury modifies the physiological responses to progressive haemorrhage such that the initial compensatory phase of the response to haemorrhage is lost and the hypotensive, bradycardic second phase is augmented and occurs after smaller volumes of blood loss. Morphine prevented the bradycardia associated with severe haemorrhage and delayed the onset of hypotension. Blast injury may therefore modify the clinical signs of blood loss in casualties and treatment with morphine may further modulate the physiological responses.

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