

Naloxone does not inhibit the attenuation of the response to severe haemorrhage seen after simulated injury in the anaesthetised rat.

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ABSTRACT

Severe haemorrhage leads to a reflex bradycardia and hypotension. This is thought to be protective, but is attenuated by both concomitant musculo-skeletal injury and exogenous morphine. The aim of this study was to determine whether the injury-induced attenuation of the response to severe haemorrhage could be blocked by naloxone. Male Wistar rats, terminally anaesthetised with alphadolone/alphaxalone (19-20 mg.kg⁻¹ i.v.) were randomly allocated to one of four groups; in Groups I and IV haemorrhage was simple (40% of estimated total blood volume (BV)) while in Groups II and III it was initiated 10 min after the onset of bilateral hindlimb ischaemia (a model of musculo-skeletal injury). Groups I and II received 20 µL of 0.9% saline intracerebroventricularly (i.c.v.) immediately before haemorrhage, while Groups III and IV received 20 µg of naloxone i.c.v., in the same volume. Group I; the bradycardia reached its peak after the loss of 32.8±0.3% BV (mean±s.e.m.). Blood pressure did not fall significantly until the loss of 15.0±3.0% BV. The response in Group IV was not significantly different from Group I. By contrast the bradycardia was absent after similar blood losses in Groups II and III, while hypotension was attenuated. These results indicate that naloxone, at a dose known to be effective in blocking opioid receptors and preventing other aspects of the response to injury, does not prevent the injury-induced attenuation of the response to severe haemorrhage. Thus the attenuation of the response to blood loss by injury is unlikely to be mediated via the µ opioid receptors.

Introduction

The body's response to a progressive simple haemorrhage (blood loss in the absence of major tissue damage) is biphasic (Barcroft *et al.*, 1944; Kirkman *et al.*, 1994; Ohnishi *et al.*, 1997). During mild haemorrhage there is a reduction in pulse pressure which results in unloading of the baroreceptors leading to a reflex tachycardia, positive inotropic effect, increase in peripheral resistance (Kircheim, 1976), all contributing to the maintenance of mean arterial blood (MAP).

As the haemorrhage progresses a second phase becomes apparent involving a bradycardia, a fall in peripheral resistance and a profound fall in blood pressure (Barcroft *et al.*, 1944). Phase II of the response to simple haemorrhage is not due to a failure of the baroreflex as sensitivity is still high (Little *et al.*, 1984) but due to the recruitment of a 'depressor' reflex. The bradycardia is mediated by activation of the vagus nerve, as this can be blocked with atropine in humans (Lewis, 1932; Barcroft *et al.*, 1944) and experimental animals (Little *et al.*, 1989). Inhibition of the sympathetic efferent nerve fibres to the vasculature mediate the fall in total peripheral resistance (Öberg & Thorén, 1973). The result of these two responses is a profound fall in blood pressure. The bradycardia is thought to be a protective mechanism to prevent high levels of cardiac work with insufficient coronary perfusion (Öberg & Thorén, 1972). However, this protective reflex is lost in the presence of concomitant musculo-skeletal injury (Little *et al.*, 1989; Mackway-Jones *et al.*, 1999); the bradycardia is prevented and the onset of hypotension is delayed (Little *et al.*, 1989).

Phase II of the response to haemorrhage is also attenuated by exogenous opioid agents (e.g., morphine) acting on the μ -opioid receptors (Ohnishi *et al.*, 1997). The endogenous opioids are also known to be responsible for the injury-induced modulation of the vagal limb of the baroreflex (Wyatt *et al.*, 1995). During injury the sensitivity of the baroreflex is reduced, this effect of injury can be blocked by naloxone and β -funaltrexamine (β -FNA), both μ opioid receptor antagonists, indicating that injury must mediate its effect on the baroreflex via activation of endogenous opioid receptors. However, blocking the δ opioid receptors with a δ specific opioid antagonist (e.g. ICI-174 856) does not inhibit the reduction in baroreflex sensitivity during injury (Wyatt *et al.*, 1995) indicating further that this effect is mediated by specific activation of the μ opioid receptors.

The aim of this study is to determine whether the effect of injury on the response to haemorrhage can be prevented by naloxone, injected into the lateral cerebral ventricles.

Methods

Ethical approval

The experiments were approved by the local ethics committee of Durham University and were conducted in accordance with the Animals (Scientific Procedures) Act, 1986.

The study was conducted on 20 male Wistar rats of the Harlan Orlac strain (body weight 233-260 g), kept on a 12 h light/dark cycle and fed on Beekay standard rat and mouse diet (B & K Universal Ltd, UK).

Implantation of cerebral ventricular guide cannulae

7-14 days prior to the study 20 rats (210-230 g body weight) were anaesthetised with sodium pentobarbitone (Sagatal, Rhone Merieux Ltd, UK; 60 mg.kg⁻¹ intraperitoneal; i.p.). When a surgical level of anaesthesia was attained, i.e. the absence of spontaneous movement and corneal and paw pinch reflexes, the rat was secured in a stereotaxic frame (stereotaxic frame model 900, Kopf, Sucedo International Ltd, UK) using atraumatic ear bars.

An incision was made along the midline of the scalp to expose the skull. A burr-hole was drilled to reveal the dura which was pierced with a needle. In each rat a guide cannula (stainless steel 22G, length 11.5 mm) was placed so that its tip lay 1mm above the right lateral cerebral ventricle at the following co-ordinates: AP -1; L -1.8; DV -2.5 (Pellegrino *et al.*, 1979), where bregma was taken as AP and L zero, while the dura was taken as DV zero. The guide cannula was secured in place with two jeweller's screws inserted into the skull with dental cement (Simplex Rapid, Associated Dental Products Ltd, UK), and sealed using a stylet.

The scalp was sutured and the animals treated prophylactically with the antibiotic oxytetracycline hydrochloride (Terramycin Q-50, Pfizer Ltd, UK; 30 mg.kg⁻¹ i.m.) injected into the left thigh muscle. The animals were allowed to recover from the anaesthetic and housed individually in cages with free access to food and water containing chlortetracycline hydrochloride (Aureomycin, Cyanamid, UK; 0.25% w/v).

By the day of the study the body weights of the rats had increased to 233-260 g. On the day of the study anaesthesia was induced in all the rats by inhalation of isoflurane (Abbott Laboratories Ltd UK; 3.5% in O₂/N₂O, FiO₂ = 0.5) in an anaesthetising chamber (Fluorovac, UK). Once the animal was surgically anaesthetised they were transferred to the operating table and the isoflurane administered by mask and co-axial scavenging (FluovacTM, International Market Supplies, UK). The percentage of isoflurane was adjusted to 2.5 – 3.5 % (0.7 L O₂.min⁻¹) to maintain a surgical level of anaesthesia.

Surgical preparation

In each rat 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 (Sensor 840, SensoNor a.s., Norway). A lateral tail vein was cannulated (2FG, Portex Ltd, UK) for drug administration. All cannulae were initially filled with heparinised saline (5 IU.ml⁻¹ in 0.9% saline) to prevent coagulation of the blood in or around the cannula. Body temperature was monitored using a rectal thermocouple (Medical precision thermometer; Allab Copenhagen DM 852). The electrocardiogram was recorded using needle electrodes placed in the skin of the ventrum, and heart period measured from the electrocardiogram. All physiological variables were amplified and recorded using a computerised Data Acquisition System (MacLab 85, ADInstruments, UK). Body temperature was maintained at 37.7 ± 0.1 °C (mean ± S.E.M.) throughout the study using a thermally-insulated operating mat and a heating lamp.

Experimental protocol

Following the surgical preparation the isoflurane was discontinued and anaesthesia maintained with alphadolone/alphaxalone (Saffan, Pitman-Moore, UK, 19-21 mg.kg⁻¹.h⁻¹; i.v.) using a pump (Harvard 22, Harvard Apparatus Ltd, UK) while the animals breathed air. The anaesthesia was adjusted within the range 19-21 mg.kg⁻¹.h⁻¹ to maintain an experimental level of anaesthesia (mild withdrawal and a pressor response of approximately 10 mmHg to a noxious pinch of the foot) with supplemental doses of 0.1 ml alphadolone/alphaxalone i.v. if required. The rats were allowed to stabilise for 60 min, before being allocated randomly to one of four groups:

- Group I; intracerebroventricular (i.c.v.) saline (20 µL), sham bilateral hindlimb ischaemia and haemorrhage
- Group II; i.c.v. saline (20 µL), bilateral hindlimb ischaemia and haemorrhage
- Group III; i.c.v. naloxone (20 µg.20 µL⁻¹ saline), bilateral hindlimb ischaemia and haemorrhage
- Group IV; i.c.v. naloxone (20 µg.20 µL⁻¹ saline), sham bilateral hindlimb ischaemia and haemorrhage.

The lower abdomen of the rats were checked for fullness of the bladder and emptied accordingly by applying light pressure to the area. Briefly, baseline (pre-injury or pre-sham injury) measurements of heart period and arterial blood pressure were made. Ten minutes before the onset of haemorrhage, bilateral hindlimb ischaemia (an established model of musculo-skeletal tissue injury (Redfern *et al.*, 1984)) was induced in groups II and III by application of rubber band tourniquets. The tourniquets were kept in place for the remainder of the study. The animals were allowed to stabilise for 10 min before a control (pre-haemorrhage) recording was made. The rats were then given heparin (Monoparin 0.1 ml intra-arterial (i.a.) of 5000 IU.ml⁻¹, solution providing approximately 2000 IU.kg⁻¹, CP Pharmaceuticals Limited, Wrexham) to prevent coagulation and blood samples taken for blood gas analysis. Following this, 20 µL 0.9% saline (Groups I and II) or 20 µg naloxone (Sigma Chemical Company, UK) in 20 µL 0.9% saline (Groups III and IV) was administered into the lateral cerebral ventricles over 20 s via the guide cannula.

Deadspace saline was then aspirated from the arterial cannula. Arterial blood was withdrawn anaerobically from the ventral tail artery in aliquots of 0.5 ml, at an overall rate of 2% estimated total blood volume (TBV) per minute until 40% of the total blood volume had been withdrawn. Total blood volume was calculated as $6.06 \text{ ml} \cdot (100 \text{ g body weight})^{-1}$ (Elebute & Little, 1978; Ohnishi *et al.*, 1997). Cardiovascular measurements were made after the withdrawal of each aliquot of blood, each blood sample subjected to blood gas analysis (ABL5, Radiometer, Denmark), and the cycle repeated until 40% of the total estimated blood volume had been withdrawn. Once the haemorrhage was complete further cardiovascular measurements were made at 100 s intervals for the following 10 min. At the end of the experiment, ink (20 μL) was injected through the i.c.v. cannula over 20 s to confirm the correct positioning of the cannula into the cerebral ventricles. The animals were then killed with an overdose of sodium pentobarbitone (Sagatal, Rhone Merieux Ltd, UK) administered i.v. without regaining consciousness. None of the animals received fluid resuscitation following the blood loss.

A *post mortem* was performed to assess whether any internal damage had occurred from the insertion of the i.A. cannula or the thermistor, and to assess the presence of any macroscopic lung pathology and the fullness of the bladder. The brain was removed and sectioned to determine the distribution of ink. Ink was found in the lateral, third and fourth ventricles in all animals.

Calculations and statistical analysis

MAP was calculated accurately using MacLab software. Data are presented as mean \pm S.E.M. Statistical comparisons were made using a 2-way analysis of variance for repeated measures (SPSS/PC v4.01) unless indicated otherwise, and the degrees of freedom adjusted using the Greenhouse-Geisser correction to minimise the risk of type 1 error (Ludbrook, 1994). Comparisons of non-repeated measurements between groups (baseline values, body weights) were made using one-way analyses of variance followed, where appropriate, by a Tukey post-hoc test (SPSS/PC v4.01). In all cases $P < 0.05$ was considered statistically significant.

Results

There were no significant differences between any of the groups in initial baseline values for heart period, MAP, body weight and temperature or arterial blood gases (Table 1).

Effects of haemorrhage on saline treated animals

A progressive simple haemorrhage in rats treated with i.c.v. saline, led to an initial fall in heart period of 16 ± 5 ms ($P=0.04$, paired t-test) after a loss of $16.4\pm 0.2\%$ blood volume (BV), from a pre-haemorrhage level of 147 ± 6 ms. Further haemorrhage then led to a progressive increase in heart period with a maximum increase of 42 ± 7 ms after the loss of $32.8\pm 0.3\%$ BV ($P=0.01$, paired t-test). MAP was initially maintained constant at a level of 102.3 ± 4.0 mmHg until the loss of $15.0\pm 3.0\%$ BV, after which further haemorrhage led to a progressive fall in MBP reaching a minimum value of 20.9 ± 1.7 mmHg ($P=0.001$) after the loss of $39.8\pm 0.4\%$ BV (Figure 1, Group I).

Arterial oxygen tension remained constant in Group I at 91.4 ± 2.9 mmHg until a loss of $19.7\pm 0.2\%$ BV after which it increased progressively to a maximum value of 112.2 ± 1.6 mmHg after a loss of $39.8\pm 0.4\%$ BV. Arterial carbon dioxide tension fell to 42.0 ± 2.4 mmHg from a pre-haemorrhagic level of 44.4 ± 1.4 mmHg, after the loss of $39.8\pm 0.4\%$ BV. Arterial pH was maintained relatively constant throughout the haemorrhage with a minimum value of 7.34 ± 0.02 after the loss of $40.0\pm 0.0\%$ BV. Arterial base excess (ABE) initially rose to a maximum value of 3.35 ± 0.48 mM after the loss of $13.1\pm 0.12\%$ BV. Thereafter it fell, reaching a minimum value of -2.5 ± 1.2 mM at the end of the haemorrhage.

Effects of injury on the response to haemorrhage in saline treated animals

The response to haemorrhage in animals subjected to bilateral hindlimb ischaemia was different to that seen in animals without ischaemia. The bradycardia was blunted ($P=0.02$) and the fall in blood pressure delayed (Figure 1, Group II). Thus, rats treated with i.c.v. saline and subject to bilateral hindlimb ischaemia preceding the haemorrhage displayed a progressive significant fall in heart period from a lower initial pre-haemorrhagic level (compared to Group I) of 132 ± 4 ms to 117 ± 2 ms ($P=0.03$) after the loss of $23.5\pm 0.3\%$ BV. Further haemorrhage then caused an increase in heart period reaching a maximum value of 142 ± 7 ms at the end of the haemorrhage, which was not different from pre-haemorrhage baseline ($P=0.15$). MAP showed a higher initial value of 121.7 ± 4.3 mmHg, compared to Group I ($P=0.01$), which was maintained constant until the loss of $23.5\pm 0.3\%$ BV where, thereafter, it showed a progressive decrease reaching a minimum value of 27.1 ± 3.3 mmHg ($P=0.001$) at end haemorrhage (Figure 1, Group II).

Arterial oxygen tension remained constant at 99.0 ± 3.5 mmHg until a loss of $23.5 \pm 0.3\%$ BV were it slowly and progressively showed a significant increase, with a maximum value of 116.2 ± 4.2 mmHg ($P=0.01$) after a loss of $40.0 \pm 0.0\%$ BV (i.e. at the end of the haemorrhage). Arterial carbon dioxide tension fell from a pre-haemorrhagic level of 40.0 ± 1.4 mmHg, to 37.4 ± 1.6 mmHg at the end of the haemorrhage. Arterial pH was maintained relatively constant throughout the haemorrhage with a minimum value of 7.39 ± 0.01 after the loss of $40.0 \pm 0.0\%$ BV. Arterial base excess was not different from that in Group I.

Effects of haemorrhage in naloxone treated animals

Progressive simple haemorrhage in the absence of injury, in animals treated with i.c.v. naloxone (Group IV) showed no significant differences in the changes in heart period, blood pressure and arterial blood gases from those seen during haemorrhage in animals treated with i.c.v. saline (i.e. Group I; Figure 1).

Effects of i.c.v. naloxone on the response to haemorrhage and injury

The change in heart period, MAP and arterial blood gases during haemorrhage and injury in naloxone treated rats (Group III) showed no difference from those in Group II treated with saline, haemorrhage and injury (Figure 1). Rats treated with i.c.v. naloxone and subjected to bilateral hindlimb ischaemia, showed no bradycardia and hypotension was delayed until 24% of the total blood volume was lost. Therefore, with respect to the bradycardic response to haemorrhage, injury was shown to have a significant effect ($P < 0.001$), while naloxone had no significant effect ($P = 0.190$) and there was no significant interaction between these two factors ($P = 0.778$), i.e. the effects of injury were unaffected by naloxone treatment.

Discussion

The primary finding of this study is that naloxone, given into the cerebral ventricles, did not modify the effects of bilateral hindlimb ischaemia on the response to haemorrhage. This suggests that the attenuation of the depressor response to blood loss by hindlimb ischaemia is not mediated by the μ opioid receptors accessible to drugs injected into the lateral cerebral ventricles. This is in contrast to the effect of bilateral hindlimb ischaemia on the baroreflex, which can be blocked by naloxone given i.c.v. (Wyatt *et al.*, 1995; Little, 1998). The results in Group I, show the biphasic pattern of cardiovascular response to a simple haemorrhage; the saline treated animals (Barcroft *et al.*, 1944; Kirkman *et al.*, 1994; Ohnishi *et al.*, 1997). The pattern of response of the blood gases is also consistent with that found in other studies (Ohnishi *et al.*, 1997; Little, 1998) and is thought to be as a result of the stimulation of the arterial chemoreceptor reflex by the reduction in blood flow through, and a sympathetically-mediated vasoconstriction of the carotid and aortic bodies, as a consequence of a reduced blood volume during the haemorrhage (Acker & O'Regan, 1981). Thus, ventilation is stimulated causing the decrease in carbon dioxide tension and the increase in oxygen tension seen.

The effects of the injury model, bilateral hindlimb ischaemia, on saline treated animals (Group II) during progressive haemorrhage was as expected (Little *et al.*, 1989); a blockade of the bradycardia and attenuation of the hypotension seen during phase II of the response to a simple haemorrhage. Moreover, MAP was maintained until a significantly larger volume of blood had been lost; it did not begin to fall until after a loss of 23% blood volume compared to 15% blood volume in the haemorrhage and sham injury group. Heart rate and MAP were also higher during haemorrhage on a background of injury than with a similar volume during a simple haemorrhage.

The opioid antagonist naloxone, when given into the cerebral ventricles, had no effect on the response to haemorrhage or its modification by injury. Animals in Group III, treated with naloxone and subjected to bilateral hindlimb ischaemia, showed no bradycardia and hypotension was delayed until 24% of the total blood volume was lost. This result shows the same pattern of response as rats treated with saline and subjected to the same injury model (Group II). The blood gas results also showed the same pattern of change throughout both experimental protocols. Animals in Groups I and IV, which were subjected to a progressive simple haemorrhage and treated with either saline or naloxone into the cerebral ventricles, showed an identical pattern of response to haemorrhage. This showed that naloxone itself had no effect on the response to a simple haemorrhage, and thus any change in the response to haemorrhage and injury would be certain to be as a result of the effect of naloxone on the response to injury. Thus, the results of this study would indicate that naloxone does not block the injury-induced modification of the response to haemorrhage utilising a pathway accessible via the i.c.v. route, unlike its effects on the injury-induced modification of the baroreflex (Wyatt *et al.*, 1995; Little, 1998). However, naloxone may have an effect on the injury-induced modification of the response to haemorrhage via pathways elsewhere in the brain, not accessible via the cerebral ventricles.

As i.c.v. administration of naloxone would only reach the periventricular areas future studies should investigate the effects of high dose intravenous or intracarotid naloxone (sufficient to block cerebral μ opioid receptors) on the injury-induced modification of the response to severe haemorrhage. Administration of a high dose of naloxone via these routes would enable the drug to reach more distant sites than adding it into the cerebral ventricles. The interpretation of results from these studies would however be difficult due to a possible peripheral site of action, but if the endogenous opioid system really is not involved in the injury-induced modification of the response to a severe haemorrhage, then naloxone would still have no effect.

In contrast to this study, high doses of naloxone administered centrally have been shown to delay the decompensatory phase of the response to haemorrhage in conscious sheep (Frithiof & Rundgren, 2006; Frithiof *et al.*, 2007) and rabbits (Evans *et al.*, 1989). However, haemorrhage-induced bradycardia is much smaller in these species compared to rat (Ohnishi *et al.*, 1997) and man (Barcroft *et al.*, 1944; Secher & Bie, 1985). In man during lower body negative pressure (LBNP), which reduces venous return (Little, 1998; van Hoeyweghen *et al.*, 2001), naloxone does not delay the onset of presyncope (Smith *et al.*, 1993). Likewise, in anaesthetised rats, centrally administered naloxone failed to attenuate the bradycardia associated with severe blood loss (Little, 1998). However, systemically administered naloxone in conscious rats is effective in reversing the decompensatory phase of the response to haemorrhage via a cerebral mechanism (Faden & Holaday, 1979) as well as virtually abolishing it if used as pre-treatment intrathecally (Ang *et al.*, 1999). However, the route of administration in this current study was different from these last two studies and hence the structures affected are likely to be very different.

Naloxone has a higher binding affinity for the μ -opioid receptor subtype than for the δ -receptors (20-fold) and the κ -receptors (10-fold) (Goldstein & Naidu, 1989). The high doses of naloxone administered in the aforementioned sheep studies (Frithiof & Rundgren, 2006; Frithiof *et al.*, 2007) are likely to delay the decompensatory phase of the response to haemorrhage by inhibition of both δ - and κ -opioid receptor subtypes (Frithiof *et al.*, 2007). However, in anaesthetised rats i.c.v. administration of high doses of naloxone, which would antagonise the δ - as well as μ -opioid receptor subtypes, failed to attenuate the bradycardia associated with severe blood loss (Little, 1998). Indeed at doses high enough to antagonise μ -, δ - and κ -opioid receptors naloxone reduced tolerance to LBNP in healthy humans (Lightfoot *et al.*, 2000), and similar observations are seen following head-up tilt, (Madsen *et al.*, 1995), i.e. the decompensated phase was apparent sooner.

In contrast to the effects of centrally administered naloxone in this study (see Figure 1) microinjections of the δ -receptor antagonist naltrindole into a more specific area of the brain; the caudal midline medulla (CMM) delayed the hypotension and attenuated the bradycardia associated with severe haemorrhage in anaesthetised rats (Henderson *et al.*, 2002). However i.c.v.

administration of the δ -receptor agonist DPDPE does not have an effect on the decompensatory phase of severe haemorrhage in the anaesthetised rat (Little, 1998). Further studies should now determine whether a δ -opioid receptor agonist, injected into a more specific area of the brain, such as the CMM, blocks the injury-induced modification of the response to a severe haemorrhage in the rat.

Conclusions

Exogenously administered naloxone given into the cerebral ventricles failed to prevent the injury-induced attenuation of the response to a severe progressive haemorrhage in the rat. But this does not mean that the endogenous opioid system is not involved in this modification, it simply means that the response is not mediated via direct activation of μ opioid receptor subtypes accessible via the cerebral ventricles. The way forward may be to take a closer look at the involvement of the δ opioid receptor subtypes in this response. Administration of a δ opioid agonist into a specific area of the brain, perhaps the CMM, may prove to block the modification of the response to haemorrhage by injury. A better understanding of the relationship of the endogenous opioids with GABA within the central nervous system may shed more light on the mechanisms underlying this response and manipulation of this relationship may prove a means for restoration of the protective reflex for the heart during the response to trauma.

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Figure 1. The effects of a progressive haemorrhage of 40% total blood volume (TBV) on heart period (HP) and mean arterial blood pressure (MBP) in anaesthetised rats.

Group I; (▲) sham bilateral hindlimb ischaemia (BHLI), haemorrhage and intracerebroventricular (i.c.v.) administration of 20µL saline, Group II; (■) BHLI, haemorrhage and 20µL saline i.c.v., Group III; (◆) BHLI, haemorrhage and 20µg naloxone per 20µL saline i.c.v. and Group IV; (○) sham BHLI, haemorrhage and 20µg naloxone per 20µL saline i.c.v. Values are mean ± S.E.M.

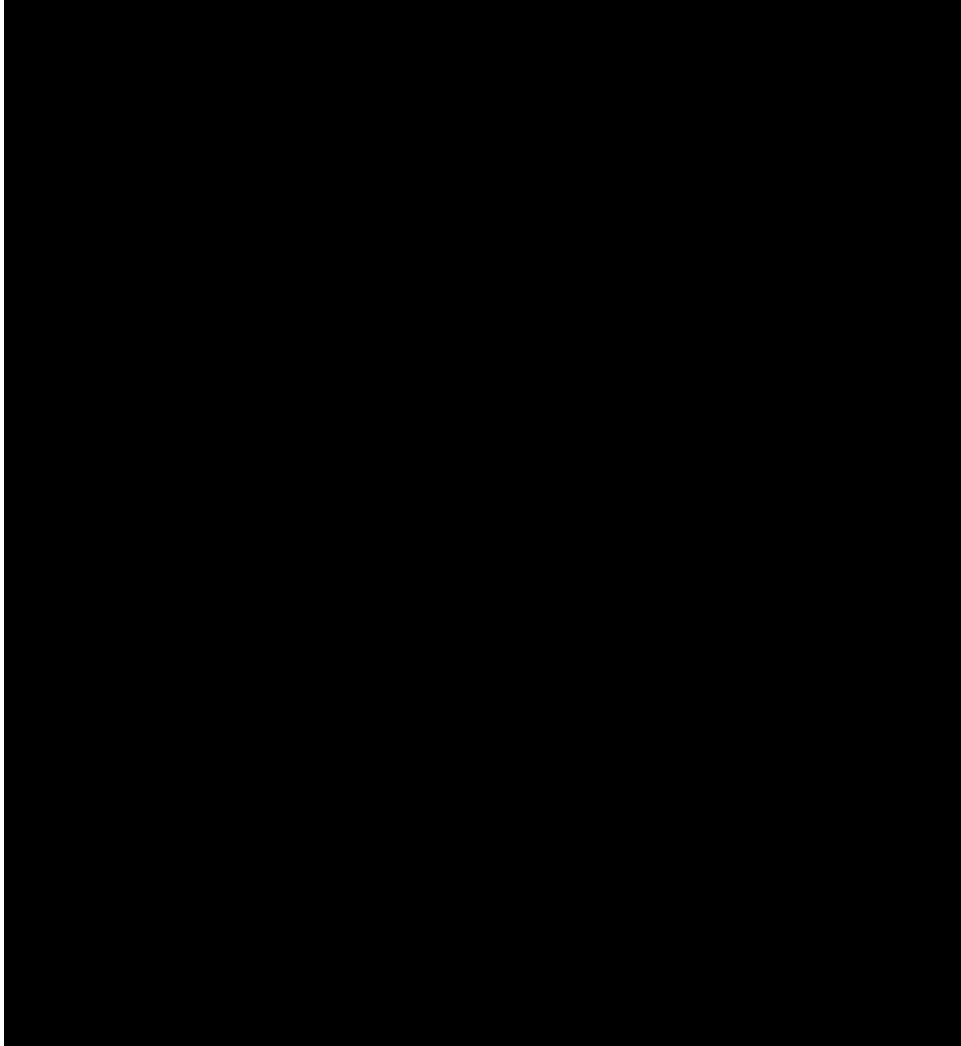


Table 1. Baseline values of all groups

	Group I	Group II	Group III	Group IV
<i>n</i>	5	5	5	5
Body wt (g)	252±2	246±3	245±4	245±3
HP (ms)	140±9	145±6	146±8	149±9
MBP (mmHg)	109±3	99±3	105±4	100±9
Pa,O₂ (mmHg)	91.4±2.9	99.0±3.5	96.2±1.5	86.8±1.6
Pa,CO₂ (mmHg)	44.4±1.4	40.0±1.4	40.4±1.2	41.4±0.9
apH	7.38±0.01	7.40±0.01	7.40±0.01	7.39±0.01
ABE (mM)	1.0±0.4	0.4±0.8	0.2±0.9	0.5±1.0
Hct (%)	39.0±1.5	37.0±2.0	38.7±0.4	37.4±0.7
Temp (°C)	37.9±0.1	37.7±0.2	37.6±1.3	37.9±0.1

Values are means ± S.E.M. Abbreviations: *n*, number of rats; Body wt, body weight; HP, heart period; MBP, mean arterial blood pressure; Pa,O₂, arterial oxygen tension; Pa,CO₂, arterial carbon dioxide tension; apH, arterial pH; ABE, arterial base excess; Hct, haematocrit; Temp, body temperature.