Effects of haemorrhage in rats lacking vasopressin (Brattleboro strain): influence of naloxone

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Summary

1. The effects of naloxone on blood pressure recovery after either rapid arterial haemorrhage or prolonged venous haemorrhage were studied in rats lacking vasopressin (Brattleboro strain) and in control (Long Evans) rats.

2. To produce similar reductions in blood pressure, less blood had to be taken from the Brattleboro rats than from the Long Evans rats.

3. After rapid arterial haemorrhage in the absence of naloxone, blood pressure recovery was slower in Brattleboro rats than in Long Evans rats. Naloxone did not affect the response to rapid arterial haemorrhage in Long Evans rats, but improved blood pressure recovery in Brattleboro rats; despite this improvement, the Brattleboro rats remained hypotensive at a time when the Long Evans rats were normotensive. These findings suggest that both the absence of vasopressin and a depressor action of β-endorphins may contribute to the poor ability of Brattleboro rats to cope with rapid haemorrhage.

4. After prolonged venous haemorrhage in the absence of naloxone, there was no difference between the recovery of blood pressure in Brattleboro rats and Long Evans rats. Naloxone improved blood pressure recovery to a similar extent in both strains of rat. These findings suggest that the absence of vasopressin does not impair blood pressure recovery after prolonged haemorrhage.

Key words: Brattleboro rats, haemorrhage, naloxone, vasopressin.

Introduction

Since haemorrhage is a potent stimulus for vasopressin release [1, 2], it is possible that the vasoconstrictor effect of this peptide is important in maintaining blood pressure during acute hypovolaemia [2]. This suggestion is consistent with the observation that, in rats with a congenital inability to synthesize vasopressin (Brattleboro strain), blood pressure recovery from rapid arterial haemorrhage is slower in the anaesthetized state than in control (Long Evans) rats, and that acute administration of vasopressin to Brattleboro rats abolishes the disorder [3].

Apart from releasing vasopressin, haemorrhage also causes release of pituitary β-endorphins, which may depress cardiovascular function [4, 5]. After haemorrhage, blood pressure recovery may, therefore, depend on a combination of the positive effects of vasopressin and the negative effects of endorphins. If so, it is feasible that, in the absence of vasopressin, the unopposed depressor effect of the β-endorphins might prolong the hypotension produced by haemorrhage. To test this possibility we have studied the effects of the opiate antagonist naloxone on blood pressure recovery in Brattleboro rats and in Long Evans rats after haemorrhage.

The original experiments describing blood pressure recovery after haemorrhage in Brattleboro rats utilized rapid arterial bleeding as the stimulus [3]. However, the experiments concerned with the involvement of endorphins in cardiovascular
responses to haemorrhage [4, 5] involved prolonged hypotension achieved by venous bleeding (in Sprague-Dawley rats). We have therefore used both experimental protocols in the present work.

Methods

Rats

Male homozygous Brattleboro rats (body weight 270-320 g) and age-matched male Long Evans rats (body weight 350-380 g) were used in this study. Long Evans rats were used as control animals because this is the strain from which the Brattleboro rat developed. It is well known that age-matched Long Evans and Brattleboro rats have different body weights, but this does not appear to be reflected in proportional differences in extracellular fluid volumes [6]. A group of male Wistar rats was also included in a preliminary study.

All catheters were implanted under barbiturate anaesthesia (sodium methohexitone, Brietal; Lilly; 60 mg/kg intraperitoneally) at least 5 h before the experiments were begun; at this time animals were fully conscious and the effects of the anaesthetic had worn off [7]. Arterial blood pressures were measured from a catheter implanted in the abdominal aorta via the caudal artery; details of the design of the catheter and recording system, which permits accurate recording of systolic and diastolic pressures, have been described elsewhere [7]. Heart rate was derived from the blood pressure recording (Devices heart rate meter). Drugs were administered via a catheter in the right jugular vein.

Two different experiments were performed: one involved rapid arterial bleeding [3] and the other involved more prolonged venous bleeding [4, 5].

Preliminary studies

Rapid haemorrhage can only be readily and reproducibly performed by bleeding from a carotid arterial catheter. However, since such a catheter occludes one carotid artery, it is necessary to ensure that animals with unilateral carotid occlusion still show baroreflex responses. We have previously shown that Brattleboro rats do not differ from Long Evans rats in their pulse-interval responses to a fall in arterial pressure induced by glyceryl trinitrate [8]. Furthermore, there is no difference between baroreflex responses obtained in Wistar and Long Evans rats (Bennett, T. & Gardiner, S. M., unpublished work). For these reasons and since Wistar rats are more readily available and very much less expensive than Long Evans rats, we therefore performed the preliminary experiments on a group of 16 male Wistar rats (body weight 270-300 g); in eight animals, the left carotid artery was ligated. To assess the adequacy of the baroreflex responses, the changes in pulse interval during increases or decreases in arterial blood pressure (induced by methoxamine, 0.4 mg/ml, 0.2 ml/min for 15 s, or glyceryl trinitrate, 0.8 mg/ml, 0.2 ml/min for 15 s, respectively) were measured. The slope of the line relating systolic blood pressure to the pulse interval of the succeeding beat was obtained by regression analysis and was used as an index of baroreflex sensitivity [7]. There was no significant difference between the baroreflex responses to an increase in pressure (control, 1.17 ± 0.07 ms/mmHg; carotid ligation, 0.97 ± 0.4 ms/mmHg), but the responses to a decrease in pressure were significantly (0.05 < P > 0.02) less in the animals with unilateral carotid ligation (control, 0.92 ± 0.09 ms/mmHg; carotid ligation, 0.68 ± 0.06 ms/mmHg). Nevertheless, these preliminary experiments demonstrated that animals with unilateral carotid ligation still showed competent baroreflexes, so we felt justified in proceeding to the main experiment, using a carotid arterial catheter to bleed from.

Experiment 1: blood pressure and heart rate responses to rapid arterial haemorrhage

Fifteen male Brattleboro rats and 13 Long Evans rats were used. Baseline measurements of systolic and diastolic blood pressures and heart rates were made for 20 min whilst the animals were undisturbed. A bolus intravenous injection of naloxone (2 mg/kg dissolved in isotonic sodium chloride solution: saline) or an equivalent volume of saline (0.1 ml flushed in with a further 0.1 ml) was then given, followed by a slow infusion of either the drug (2 mg h⁻¹ kg⁻¹) or saline (1.5 ml/h) for 40 min. Blood was then rapidly withdrawn from a catheter in the left carotid artery until systolic blood pressure fell to approx. 70 mmHg; the volume of blood withdrawn was noted. Blood pressures and heart rates were then recorded continuously for the following 60 min whilst the infusions were continued.

Experiment 2: blood pressure and heart rate responses to prolonged venous haemorrhage

Twelve Brattleboro rats and ten Long Evans rats were used. After a 20 min period of control recordings, blood was withdrawn from a catheter in the right jugular vein until mean arterial
pressure fell to approx. 50 mmHg; the arterial pressure was then kept at this level for 20 min by intermittent bleeding and the total volume of blood withdrawn was noted. Naloxone (3 mg/kg) or an equivalent volume of saline (0.1 ml flushed with a further 0.1 ml) was then administered intravenously through a second catheter in the right jugular vein and blood pressures and heart rates were monitored for the following 120 min.

**Statistical analysis**

Results are expressed as the mean ± SEM; n is the number of animals. Analysis of variance was used to compare results across different groups and treatments. Individual differences were tested for statistical significance by using the Mann-Whitney U-test (unpaired) or Wilcoxon rank-sum test (paired) as appropriate.

**FIG. 1.** Effect of rapid arterial haemorrhage (H) on arterial blood pressures and heart rates in Long Evans rats and Brattleboro rats in the presence of saline or naloxone. In the presence of saline, blood pressure recovery was slower in Brattleboro rats (b; n = 7) than Long Evans rats (a; n = 6); in the latter, blood pressures had returned to resting levels within the first 20 min after blood withdrawal, whereas the Brattleboro rats were still hypotensive at the end of the experiment. Naloxone did not influence blood pressure recovery in Long Evans rats (c; n = 7), but improved blood pressure recovery in Brattleboro rats (d; n = 8). Despite the beneficial influence of naloxone, the Brattleboro rats were still hypotensive at the end of the experiments. Values are means ± 1 SEM.
Results

Experiment 1: blood pressure and heart rate responses to rapid arterial haemorrhage

There were no significant differences between the arterial blood pressures in Brattleboro rats and Long Evans rats at the start of the experiment (Fig. 1); resting heart rate was significantly (0.01 > P > 0.001) higher in the Brattleboro rats.

Saline-treated rats. The bolus injection of saline, followed by the slow saline infusion, did not affect baseline measurements (Fig. 1). In order to achieve the same fall in arterial blood pressure (Figs. 1a and 1b), it was necessary to take less blood from the Brattleboro rats (4.3 ± 0.3 ml; n = 7) than from the Long Evans rats (7.1 ± 0.6 ml; n = 6; P < 0.001).

Blood pressure in the Long Evans rats returned to resting levels within 20 min after bleeding (Fig. 1a), whereas the Brattleboro rats still showed a significant hypotension (0.01 > P > 0.001) 60 min after blood withdrawal (Fig. 1b). The heart rate response to haemorrhage was variable, some animals showing a tachycardia and others a bradycardia.

Naloxone-treated rats. The bolus injection and subsequent infusion of naloxone did not affect arterial blood pressures, but caused a significant reduction in heart rate in Brattleboro rats (P < 0.001; n = 8) and Long Evans rats (0.02 > P > 0.01; n = 7; Figs. 1c and 1d). As before, in order to achieve a similar fall in arterial blood pressure (Figs. 1c and 1d), it was necessary to take less blood from the Brattleboro rats (3.8 ± 0.2 ml; n = 8) than from the Long Evans rats (7.2 ± 0.2 ml; n = 7; P < 0.001). Within a group, there was no significant difference between the volumes withdrawn in the presence of naloxone or saline.

Blood pressure recovery was not affected by naloxone in Long Evans rats (Fig. 1c). In the Brattleboro rats given naloxone, systolic and diastolic blood pressures were significantly (P < 0.05) higher than in the group given saline at all time points from 20 min after haemorrhage (Fig. 1d). However, there was still a significant hypotension (0.05 > P > 0.02) at the end of the experiment (Fig. 1d).

Experiment 2: blood pressure and heart rate responses to prolonged venous haemorrhage

Before haemorrhage, there were no significant differences in the arterial blood pressures of the Long Evans rats compared with the Brattleboro rats, but the latter had significantly (0.02 > P > 0.01) higher heart rates (Fig. 2). During the period of haemorrhage, heart rate was significantly (P < 0.001) reduced in both groups but the change was greater in the Brattleboro rats than in the Long Evans rats (Fig. 2).

To reduce mean arterial pressure to 50 mmHg, in the Brattleboro rats 2.7 ± 0.3 ml of blood had to be withdrawn and this took 2.9 ± 0.3 min, whereas in the Long Evans rats significantly more blood (6.3 ± 0.3 ml; P < 0.001) needed to be withdrawn and this procedure took 7.4 ± 0.5 min; thus the rate of bleeding was similar in the two strains (Brattleboro = 0.93 ml/min; Long Evans = 0.85 ml/min). To keep mean arterial pressure at 50 mmHg for the subsequent 20 min, similar volumes needed to be taken from the Brattleboro (1.5 ± 0.2 ml) and Long Evans (1.7 ± 0.2 ml) rats. Thus the total volume of blood withdrawn from the Brattleboro rats (4.1 ± 0.3 ml) was significantly (P < 0.001) less than that taken from the Long Evans rats (8.0 ± 0.3 ml).

Saline-treated rats. There was no significant difference in the recovery of blood pressure in the Long Evans rats given saline from that of the Brattleboro rats given saline (Figs. 2a and 2b); both groups of animals were still significantly hypotensive (P < 0.05) 120 min after the end of the bleed.

Naloxone-treated rats. Naloxone improved the blood pressure recovery after haemorrhage in Long Evans rats and Brattleboro rats to a similar extent (Figs. 2c and 2d). At all time points starting 1 min after drug administration, the arterial pressures were significantly (P < 0.05) higher in the groups given naloxone than in the groups given saline. Naloxone did not influence the heart rate during the recovery of blood pressure after haemorrhage (Figs. 2c and 2d).

Discussion

In the present work we considered two aspects of the response of conscious Brattleboro and Long Evans rats to haemorrhage: (i) the sensitivity to haemorrhage, i.e., the volume of blood that had to be withdrawn to reduce systemic arterial blood pressure to a particular level, and (ii) the recovery of arterial pressure after its reduction by removal of blood.

Sensitivity to haemorrhage

The body weight, the volume of blood withdrawn and the change in blood pressure during a haemorrhage all affect the level of "shock" experienced by the animal [5]. In experiments on hypophysectomized rats that were lighter and had a lower mean arterial blood pressure in the resting state than did sham-operated rats,
Holaday et al. [5] adjusted the level to which mean arterial pressure was reduced, by bleeding the two groups in such a way that all animals experienced the same 'shock index' [expressed as volume removed (100 g body weight)$^{-1}$ (mmHg fall in mean arterial pressure)$^{-1}$. Although, in the present study, the Brattleboro rats were lighter than the Long Evans rats, mean arterial pressures in the resting state were not different in the two groups. Thus it seemed reasonable to bleed the animals to the same level of blood pressures and to compare their responses. When this was done, it was found that Brattleboro rats were more sensitive than Long Evans rats to arterial and to venous haemorrhage, inasmuch as less blood needed to be withdrawn from the Brattleboro rats than from the Long Evans rats to achieve similar reductions in arterial blood pressure. One plausible explanation for this finding is that the congenital absence of vasopressin leads to a chronic state of dehydration and hence a reduced blood volume; the smaller volume removed from the Brattleboro rats might, therefore, have represented a percentage of the blood volume similar to that removed from the control animals. However, Friedman & Friedman [7] have shown that extracellular fluid volume (expressed per 100 g body weight) is higher in Brattleboro than in Long Evans rats. If their data are applied to our animals, then the mean extracellular fluid volume of the Brattleboro rats in the
present study was less than that of the Long Evans rats (50 vs 59 ml/rat), but this difference was much smaller than the difference in volumes of blood withdrawn to cause similar degrees of hypotension. On the basis of values estimated from Friedman & Friedman's [7] data, the Brattleboro rats experienced a haemorrhage amounting to 31% of their blood volume, whereas in Long Evans rats the corresponding value was 43%.

It is possible that the greater sensitivity of Brattleboro rats to haemorrhage was due to the absence of the pressor action of vasopressin. However, at present, it is not clear to what extent reduced [9] or increased [10] activation of the renin-angiotensin (ANG II) system contributed to the response seen.

**Recovery from haemorrhage**

After blood had been rapidly withdrawn from the carotid artery, blood pressure recovery was slower in Brattleboro rats than in Long Evans rats. In the latter, there was no evidence of an effect of naloxone on the response to haemorrhage, whereas in the Brattleboro rats, naloxone improved blood pressure recovery. This indicates that, in part, the poor ability of these animals to recover from the effects of rapid bleeding might have been due to an unopposed depressor action of endorphins. But, even in the presence of naloxone, blood pressure recovery was slower in the Brattleboro rats than in the Long Evans rats. This could be taken to mean that the residual defect in blood pressure recovery was due to the absence of vasopressin. However, differential activation of the renin-ANG II system [9, 10] might have contributed to the differences in the recovery from haemorrhage seen in Brattleboro and Long Evans rats. Furthermore, since Pendleton et al. [11] have provided some evidence that adrenaline may function as an important central neurotransmitter in the cardiovascular response to haemorrhage, and others [12] have shown reduced concentrations and metabolism of adrenaline in several brain nuclei of Brattleboro rats, then a disturbance in this system could affect the recovery of blood pressure after haemorrhage.

The question which arises from the experiments in which haemorrhage was performed rapidly is why a reduction of arterial blood pressure to the same level appeared to cause opioid release in Brattleboro rats but not in Long Evans rats. The most likely explanation of the difference is that although blood pressures were reduced to a similar level in the two groups, the duration of the stimulus experienced by them was different. In the Brattleboro rats, blood pressures remained low for a longer period of time than in Long Evans rats. Thus it is feasible that blood pressures must be low for some time before endorphins are released. This is consistent with our finding that a beneficial effect of naloxone in Brattleboro rats was not apparent until 20 min after arterial haemorrhage. Furthermore, in the second experiment, when both groups of animals experienced 20 min of hypotension before receiving naloxone, there was no difference between the two strains in the extent to which the opioid antagonist improved blood pressure recovery.

Two other important observations from the experiments in which the hypotension was more prolonged were: (i) after the initial haemorrhage, there was no significant difference between the extra volumes of blood removed (to keep mean arterial blood pressure at 50 mmHg or 20 min) from Brattleboro and Long Evans rats, and (ii) there was no difference between the pattern of blood pressure recovery in the two strains of rat. These findings suggest that vasopressin plays no role in the adaptation to prolonged haemorrhage, and are consistent with the demonstration in dogs [13] and rats [14] that the peak plasma levels of vasopressin occur within the first few minutes after the start of a haemorrhage and thereafter decline, despite continued hypotension [14], owing to exhaustion of the neurohypophysial pool of vasopressin [15]. On the basis of those observations, Feuerstein et al. [14] concluded that “a pressor effect of vasopressin due to high plasma concentrations is not anticipated during protracted hypovolaemic-hypotensive stimuli”.

The heart rate responses to haemorrhage which we observed deserve some comment. During the rapid arterial bleed there was a prompt reflex tachycardia in both strains of rat and thereafter some animals showed a sustained tachycardia, whereas in others there was a bradycardia. This bimodal distribution of heart rate response to haemorrhage in rats has been reported previously [4, 16, 17], and may depend to some extent on the strain of rat, volume withdrawn and prevailing autonomic balance. The prolonged hypotension after venous haemorrhage consistently produced a bradycardia in all animals, the change being markedly greater in the Brattleboro rats. Sjostrand [18] proposed that the bradycardia caused by haemorrhage (in Sprague-Dawley rats) was due to excitation of cardiac vagal afferent fibres which stimulated the release of vasopressin, the latter causing bradycardia through excitation of the hypothalamic depressor area [18]. However, the central component of this proposed reflex cannot explain the profound bradycardia which occurred in our Brattleboro rats.
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References


