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NEUROHUMORAL MECHANISMS AND THE ROLE OF ARTERIAL BARORECEPTORS IN THE RENO-VASCULAR RESPONSE TO HAEMORRHAGE IN RABBITS

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SUMMARY

1. Conscious rabbits, with implanted renal artery Doppler flow probes were bled at a constant rate (4 ml min^{-1}). We assessed the contribution of autonomic, hormonal and local factors to the renal vasoconstrictor response to 20% loss of blood volume (BV) and the role of the sinoaortic baroreceptors in the neurohumoral response.

2. With intact autonomic effectors, 20% BV loss was associated with a small fall in vascular conductance, which was completely unaffected by inhibition or blockade of the combined effects of the two major pressor hormones angiotensin II (AII) and arginine vasopressin (AVP). Combined blockade of the autonomic effects plus those of the two pressor hormones resulted in marked elevation of vascular conductance, considered to be due to the local effects of haemorrhage. This response provided the baseline for assessing the constrictor response in the intact animal which, during 20% BV loss, was entirely due to reflex activity through the sympatho-adrenal system.

3. In contrast to the early phase of haemorrhage (< 20% BV removal) both hormones played a role in the maintenance of mean arterial pressure immediately after haemorrhage and in the maintenance of renal vascular tone. This suggested that the contribution by hormones occurs only after more pronounced blood loss and hypotension.

4. In the presence of autonomic blockade with mecamylamine plus methscopolamine (plus a constant infusion of noradrenaline to maintain resting blood pressure) the renal vasoconstrictor response was similar to that of the intact animal. We have previously found that this regime is associated with greatly enhanced release of AVP and plasma renin activity. Sinoaortic denervation had no effect on this hormonally mediated vasoconstriction.

5. When the autonomic nervous system was intact but the effects of AII and AVP were blocked to prevent the accentuated hormonally mediated vasoconstriction, sinoaortic denervation completely abolished the normal autonomic renal constrictor response, which is thus largely under control of the arterial baroreceptors.

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INTRODUCTION

Recent work from our laboratory assessed the autonomic, hormonal and local factors involved in the hindlimb and mesenteric blood flow response to haemorrhage in conscious rabbits using various combinations of hormonal blockade and total autonomic blockade (Korner, Oliver, Zhu, Gipps & Hannenman, 1990). During haemorrhage at a constant rate, up to removal of 20% of the blood volume, regulation was entirely through neural autonomic mechanisms rather than through angiotensin II, vasopressin and autoregulation. The roles of these mechanisms on the vasculature of the kidney were not examined. We wondered whether the kidney vasculature might be more susceptible (compared to the hindquarter or mesenteric beds) to the vasoconstrictor action of angiotensin II and perhaps also vasopressin released during haemorrhage. One purpose of this study therefore, was to examine the importance of autonomic neural mechanisms and of the two major pressor hormones on the renal vascular response to haemorrhage. In addition, we examined the role of the sinoaortic baroreceptors in the neurohumoral response during and immediately after haemorrhage.

We have shown previously that in conscious rabbits during total autonomic blockade (TAB) there is an augmented hormonal (vasopressin and angiotensin II) response to haemorrhage (Oliver, Korner, Woods & Zhu, 1990). In the present study we have used this as an 'augmented hormone secretion model' to investigate the hormonal component of the renal vasoconstrictor response during haemorrhage and to assess the role of the sinoaortic baroreceptors in this response.

METHODS

Animals and operations

All rabbits underwent a preliminary operation under halothane anaesthesia after induction with Pentothal sodium (10–15 mg kg^{-1}) for the implantation of a renal Doppler flow probe. The flow probe was enclosed in a dacron cuff as described elsewhere (Wright, Angus & Korner, 1987). The left renal artery was isolated through a mid-line incision and, with the aid of a dissecting microscope, the visible renal nerves were dissected free from a 1 cm length of the renal artery. The Doppler cuff was placed around the cleared section of the renal artery and the Doppler wire was tunnelled under the skin and buried in the mid-iliac region. The incision was closed and the rabbit returned to the animal house for a 2 week recovery period.

Following this recovery period the rabbits were subjected to a second operation (same induction and anaesthesia as above) which involved a sinoaortic denervation (SAD) or sham denervation (Chalmers, Korner & White, 1967) and installation of an inferior vena cava (IVC) 'bleed' cannula. The IVC cannula was filled with a mixture of 10% (Macrodex-70) dextran saline containing heparin and the end of the cannula was tunnelled subcutaneously and buried in the mid-iliac region. The rabbits were then allowed a further 1 week recovery period before the first experiment.

Haemodynamic measurements

Mean arterial pressure (MAP) was measured with a Statham P23dc transducer, and heart rate (HR) with a heart rate meter. We used the Iowa pulsed Doppler system (Haywood, Shaffer, Fastenow, Fink & Brody, 1981) to measure renal blood flow (RBF), which was recorded in kHz Doppler shift. The analog signals for each variable were digitized in an Olivetti computer and stored on disc for subsequent processing and data analysis as described earlier (Korner *et al.* 1990). We used the nasopharyngeal reflex to test the integrity of the sympathetic innervation to the renal artery – a puff of cigarette smoke transiently reduced renal blood flow and conductance to close to zero. This dramatic vasoconstriction was due to an increase in sympathetic nerve activity and was completely abolished by total autonomic blockade.

Blood volume relationships

An on-line computer program digitized the phasic and mean arterial pressure (MAP), renal blood flow (RBF) and heart rate (HR) signals every 40 ms. Renal vascular conductance (RVC) units were calculated as the ratio of (RBF/MAP × 100). The values of all variables were averaged every 20 s over the course of the experiment. The blood volume (BV) was withdrawn at a constant rate in every experiment, and an on-line program calculated the relationship between the nominal BV (initial BV − BV withdrawn) and the circulatory variables throughout bleeding. The initial BV was calculated as 50 ml kg^{-1} (Korner *et al.* 1990). The nominal BV was then expressed as a percentage of the starting BV (100%) on the day we determined the relationship between BV and MAP, HR, RBF and RVC. The BV–circulatory response during the initial phase of haemorrhage (−20% BV) and after total autonomic blockade were adequately described by linear regression equations of the type $Y = a + b$ BV where Y = variable; BV is the nominal BV expressed as a percentage of the starting BV; b = slope of the regression of Y on BV; a = intercept of the regression line at zero BV.

Plan of study and protocol

The study was approved by the Baker Institute–Alfred Hospital Animal Ethics Committee, as conforming to the 'Australian Code of Practice for the Care and Use of Animals for Scientific Purposes' of the Australian National Health and Medical Research Council, the Commonwealth Scientific and Industrial Research Organization and the Australian Agricultural Council.

Rabbits were divided into two groups: those with intact sinoaortic baroreceptors (intact SAB, $n = 8$) and those which had previously undergone sinoaortic denervation (SAD, $n = 6$). Both groups of rabbits underwent two experiments, approximately 7–10 days apart, in which they were subjected to four different combinations of efferent blockade (two combinations per experiment) as follows: control, no treatment; HB, hormonal blockade; TAB, total autonomic blockade; and TAB + HB, combined TAB and HB.

On each experimental day the rabbits underwent minor operative procedures under local anaesthesia (Xylocaine, 2%) for the retrieval of Doppler wires, catheters and for the cannulation of the central ear artery and vein. The rabbits were then allowed a 60 min rest period, followed by pharmacological induction of the effector blockade. In animals subjected to total autonomic blockade with mecamylamine and methscopolamine, resting blood pressure was maintained close to the animal's pressure level under normal (control) conditions, as described previously (Korner *et al.* 1990).

The protocol consisted of (1) a 5 min pre-haemorrhage control period, (2) a haemorrhage period lasting 5–12 min, (3) an interval period of 2 min and (4) a reinfusion period of 5–12 min, with recording continued for a further 15 min. The second haemorrhage began approximately 120 min later, when the rabbits' circulatory variables were close to initial control values. In all experiments, the rabbits were bled and blood was subsequently reinfused at a fixed rate of approximately 4 ml min^{-1}, using a Harvard Instruments Series 960 Infusion/Withdrawal pump.

The rabbits tolerated bleeding well and in none of the preparations was there any sign of distress, either during haemorrhage or reinfusion. During the haemorrhage phase, bleeding was stopped as soon as the blood pressure had definitely fallen below 50 mmHg (i.e. to about 45–48 mmHg). The reason why the procedure was so well tolerated by the rabbits may have been the relatively brief period during which blood pressure was low; in most animals it tended to rise spontaneously soon after haemorrhage was stopped (see Results; *Post-haemorrhage response*). Reinfusion began after the interval phase of 2 min, which was necessary to thoroughly mix the heparinized blood withdrawn during bleeding. One hour after reinfusion the rabbit received 10 mg protamine zinc sulphate and heparinized Dextran–saline as described previously (Oliver *et al.* 1990). In between experimental days the rabbits remained in good health, moving about their cage similarly to unoperated rabbits and eating and drinking normally. In the present series they maintained their body weight, which averaged 2·6 ± 0·1 kg ($n = 14$) at both the start and the end of the study. The present group of rabbits looked similar in appearance to those of our earlier study, where over a 5–6 week experimental period the animals showed a 3–4% increase in body weight and maintained haematocrit within 1–2% of the starting value.

Drugs

Hormonal blockade was produced with the converting-enzyme inhibitor captopril (CEI; E. R. Squibb & Sons; 10 $\mu g\ kg^{-1}\ min^{-1}$ I.V.) and the arginine vasopressin (AVP) antagonist pen-

icillamine-*o*-methyl tyrosine AVP (AVPA, Bachem Inc. Torrance, CA, USA; 30 μg hr^{-1} I.V.). In conscious rabbits this dose of captopril shifted the AII–blood pressure dose–response curve 100-fold, while the dose of the AVP vascular antagonist abolished the pressor response to a bolus dose of 100 ng AVP (Woods, Oliver & Korner, 1989). Angiotensin II (AII; 5 ng kg^{-1} min^{-1} I.V.) was infused to establish a baseline level of AII in the captopril-treated rabbits. This was to ensure MAP and renal haemodynamics were similar to pre-blocked levels. AVP was not infused in the HB rabbits since it has only a modest direct renal vasoconstrictor effect and reducing AVP to zero levels has no appreciable effect on renal haemodynamics.

Total autonomic blockade was produced with mecamylamine (Mevasive, Merck Sharp & Dohme), 10 mg kg^{-1} min^{-1} and methscopolamine (Upjohn Pty, Ltd), 1·0 μg kg^{-1} min^{-1}. To compensate for the fall in arterial pressure which accompanied infusion of mecamylamine, we infused noradrenaline (Levophed, Stearns). The dose of noradrenaline was chosen for each rabbit such that arterial pressure was restored to levels which were similar to the pre-mecamylamine level (range, 0·1–0·25 μg kg^{-1} min^{-1}). This was in an effort to maintain the same pre-haemorrhage blood pressure treatment between different treatment groups. The rabbits which underwent combined TAB + HB treatment received mecamylamine, methscopolamine, captopril and AVPA as described above. Blood pressure was maintained with noradrenaline and AII (as above).

Data analysis

For analysis of the data we used linear regression and two-way analysis of variance. The individual degrees of freedom were orthogonally partitioned to assess the statistical significance of differences of the various treatment combinations within animals (Snedecor & Cochran, 1980).

RESULTS

Baseline data

The baseline pre-haemorrhage values of mean arterial pressure (MAP), heart rate (HR), renal blood flow (RBF) and renal vascular conductance (RVC) in two groups of rabbits with different combinations of efferent blockade are summarized in Table 1. The rabbits in group I had intact sinoaortic baroreceptors (intact SAB). During hormonal blockade (HB) all the variables were similar to those in rabbits which received no treatment (control). Total autonomic blockade (TAB), however, significantly increased baseline HR and RVC. The resting MAP in rabbits with combined TAB + HB was slightly, although not significantly, higher than the other treatment groups. This was probably due to the background noradrenaline and AII infusions, which were given to restore MAP to close to pre-blocked levels. The rabbits which had undergone sinoaortic denervation (group II, SAD) experienced no significant alterations in haemodynamic variables with any of the combinations of efferent blockade.

Haemorrhage data

In general we have compared the various haemodynamic response patterns under the different conditions of efferent and afferent activity at 80% BV, because this level of haemorrhage was within the data limits of all the preparations. Because of differences in control mechanisms the BV withdrawn differed in the various preparations, as explained below.

Figure 1 and Table 2 contain averaged data collected throughout the entire bleed period and have been included to emphasize the haemodynamic pattern during bleeding (Fig. 1) and the time course of blood pressure maintenance under different conditions of blockade. In all rabbits blood was withdrawn at a constant rate until MAP fell from a control value of approximately 85 mmHg (1 mmHg = 0·133 kPa) to

a level below 48 mmHg (see Methods; *Plan of study and protocol*). In rabbits with intact sinoaortic baroreceptors the time taken to reach that level of hypotension varied between combinations of efferent blockade (Table 2). In control rabbits, blood pressure was well maintained until 30–40% BV had been removed; then it fell abruptly (Fig. 1). On average, under these conditions, bleeding was stopped after

TABLE 1. Baseline haemodynamic variables in two groups of conscious rabbits during four combinations of efferent blockade

	Control	HB	TAB	TAB+HB	±S.E.D.
Group 1 (intact SAB)					
MAP (mmHg)	85·2	86·0	84·6	96·2	4·7
HR (beats min^{-1})	224·9	220·3	292·9*	291·0*	11·4
RBF (kHz)	9·6	8·6	11·7	9·1	1·3
RVC (units)	11·4	10·1	13·9*	9·6	1·0
Group 2 (SAD)					
MAP (mmHg)	102·1	105·2	92·1	90·3	7·4
HR (beats min^{-1})	279·9	285·7	261·6	273·6	16·1
RBF (kHz)	7·8	7·6	9·3	8·6	0·9
RVC (units)	7·8	6·9	10·4	9·1	1·4

The four combinations of efferent blockade were as follows: control, no treatment; HB, hormonal blockade; TAB, total autonomic blockade; TAB+HB, combined total autonomic and hormonal blockade. Rabbits were divided into two groups; group I had intact sinoaortic baroreceptors (intact SAB) while group II had undergone sinoaortic denervation (SAD). RVC was calculated as follows: RBF/MAP × 100. S.E.D. is the standard error of the difference calculated from the ANOVA of the different treatment groups. *$P < 0·05$ as compared to control.

11·2 min (Table 2), when 44·8 ml of blood had been withdrawn. This amounted to 34·5% BV (assuming an average BV of 50 ml kg^{-1}). In rabbits with hormonal blockade (HB) the bleeding time was very similar to that seen in control rabbits (Table 2). However, during TAB or TAB+HB, blood pressure declined more rapidly right from the start of haemorrhage (Fig. 1, TAB); bleeding was not stopped earlier (Table 2) with removal, on average, of 29% BV (TAB) and 25% BV (TAB+HB). In sinoaortically denervated rabbits, about 30% BV was removed under control, HB and TAB conditions (Table 2). But with combined TAB+HB the fall in pressure was more rapid, accounting for the shorter bleeding time (Table 2), which corresponded to removal of 19·4% BV. The haematocrits were measured immediately prior to the bleed and at the end of the bleed (in all groups) to give an estimate of fluid shift in each animal and are shown in Table 3.

Under control conditions, the good maintenance of arterial pressure was accompanied by a rise in heart rate and a fall in RVC, the latter signifying renal vasoconstriction (Fig. 1, control). Following the abrupt fall in pressure, at the end of this phase, there was an increase in conductance, indicating that vasodilatation had supervened. After TAB there was no appreciable change in HR, a pronounced fall in RBF, and RVC fell after an initial rise early in the haemorrhage (Fig. 1). The haemodynamic pattern exhibited by this rabbit was characteristic of the general response to haemorrhage in control and TAB rabbits with one exception; in TAB rabbits the initial rise in RVC was not always present and the fall in RVC was often more pronounced.

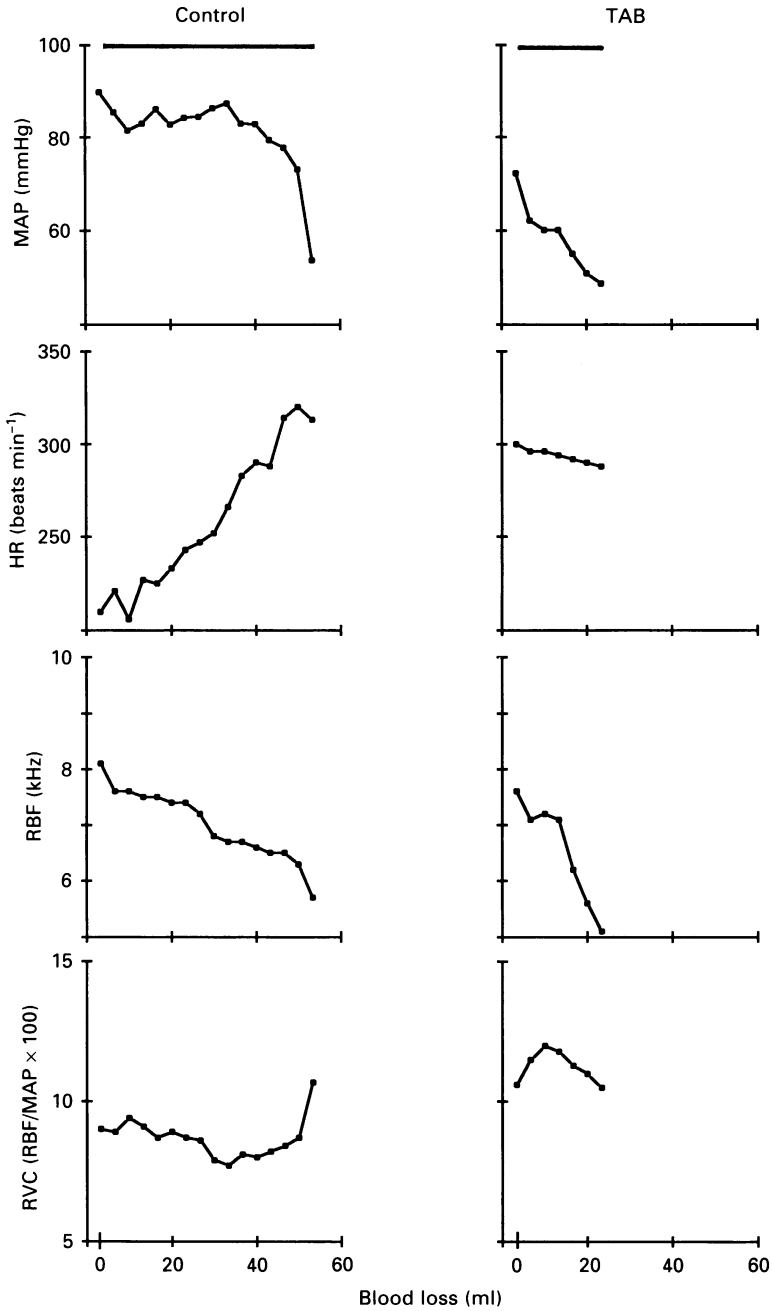

Fig. 1. Haemodynamics variables in two representative rabbits during blood loss at a constant rate of 4 ml min^{-1}; one with no treatment (control) and one after total autonomic blockade (TAB). The bars at the top of the panels represent the duration of the blood loss. Measured variables were mean arterial pressure (MAP), heart rate (HR), renal blood flow (RBF) and renal vascular conductance.

TABLE 2. Bleed time (min) in conscious rabbits to decrease arterial pressure to below 48 mmHg

	Bleed time (min)				
	Control	HB	TAB	TAB+HB	±S.E.D.
Intact SAB	11·2	11·1	9·3*	8·0*	1·2
SAD	9·7	9·0	10·5	6·3*	1·3

Rabbits were bled at a constant rate of $\simeq 4$ ml min^{-1} in four separate haemorrhages during four combinations of efferent blockade. The explanation of groupings (Intact SAB or SAD) and the four combinations of efferent blockade are as in Table 1. S.E.D. is the standard error of the difference calculated from ANOVA of the different treatment groups. $*P < 0{\cdot}05$ as compared to control.

TABLE 3. Haematocrits (%) measured in conscious rabbits before (B) and immediately after (A) haemorrhage

	Haematocrit (%)								
	Control		HB		TAB		TAB+HB		±S.E.D.
	B	A	B	A	B	A	B	A	
Intact SAB	35·0	31·1*	34·7	29·6*	32·3	29·2*	33·8	31·2*	0·94
SAD	37·5	32·5*	35·8	30·5*	35·1	31·2*	32·3	30·5*	0·97

Rabbits were bled at a constant rate of 4 ml min^{-1} in four separate haemorrhages under four combinations of efferent blockade. The explanation of groupings (Intact SAB or SAD) and the four combinations of blockade are as in Table 1. S.E.D. is the standard error of the difference calculated from the ANOVA of the different treatment groups. $*P < 0{\cdot}05$ A as compared to B.

Autonomic component of the response to haemorrhage

The haemodynamic variables in the following figures (except 3 and 6) have been calculated at 80% BV (or 20% BV removal). The value of 20% was chosen because this level of haemorrhage was within the data limits of all the preparations. In rabbits which received no treatment (control), MAP was well maintained and accompanied by a fall in RVC during haemorrhage of 20% of the blood volume (Fig. 2). Hormonal blockade (HB) did not alter the haemodynamic response to withdrawal of 20% of the blood volume. Combined total autonomic blockade plus hormonal blockade (TAB+HB) resulted in a significantly greater fall in MAP and a rise in RVC as compared to the rabbits which received no treatment (control). The combined TAB+HB represented the response to removal of 20% of the blood volume which was used as the baseline for assessing the magnitude of the autonomic component to maintenance of MAP and the renal vasoconstrictor response (Fig. 2). With respect to the renal vascular conductance one can calculate the estimated renal constrictor response as the difference between control and TAB+HB rabbits or $152-95 = 57$ estimated constrictor units (ECU). This value was very similar to that after hormonal blockade when it averaged $152-94 = 58$ ECU. Thus under normal conditions the constriction was entirely due to neural autonomic activity.

Renal blood flow (RBF) fell significantly ($89{\cdot}9 \pm 2{\cdot}4$% of control) during haemorrhage of 20% of the blood volume and was not significantly affected by hormonal blockade (HB, $88{\cdot}1 \pm 2{\cdot}2$%). Both TAB and combined TAB+HB further

augmented the fall in RBF during this stage of haemorrhage (73·9 ± 5·1; 66·5 ± 7·9 % respectively).

Post-haemorrhage response

With the present protocol haemorrhage was terminated once the mean arterial pressure had fallen to a level below 48 mmHg. During the 2 min period immediately

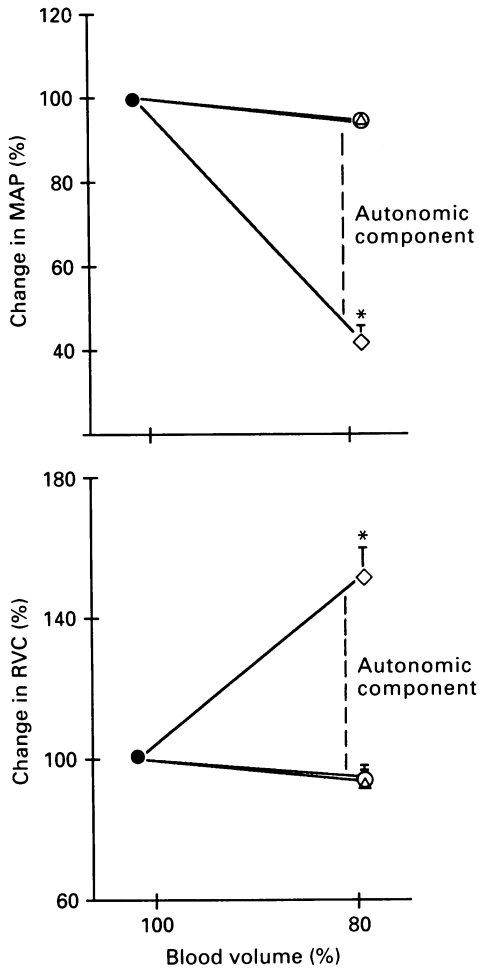

Fig. 2. The percentage change (from pre-haemorrhage values) in MAP and RVC during removal of 20% of the blood volume. Points were calculated from a linear regression equation generated during the haemorrhage (see Methods). Conditions of efferent blockade were as follows: ○, no treatment; △, HB (hormonal blockade); ◇, TAB + HB (combined total autonomic blockade and hormonal blockade). With respect to RVC, values under 100% represent vasoconstriction and over 100% represent vasodilatation. The difference in the MAP or RVC between control (no treatment) and TAB + HB is that due to the action of the autonomic nervous system. Same abbreviations as Fig. 1. $*P < 0{\cdot}05$ as compared to control. Values represented as means + S.E.M. throughout.

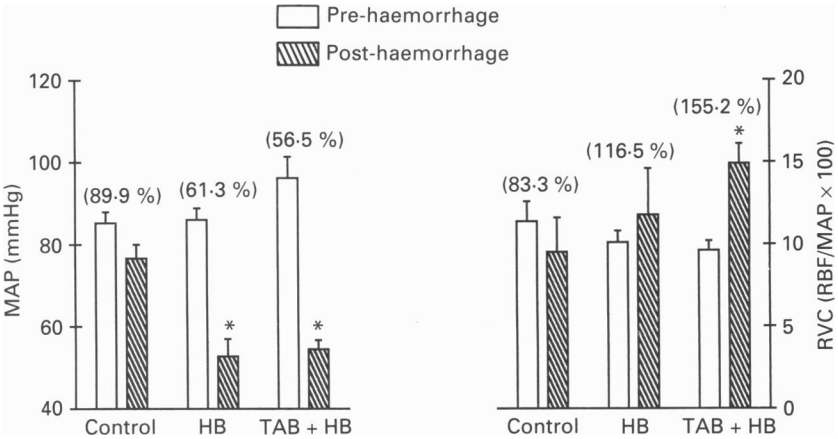

Fig. 3. The average MAP and RVC measured over 5 min prior to the beginning of haemorrhage and in the 2 min post-haemorrhage interval period. The rabbits were studied under the following conditions of efferent blockade: control, HB and TAB+HB. The values in parentheses represent the percentage change from the pre-haemorrhage control as measured during the 2 min post-haemorrhage interval. Abbreviations as from Figs 1 and 2. $*P < 0.05$ as compared to pre-haemorrhage.

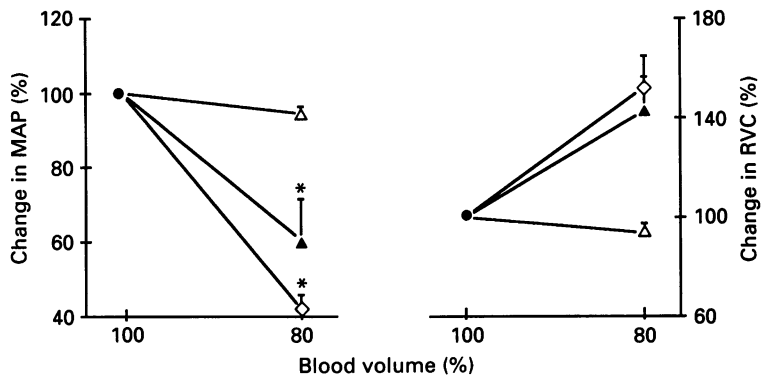

Fig. 4. The role of the sinoaortic baroreceptors in the MAP and RVC response to removal of 20% of the blood volume. To isolate the autonomic component of this response the rabbits all underwent hormonal blockade and the response to haemorrhage was compared before (△) and after (▲) sinoaortic denervation. For comparison these values were plotted with the values from rabbits with combined TAB+HB (the local response, ◇). Abbreviations as for Figs 1 and 2. $*P < 0.05$ as compared to before sinoaortic denervation.

following termination of haemorrhage, hormonal blockade (HB) significantly attenuated the recovery of MAP (Fig. 3). The post-haemorrhage MAP in rabbits which received no treatment (control) was 89·9% of pre-haemorrhage levels compared to only 61·3% recovery after blockade of the vascular actions of vasopressin and AII (HB). In addition the fall in RVC (83·3% of pre-haemorrhage) seen in rabbits which received no treatment (control) was abolished and reversed by a rise in RVC (116·8%) after hormonal blockade (HB).

Sinoaortic denervation

To assess the role of the sinoaortic baroreceptors in the autonomic response we compared the responses of rabbits treated with hormonal blockade before (HB) and after sinoaortic denervation (HB(SAD), Fig. 4). After sinoaortic denervation MAP

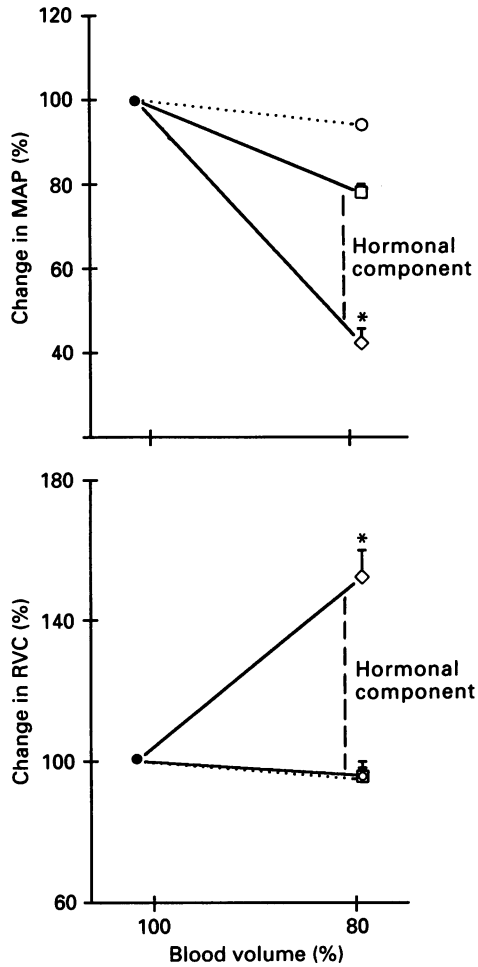

Fig. 5. The percentage change (from pre-haemorrhage values) in MAP and RVC during removal of 20% of the blood volume. Conditions of efferent blockade were as follows: ○, control; □, TAB; and ◇, TAB+HB. The difference of MAP or RVC between TAB and TAB+HB is that due to the action of the hormones vasopressin and AII. Abbreviations as for Figs 1 and 2. $*P < 0{\cdot}05$ as compared to TAB.

was much less well maintained and fell nearly as much during combined total autonomic and hormonal blockade (TAB+HB) the renal vasoconstriction (fall in RVC), observed in rabbits with hormonal blockade (HB) was abolished after sinoaortic denervation (HB(SAD)) and there was now a significant rise in RVC (vasodilatation), which was closely similar to that observed after neural and humoral

blockade (TAB+HB). Thus during 20% blood volume removal the role of the sinoaortic baroreceptors was of paramount importance in the maintenance of MAP and renal vasoconstrictor response.

Augmented hormone secretion model

We know from previous studies in our laboratory that after TAB levels of vasopressin and renin were significantly enhanced during haemorrhage (Oliver *et al.*

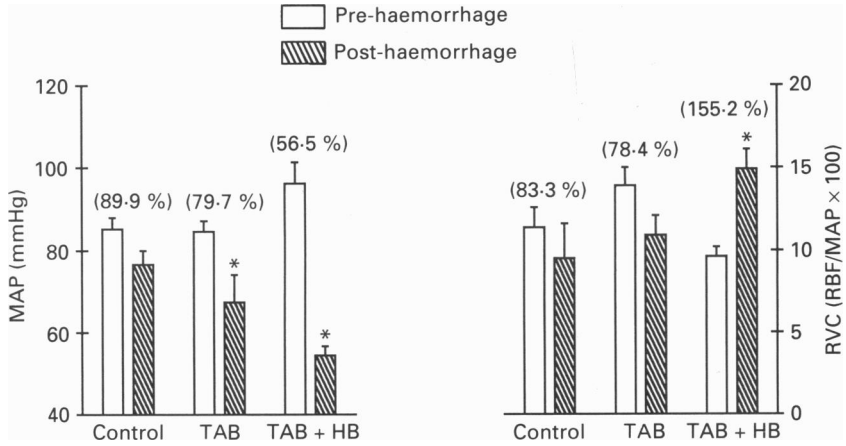

Fig. 6. The average MAP and RVC measured over 5 min prior to the beginning of haemorrhage and in the 2 min post-haemorrhage interval period. The rabbits were studied under the following conditions of efferent blockade: control, TAB and TAB+HB. The values in parentheses represent the percentage change from the pre-haemorrhage control as measured during the 2 min post-haemorrhage interval. Abbreviations as for Figs 1 and 2. $*P < 0{\cdot}05$ as compared to pre-haemorrhage.

1990). In the present study we examined the effect on MAP and RVC responses and the role of sinoaortic baroreceptors. Figure 5 shows the percentage change in MAP and RVC during removal of 20% of the blood volume under three different treatment combinations. After TAB the slope of the blood volume–MAP relationship was 3 times that observed in rabbits with an intact autonomic nervous system (control). In contrast the slope of the blood volume–RVC relationship had changed little from the control response during the studied 20% of blood volume removal (Fig. 5). The fall in MAP was significantly increased during TAB+HB as compared to TAB alone (Fig. 5). The fall in RVC (seen in TAB rabbits) was abolished after TAB+HB and replaced by a rise in RVC (vasodilatation). The estimated vasoconstrictor response due to augmented hormone secretion therefore was the difference between TAB rabbits and the response of TAB+HB rabbits or $152-96 = 56$ ECU.

Post-haemorrhage response

We also investigated the role of the hormones in mediating renal vasoconstriction during the immediate post-haemorrhage interval in the augmented hormone secretion mode. At this time the enhanced hormone secretion helped to maintain

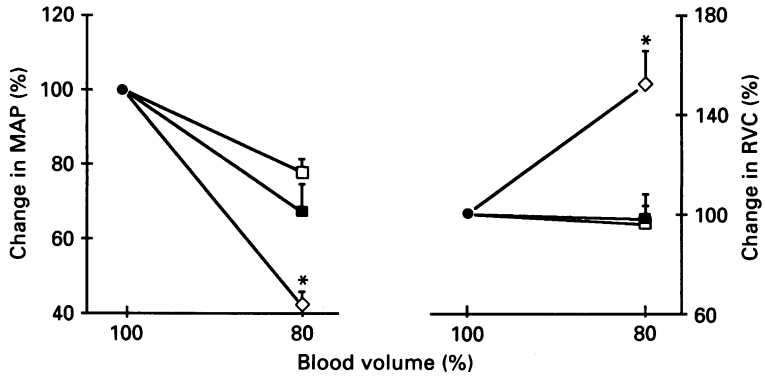

Fig. 7. The role of the sinoaortic baroreceptors in MAP and RVC response to removal of 20% of the blood volume. To isolate the hormonal component of this response the rabbits all underwent TAB and the response to haemorrhage was compared before (□) and after (■) SAD. For comparison these values were plotted with the values from rabbits with combined TAB + HB (◇, the local response). Abbreviations as for Figs 1 and 4. $*P < 0.05$ as compared to before SAD.

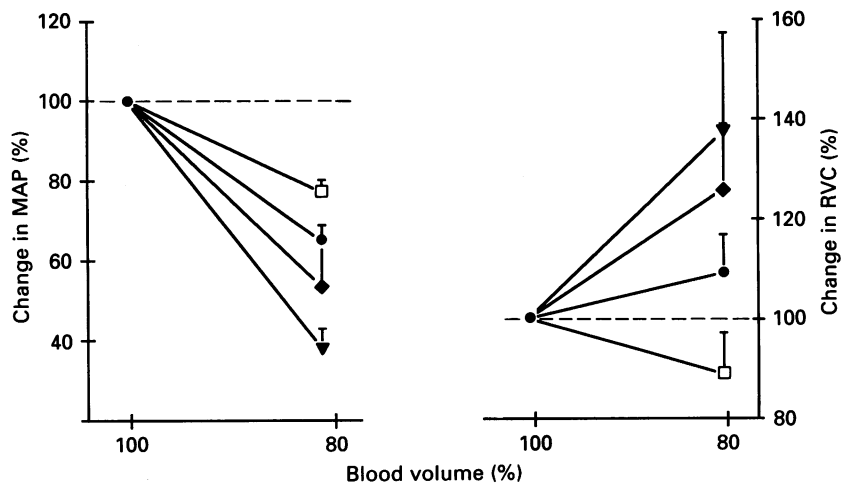

Fig. 8. The percentage change (from pre-haemorrhage values) in MAP and RVC (RBF/MAP) during withdrawal of 20% of the blood volume. Haemorrhages were carried out in rabbits which had undergone TAB alone (□) and TAB with blockade of the vascular actions of either arginine vasopressin, using arginine vasopressin antagonist, (●) AII, using CEI (◆) or combined blockade of both AVP and AII (▼). Abbreviations as for Figs 1 and 2.

MAP and to constrict the renal vasculature. This was evident by comparing the responses of rabbits subjected to TAB alone with those subjected to TAB + HB (Fig. 6). In the TAB rabbits during the post-haemorrhage interval the MAP was 79·7% of the pre-haemorrhage value compared to 56·5% after TAB + HB. In addition the RVC during the post-haemorrhage interval was 78·4% of the pre-haemorrhage level in TAB rabbits (vasoconstriction) *vs.* 155·2% after TAB + HB (vasodilatation).

Sinoaortic denervation

After sinoaortic denervation in the TAB rabbits (TAB(SAD)) the fall in MAP was only slightly augmented compared to the response in rabbits with intact sinoaortic baroreceptors (TAB) (Fig. 7). In contrast, sinoaortic denervation had no effect on the renal vasoconstrictor response to haemorrhage in the TAB rabbits as evidenced by the very similar slopes of the blood volume–RVC relationship between TAB and TAB(SAD) rabbits (Fig. 7).

To further elucidate the hormonal component of the MAP and RVC response to haemorrhage each hormone was blocked individually. This resulted in an intermediate response in MAP and RVC compared to that with both hormones intact (TAB) or both blocked (TAB+AVPA+CEI) (Fig. 8).

DISCUSSION

Normal renal vascular response

In normal conscious rabbits removing 20% of the blood volume was associated with good maintenance of mean arterial pressure and renal vasoconstriction. We found no evidence of a contribution by the two pressor hormones vasopressin or AII to the renal vasoconstrictor response during this phase of haemorrhage which suggests that it was entirely mediated through autonomic mechanisms involving the sympatho-adrenal system. This agrees with the observations found in anaesthetized dogs where small decreases in carotid sinus pressure decreased renal blood flow and this effect was mediated entirely by renal sympathetic nerves (independent of any extrarenal humoral factors) (Karim, Poucher & Summerhill, 1989). Combined autonomic and hormonal blockade was associated with marked renal vasodilatation and we have assumed that this was due to the local response to haemorrhage, as discussed earlier (Korner *et al.* 1990). This provides the baseline for assessing the estimated constrictor effects of haemorrhage which amounted to 57 ECU mediated entirely through the autonomic nervous system.

Both the local and autonomic renal vascular responses to haemorrhage were similar in magnitude to those we have previously observed in the mesenteric bed and greater than those in the hindquarter bed (Korner *et al.* 1990). We had expected some contribution to the vasoconstrictor response by AII, since plasma renin activity has begun to rise well before 20% of the blood volume had been removed (Oliver *et al.* 1990). However, the present findings suggest that this is not the case. This is in accord with previous findings in mesenteric and hindlimb beds and suggests that intrarenal production of AII played no special role in intrarenal vasoconstriction during this phase of good arterial pressure maintenance. Plasma vasopressin does not rise until after 25% of the blood volume has been removed and is thus unlikely to contribute to the early vasoconstrictor response. However, both hormones played a role in the maintenance of MAP immediately after haemorrhage and in the maintenance of renal vascular resistance, at a time when there was pronounced inhibition of renal sympathetic activity (Burke & Dorward, 1988).

The neural renal vasoconstrictor response during haemorrhage was completely abolished by sinoaortic denervation. To avoid the complicating effects of hormone

release when there was a greater fall in MAP in the SAD preparation, we used rabbits in which the effects of both pressor hormones were blocked. In this way the effects of SAD in this preparation affected only the neural response. In the present study we did not examine the role of the cardiac or other low pressure baroreceptors. Burke & Dorward (1988) showed that cardiac receptors contribute to the depression of the renal baroreflex during hypotensive haemorrhage. The present study suggests their effect on renal sympathetically mediated vasoconstriction is probably small. Any effect would appear to depend on the integrity of the sinoaortic baroreceptors so that their role, if any, may be for fine tuning of the reflex response.

Augmented hormone secretion model

The autonomic blockade was of a high order, sufficient to block the effect of strong renal vasoconstrictor reflexes. Hence the renal vasoconstriction response, in the rabbits which received total autonomic blockade, was due entirely to the vascular actions of vasopressin and AII and was completely abolished by superimposing hormonal blockade on total autonomic blockade. It is interesting that the enhanced release of vasopressin and angiotensin (Oliver *et al.* 1990) produced similar vasoconstriction as was mediated normally through the autonomic nervous system in the intact animal.

The hormonally mediated renal vasoconstrictor response was completely unaffected by sinoaortic denervation. Previously we have shown that vasopressin is significantly increased at this stage of blood removal (Oliver *et al.* 1990), and unloading of cardiac and/or pulmonary receptors has been shown to be a powerful stimulant for vasopressin release during haemorrhage in normal rabbits (Quail, Woods & Korner, 1987). We have not examined in this study to what degree the cardiac receptors contribute to the haemorrhage-induced hormone release. From an earlier study, however, renin release in haemorrhage was independent of the integrity of the reflex baroreceptors. There is ample evidence for the existence of a renal vascular receptor mechanism capable of causing renin release during haemorrhage. Blaine, Davis & Witty (1970) isolated the renal vascular receptor from other factors which could influence renin release using dogs with non-filtering kidneys that had previously been adrenalectomized and had undergone renal denervation. In this isolated vascular receptor preparation, haemorrhage produced a significant increase in plasma renin secretion. During haemorrhage therefore, the renal vascular receptor is thought to respond to a fall in afferent arteriolar wall tension and stimulate renin release (Witty, Davis, Shade, Johnson & Prewitt, 1971; Davis & Freeman, 1976).

In conclusion, the present study suggests that during moderate haemorrhage the renal vasoconstrictor response was entirely mediated by the sympatho-adrenal system and largely under baroreceptor control. In normal rabbits vasopressin and AII contributed to the maintenance of arterial pressure and renal vasoconstriction only immediately after haemorrhage when blood pressure was low. In the augmented hormone secretion model (TAB) renal vasoconstriction was almost of the same magnitude as that in rabbits with an intact autonomic nervous system, but the vasoconstrictor response was due to the vascular actions of vasopressin and AII and was not dependent on the integrity of the arterial baroreceptors. This latter observation emphasizes the presence of overlapping pressor systems (hormonal and autonomic) one of which, under normal conditions, may be quiescent. However,

during a hypotensive episode, and in the absence of normally contributing pressor systems, it may be called into play.

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