Effects of acute blood volume expansion on vascular resistance and reactivity in anaesthetized dogs

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(Received 17 September/20 December 1982; accepted 19 January 1983)

Summary

1. The effects of acute volume expansion on vascular resistance and reactivity to noradrenaline and angiotensin II are reported in this study. The estimated circulating blood volume of pento-bartibal-anaesthetized dogs was expanded by about 35% with equilibrated donor blood. The animals were bilaterally nephrectomized to sustain expanded volume.

2. Functional changes in vascular smooth muscle were determined in the flow controlled, vascularly isolated, denervated, perfused hind limb preparation in the same animal.

3. Systemic volume expansion per se had no immediate influence on vascular resistance. However, resistance in the hind limb, as determined by the shift of the pressure-flow curves, progressively increased 60 and 120 min after volume expansion. The changes noted after 120 min were significantly greater than those observed after 60 min.

4. The changes in vascular resistance were accompanied by potentiation of the vascular responses to noradrenaline but not to angiotensin II. Significant shifts which occurred in the noradrenaline dose-response curves were similar to those of the resistance curves.

5. In closely simulated control experiments in dogs whose kidneys were intact or had been removed, and whose blood had or had not been equilibrated with donor blood, the above-mentioned vascular changes were not observed in the absence of volume expansion.

6. It is suggested that the functional changes observed in the hind limb vasculature after volume expansion are related to the presence of a circulating substance. From the data obtained from the experimental model used in this study, it can be concluded that such a substance is not released from the kidney.

Key words: blood volume expansion, vascular resistance.

Introduction

Several researchers concerned with the pathophysiology of hypertension have shown that a circulating 'digitalis-like' substance could be responsible for suppression of \( \text{Na}^+,\text{K}^+ \)-stimulated ATPase in various cardiovascular tissues of volume-expanded, low renin types of hypertensive model [1, 2]. It is further suggested that sodium pump inhibition by an endogenous substance would contribute to enhanced contractility and reactivity of vascular smooth muscle [3, 4]. A number of other investigators have provided evidence for the formation and/or release of an endogenous sodium pump inhibitor, often referred to as 'natriuretic hormone', after acute volume expansion in experimental animals [5]. It should therefore be possible to demonstrate that acute expansion of the circulating blood volume of experimental animals will lead to functional changes in vascular smooth muscle which could be attributed to a circulating humoral substance. The present studies are designed to evaluate the consequences of acute volume expansion on vascular resistance and reactivity in anaesthetized mongrel dogs.

Methods

Dogs

Mongrel dogs of either sex (15–18 kg) were anaesthetized with sodium pentobarbital, 35
mg/kg intravenously, and placed on positive pressure ventilation (Bird Respirator). In order to maintain a stable anaesthetic level, a small dose of the anaesthetic (4 mg h⁻¹ kg⁻¹) was continuously infused throughout the period of the experiment (Harvard infusion pump). Arterial blood pressure was recorded from a catheterized brachial artery (Statham P23 Db) and heart rate was monitored from the pressure pulse (Grass Tachograph). A polyethylene catheter was introduced into the right atrium via the right brachial vein to monitor central venous pressure (Statham P23 V). All the above variables were recorded on a Grass polygraph.

Donor-blood equilibration and volume expansion

After a midline incision into the abdominal cavity, the dogs were acutely nephrectomized so that the expanded volume could be sustained. Blood for expansion was freshly collected from pentobarbital anaesthetized and heparinized (500 units/kg) donor dogs. The donor blood was placed in a reservoir maintained at 37°C and continuously oxygenated. The recipient animal was also similarly heparinized and the blood in the reservoir equilibrated with that of the recipient as follows.

By use of a dual channel Harvard peristaltic pump (model 1203), blood in the reservoir was slowly infused at a rate of 12 ml/min into the left femoral vein of the recipient and blood from the recipient was simultaneously withdrawn into the reservoir via the left femoral artery at exactly the same rate. In this manner the contents of the reservoir were thoroughly mixed and equilibrated with that of the recipient in about 60 min, without producing any alteration in the volume of blood in the reservoir. In order to produce volume expansion, withdrawal of the blood from the recipient was stopped (Fig. 1).

Evaluation of vascular resistance and reactivity

The right hind limb of the dog was acutely denervated by section of the ipsilateral lumbar sympathetic chain (at L-5), and the sciatic and femoral nerve trunks. Collateral circulation to the limb was minimized by ligation of the internal iliac artery. The distal end of the femoral artery was catheterized and perfused with blood drawn from the central end of the same vessel in Tygon tubing. A peristaltic pump was placed with the circuit so that flow rate could be controlled. Perfusion pressure was monitored via a T-tube placed in the circuit distal to the pump and close to the limb. An arterial pressure transducer (Statham P23 Db) was used to record perfusion pressure on the polygraph (Fig. 1). In order to evaluate vascular resistance in the limb, pressure-flow curves were constructed by recording perfusion pressures at

FIG. 1. Schematic diagram of the experimental model. Blood vessels in the left leg were used for donor blood equilibration and volume expansion. The right limb was denervated, vascularly isolated and perfused at controlled flows.
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Protocol

After a stabilizing period of not less than 60 min, during which donor blood was completely equilibrated with that of the recipient, control pressure–flow curves and vascular responses to noradrenaline and angiotensin II were established. The withdrawal of blood from the recipient was then stopped and blood in the reservoir infused into the recipient at a rate of 24 ml/min. The blood volume of the recipient was estimated as 85 ml/kg body weight and this volume was expanded by about 35%. In a typical experiment, volume expansion took place over a period of 20 min. Within the first 10–15 min after completion of volume expansion, pressure–flow curves were established. Again at 60 min and 120 min after completion of volume expansion, both the pressure–flow curves and vascular responses to noradrenaline and angiotensin II were obtained. Blood samples were also collected before, and at different times after, volume expansion for determination of packed cell volume. Three determinations were made for each sample with a micro-haematocrit centrifuge (Clay-Adams) and the average was calculated.

In five additional experiments, the effect of volume expansion on vascular resistance and on vasoconstrictor responses to three doses of intra-arterial angiotensin II (0.2, 0.4 and 0.8 μg) was studied.

Control experiments

Since there are several variables in this experimental model, the above experiments required adequate controls. Three different groups of control experiments were conducted:

(a) an intact denervated, perfused hind limb preparation in which the kidneys were intact and donor blood was not equilibrated (n = 5);
(b) a denervated, perfused hind limb preparation in nephrectomized dogs in which donor blood was not equilibrated (n = 5);
(c) a denervated, perfused hind limb preparation in nephrectomized dogs in which donor blood was equilibrated but the volume was not expanded (n = 5).

In all these control experiments, the original experimental procedures were simulated as closely as possible, taking into consideration the time required for stabilization, donor blood equilibration and for volume expansion. Thus the overall duration of the experiments, and the times at which the various variables were measured, were essentially similar in all the experiments, except that in these groups of controls, volume was not expanded.

All the data are presented as means ± SEM and the statistical significance of a particular change which occurred at different time intervals after expansion was determined by Dunnet’s test [7]. Significant differences between the various pressure–flow and dose–response curves were established by using the method of analysis of variance (Digital minicomputer system, MINC-11).

Results

Packed cell volumes, which were determined at different time intervals, were as follows (%): after blood equilibration: 36.3 ± 2.28; after volume expansion: 0–5 min, 38.0 ± 1.17; after 60 min, 41.3 ± 1.06; after 120 min, 44.1 ± 1.70.

Volume expansion resulted in significant elevations in both systolic and diastolic blood pressure (Fig. 2). During the first 60 min after expansion there was a further rise in diastolic pressure. Both systolic and diastolic pressure remained stable at higher levels during the rest of the experimental period. These changes in blood pressure were accompanied by significant reductions in heart rate, which stabilized at the reduced levels. There was a gradual and significant increase in central venous pressure during expansion; however, central venous pressure gradually returned to pre-expansion levels within 120 min.

Either during volume expansion or within the first 10–15 min after its completion, there were no changes in pressure–flow curves. After this period there was a progressive increase in hind limb vascular resistance, reflected by a shift of the pressure–flow curves to the left after 60 and 120 min (Fig. 3). These shifts differed significantly from the pre-expansion curves (P < 0.05 at 60 min; P < 0.01 after 120 min). In Fig. 4, poly-
FIG. 2. Effects of acute volume expansion on (a) blood pressure, (b) heart rate and (c) central venous pressure are shown. In (a): $\circ$--$\circ$, systolic pressure; $\oplus$--$\oplus$, diastolic pressure; $\ldots \ldots \ldots \ldots$, mean blood pressure. Mean values ± SEM (bars) are shown. Stars indicate that the changes are significantly different ($P<0.05$) from pre-expansion values ($n=7$).

Graph tracings from one experiment show the effects of intra-arterial noradrenaline on perfusion pressure, before and after volume expansion. The pressor effects of noradrenaline were potentiated after 60 and 120 min. The dose–response curves to noradrenaline were shifted significantly to the left, when compared with the pre-expansion curves ($P<0.05$ after 60 min; $P<0.01$ after 120 min). However, vasoconstrictor effects of intra-arterial angiotensin II were not significantly affected by volume expansion (Fig. 5).

In the additional experiments, in which the vascular responses to three doses of angiotensin II were measured, volume expansion resulted in significant shifts of the pressure–flow curves. These changes were qualitatively as well as quantitatively similar to those shown in Fig. 3. The dose-dependent vasoconstrictor effects of angiotensin II are shown in Table 1. These data further confirmed that the vascular effects of angiotensin II were not significantly altered by volume expansion.

The data from three groups of control experiments are shown in Figs. 6–8. The results indicate that, in the absence of volume expansion, neither the pressure–flow nor the noradrenaline dose-response curves were altered in these simulated controls. In fact, in some preparations the noradrenaline responses were slightly depressed.

Discussion

During volume expansion there were predictable increases in both systolic and diastolic blood pressure accompanied by significant reductions in heart rate. The reduction in heart rate may have resulted from a reflex compensation to the elevated arterial pressure and it is also possible that a similar reflex decrease in sympathetic neuronal outflow to the vasculature may have occurred. However, there was a further increase in diastolic pressure during the first hour after expansion, which may have been indicative of an increase in vascular smooth muscle tone. The elevated central venous pressure occurring during volume expansion returned to control level after 60 min. Thus it appears that the myocardium of these animals functioned effectively in handling an enhanced pre-load. Arterial pressures remained
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**FIG. 4.** Polygraph tracings from an experiment to illustrate perfusion pressure responses to intra-arterial administration (i.a.) of various doses of noradrenaline before (control) and after volume expansion. Vascular effects of noradrenaline were progressively enhanced.

**FIG. 5.** Effect of acute volume expansion on the vascular effects of intra-arterial (i.a.) noradrenaline and angiotensin II. (a) Dose-response curves to noradrenaline shifted significantly to the left after 60 min ($P<0.05$) and after 120 min ($P<0.01$) when compared with the pre-expansion control curve (C). Mean values ± SEM (bars) are shown. Stars indicate that the shifts are statistically significant. (b) Perfusion pressure responses to intra-arterial angiotensin II were not significantly altered by volume expansion. $n = 7$.

stable at elevated levels during the rest of the experimental period. It should be noted that whereas arterial pressures rose, pressure in the flow-controlled perfused hind limb did not alter either during expansion or within the first 15 min after expansion. These observations suggested that the collateral circulation to the limb was minimal and the perfusion pressure was relatively independent of physical changes in volume and pressure in the systemic circulation. Fifteen
TABLE 1. Effects of volume expansion on perfusion pressure responses to intra-arterial administration of angiotensin II

Mean values ± SEM are shown.

<table>
<thead>
<tr>
<th>Doses of angiotensin II (µg)</th>
<th>Changes in perfusion pressure (mmHg)</th>
<th>Before expansion</th>
<th>After expansion*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60 min</td>
<td>120 min</td>
</tr>
<tr>
<td>0.2</td>
<td>15 ± 2.54</td>
<td>18 ± 2.42</td>
<td>16 ± 1.23</td>
</tr>
<tr>
<td>0.4</td>
<td>20 ± 2.85</td>
<td>23 ± 1.90</td>
<td>24 ± 2.48</td>
</tr>
<tr>
<td>0.8</td>
<td>32 ± 3.72</td>
<td>33 ± 4.69</td>
<td>35 ± 4.57</td>
</tr>
</tbody>
</table>

* Changes which occurred in perfusion pressures at 60 and 120 min after expansion were not significantly different from control values (n = 5).

However, it should be pointed out that only noradrenaline responses and not those of angiotensin II were augmented. Since in the first group of animals only one dose of angiotensin II was employed, additional experiments were conducted to determine the influence of volume expansion on the vasoconstrictor effects of different doses of angiotensin II. The data from these studies confirmed the initial observation that the vascular responses to this agent were not altered by volume expansion. Thus any influence of perfusion on the vascular responses can be discounted in these studies.

In the control groups, in which the experimental procedures were closely simulated but in which blood volume was not expanded, no significant changes were observed either in hind limb vascular resistance or in reactivity. Thus it can be concluded that circulating volume expansion activated certain physiological mechanisms, which lead to enhanced vascular tone and reactivity to noradrenaline. An increase in viscosity of the blood resulting from an increase in packed cell volume could have contributed to alterations in vascular resistance. Previous studies have demonstrated that in the hind limb vasculature of dogs, a rise in packed cell volume from 45% to 70% results in more than a twofold increase in apparent viscosity; however, these studies also showed that changes in packed cell volume within a lower range similar to that obtaining in the current studies (36–44%)
would not alter significantly either the apparent viscosity or the resistance of the small vessels contributing to net vascular resistance [8, 9]. Even if these changes in packed cell volume contributed partly to increases in resistance, they could not be accounted for the specific potentiation of the noradrenaline responses. Since the hind limb was denervated, neurogenically mediated vasoconstric-
vasopressin. Gruber & Buckalew [13] and Plunkett et al. [14] isolated two natriuretic fractions from the plasma of volume-expanded dogs; although both these fractions, which inhibited Na⁺,K⁺-ATPase, potentiated the vasoconstrictor effects of noradrenaline on rat cremaster arterioles, one of the fractions produced its peak effects at 40 min after injection whereas the second fraction produced its peak effects after a delay of 130 min. The duration of these effects was 130 and 170 min respectively. In the present studies, alterations in the vascular resistance and reactivity to noradrenaline were demonstrated in the same animal whose blood volume was expanded. The time course of these effects appeared to be similar to that of the above studies as well as to that of other reports in which natriuretic effects have been demonstrated after volume expansion [5]: peak natriuretic effects were usually observed after about 60 min.

Several investigators have conducted experiments to demonstrate the presence of natriuretic substance(s) in blood after volume expansion and these studies have been comprehensively reviewed by de Wardener [5]. It is evident from these studies that in order to demonstrate the presence of these substances, the expansion in volume must be sustained. For this purpose, different techniques have been employed by different workers; these include nephrectomy [15], urine re-infusion [16, 17] and slow intravenous infusion of Locke-Ringer to compensate for loss of fluids [13]. Knock & de Wardener [16] and Knock [17], using the urine re-infusion technique, have demonstrated natriuresis in a recipient rat when it was cross-circulated with a donor whose circulating volume was expanded with equilibrated blood. Their data indicated that urine re-infusion into the donor was essential to demonstrate the above effect. They further suggested that urine re-infusion not only prevented volume loss but also enhanced the level of the natriuretic substance(s) in the circulation. Such conclusions are consistent with evidence that the natriuretic substances are excreted in urine [18-20]. Knock [17] in a separate study could not demonstrate natriuretic activity in the plasma of volume-expanded rats in which fluid loss had been prevented by bilateral nephrectomy and thus concluded that these substances may be produced by the kidney. Godon has also identified a natriuretic substance in the kidney [21]. However, these observations of Knock are not in agreement with those of other workers, who have shown circulating natriuretic agents in the plasma of nephrectomized, volume-expanded rats [15, 22]. Likewise, in the present studies the dogs were nephrectomized and thus any circulating factors responsible for the vascular functional changes could not have originated from the kidneys. However, nephrectomy may have caused progressive increases in the levels of endogenous substance(s) in the circulation during the course of the experiment, since their excretion was prevented. Such a possibility is consistent with the finding that the vascular changes noted at the end of 120 min were consistently greater than those at 60 min.

Several reports indicate that the humoral substance may be very similar to digitalis. Serum preparations from volume-expanded dogs selectively inhibited ouabain-sensitive ⁸⁶Rubidium uptake in the tail arteries of rats [23]. Gruber et al. [24] have isolated a natriuretic factor which was a sodium pump inhibitor from the plasma of volume-expanded dogs. This substance competed with digoxin for two specific digoxin antibodies, suggesting that it may have a digitalis-like structure. In this context, it should be pointed out that ouabain has been shown to potentiate nonspecifically the effects of various vasoconstrictor agents [11, 12]. But in these studies we were unable to document any significant changes in the vasoconstrictor effects of angiotensin II. This is surprising since it has been shown recently that angiotensin II promotes sodium influx and enhances intracellular sodium in aortic smooth muscle cells, especially in the presence of ouabain [25]. Such an effect is expected to enhance the vasoconstrictor actions of angiotensin II. Although no definitive conclusion
may be drawn at this time, it should be considered that certain physiological properties of the endogenous humoral substance may differ from that of digitalis glycosides. The experimental procedures employed in the current studies closely follow those of other investigators, who have provided evidence for the formation and/or release of circulating natriuretic hormone(s) in animals after blood volume expansion. Without using cross-circulating recipient preparations, the current studies demonstrate that acute volume expansion and maintenance of the expanded volume will lead to an increase in vascular resistance and reactivity to noradrenaline in the same animal. It is suggested that these changes in vascular function can be attributed to a circulating humoral substance(s). These data further support the view that a circulating substance may play an important role in the pathogenesis of hypertension associated with volume expansion.

References