Hemodilution-Induced Inhibition of Cardiovascular Responses to Some Vasoactive Agents in Anesthetized Cats

A. TALWAR, M. E. HUSSAIN, and M. FAHIM*

Department of Physiology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi 110007, India

Abstract Cardiovascular responses to adrenaline and acetylcholine (ACh) were investigated in anesthetized, artificially ventilated cats in control and after induction of acute normovolemic hemodilution. Progressive replacement of blood by high molecular weight dextran was performed in three steps of 20% each of the total estimated blood volume. Hemodynamic responses were recorded at four stages: the control stage and after the 1st, 2nd, and 3rd exchanges of blood for dextran. With the fall in hematocrit (Ht) there was a corresponding significant (p < 0.05) increase in heart rate (HR), cardiac output (CO), and stroke volume (SV), and a decrease in systemic vascular resistance (TPR). However, left ventricular systolic pressure (LVSP), left ventricular contractility (LVdP/dt max), mean arterial pressure (MAP), and right atrial pressure (RAP) did not show any significant (p > 0.05) change due to hemodilution. The cardiovascular responses of intravenously administered adrenaline and ACh were significantly (p < 0.05) attenuated. Responses to sodium nitroprusside (SNP), a potent vasodilator and an exogenous source of nitric oxide, were also attenuated after hemodilution. The increase in SV and HR seem to be the contributing factors to the CO response. Our results indicate that the cardiovascular responsiveness to adrenaline, ACh and SNP is reduced during acute hemodilution which could be due to inadequate myocardial and vascular O2 supply. The possibility of a modulatory role of an endothelium-dependent mechanism and reflex regulatory responses by arterial baroreceptors during hemodilution also exists.

Key words: hemodilution, anemia, adrenaline, acetylcholine, sodium nitroprusside.

Received on November 2, 1994; Accepted on April 3, 1995
* To whom correspondence should be addressed.

Correspondence should be addressed to: M. Fahim, Department of Physiology, V. P. Chest Institute, University of Delhi, P. O. Box 2101, Delhi 110007, India
The oxygen-carrying capacity of blood is severely reduced by rapid induction of an anemic state by normovolemic hemodilution [1, 2]. The performance of the left ventricle may also be disturbed due to inadequate myocardial oxygen supply [3]. The possible compensatory mechanisms to meet oxygen demand by the tissues under such conditions include an increase in cardiac output (CO) and redistribution of blood flow to essential organs [3-8]. Studies in anesthetized dogs have demonstrated that hemodilution reduces cardiovascular responsiveness to selective α- and β-adrenergic receptor stimulation and to certain drugs [2, 9-11]. However, variable and inconsistent vascular responsiveness of these drugs during hemodilution has been observed in earlier studies. Normovolemic hemodilution to Ht 16% diminishes the chronotropic response to isoproterenol, whereas the inotropic response was not altered significantly [9]. Mahdi et al. [10] related the reduced cardiovascular responsiveness to phenylephrine during hemodilution to antagonism by local vasodilating effects of anemic hypoxia. They also suggested that hemodilution per se activates peripheral α-adrenergic receptors reflexly, thus attenuating pharmacological activation. However, such a mechanism remains uncertain in the absence of experimental evidence for reduced responsiveness to the drugs during hemodilution. The earlier studies do not provide any information regarding the cardiovascular effects of depressor agents such as acetylcholine (ACh), which is known to be involved in cardiovascular regulation [12].

Therefore, the present study was undertaken in anesthetized cats to evaluate i) the myocardial and hemodynamic responses during normovolemic hemodilution, ii) cardiovascular effects of certain important drugs such as adrenaline and ACh before and after graded acute hemodilution. To look into the endothelium-dependent mechanism(s) in reduced responsiveness to adrenaline and ACh, the influence of hemodilution on the cardiovascular responsiveness to sodium nitroprusside, an agent known to produce a vasodilatory effect by acting directly on the vascular smooth muscle and an exogenous source of nitric oxide [13] was also studied.

MATERIALS AND METHODS

Experiments were performed on 24 adult cats of either sex weighing 3-6 kg, anesthetized with a mixture of 70 mg/kg chloralose (BDH) and 350 mg/kg urethane (Merck). The trachea was cannulated and the cats were ventilated with a respiratory pump (Inco, India). A polyethylene catheter was placed in the descending aorta through a femoral artery to record arterial blood pressure with a pressure transducer (Statham P23 Db). Blood samples were withdrawn in heparinized syringes anaerobically through this catheter to measure arterial blood $P_{O_2}$, $P_{CO_2}$ and pH with a blood gas monitor (Radiometer BMS-3 MK-2 microsystem and PHM-73 pH electrodes). The arterial blood samples were also used to measure hematocrit (Ht) with a microcentrifuge (Janetzki TH12) and hemoglobin (Hb) with a hemometer (Shandilya, India). The right femoral vein was cannulated for
intravenous injections and infusion of dextran. A polyethylene catheter was placed in the right atrium through an external jugular vein to record right atrial pressure (RAP) with a pressure transducer (Statham P23 Gb). The mixed venous blood samples were withdrawn through this catheter to measure blood gas tensions. To ensure the position of the catheter in the right atrium, the catheter was initially pushed into the right ventricle and was withdrawn gradually until it displayed an atrial pressure waveform. After completion of the experiment, position of the catheter was confirmed by post-mortem observation. Another polyethylene catheter was placed into the left ventricle through the left carotid artery for recording left ventricular pressure (LVP) with a pressure transducer (Statham P23 Db). The left ventricular pressure pulse was differentiated electronically with a differentiator (Lectromed Model 5270) to record LV dP/dt and was also used to drive a cardiograph (Lectromed Model 5260) for recording heart rate (HR). All these variables were recorded on a polygraph (Lectromed, U.K.). CO was measured by a thermodilution technique using a Swan-Ganz flow-directed thermodilution catheter (Model 93A-131-7K). Three milliliters of cold saline was injected into the right atrium and CO was measured by a cardiac output computer (COM 1 Edward Co., USA) before and after each exchange of blood. Three consecutive measurements of CO were made under each stage to get variation less than 5% [14].

Cardiovascular measurements were made 30 min after completion of the surgical procedure. Normovolemic hemodilution was induced by dextran (mol. wt. 150,000) for blood exchange. It is well documented that substances which are specifically designed to restore plasma volume must have colloid osmotic pressure comparable to plasma and the viscosity characteristics should be suitable for infusion. Dextran has suitable oncotic properties but no oxygen-carrying capacity, and it is known to possess most of the attributes of an ideal plasma expander for clinical use [15]. A 6% solution of dextran (Rallis, India) in 5% w/v dextrose was warmed to 37°C before infusion through the femoral vein catheter. The viscosity of 6% dextran solution is approximately equal to that of plasma. Blood was replaced by dextran in steps of 20% each of the total estimated blood volume (5% of body weight) [16]. Cardiovascular variables were recorded after a stabilizing period of 30 min following each exchange. The body temperature of the animal was maintained at 37–38°C by surface heating with the help of heating pads.

Drugs. Stock solutions of adrenaline (100 μg/ml), acetylcholine (100 μg/ml), and sodium nitroprusside (200 μg/ml), all from Sigma were freshly prepared in normal saline on the day of the experiment. Drugs (0.1 ml/kg) were administrated through the femoral vein catheter which was flushed quickly (less than 5 s) with 2 ml of saline. Injection of a similar volume of normal saline acted as a control. The time of injection was marked at the beginning of flushing.

Experimental protocol. Two drugs, adrenaline and acetylcholine, were studied in 14 cats and sodium nitroprusside was studied in another series of experiments (10 cats). In preliminary experiments, the dose-response of each drug was tested and the dose producing a submaximal effect was selected for the study. A recovery
period of 30 min was given after administration of each drug and the total time taken for a single experiment was 8–10 h.

**Statistical analysis.** The statistical analysis was done for each drug separately. All the variables were subjected to log transformation in order to achieve variance stability and normality before the data was subjected to statistical analysis. The data on initial findings (control level) was subjected to two-way analysis of variance (ANOVA) after ascertaining homogeneity of variance and normality on evidence of significant variation amongst various anemic stages and other appropriate comparisons were done through linear contrasts [17].

**RESULTS**

Cardiovascular effects of hemodilution are summarized in Tables 1 and 2. Normovolemic hemodilution was done in three steps of 20% estimated blood volume each. After the last exchange of blood for dextran (total 60% of estimated

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<tr>
<th>Table 1. Hematocrit, hemoglobin, blood gas tension and pH of arterial blood before and after three stages of hemodilution.</th>
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<td>Control</td>
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<tr>
<td>Hematocrit (%)</td>
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<td>Hémoglobin (g/dl)</td>
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<td>pH</td>
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Values are mean±SEM. *p<0.05. \(P_{O_2}\), partial pressure of oxygen in arterial blood; \(P_{CO_2}\), partial pressure of carbon dioxide in arterial blood.

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<th>Table 2. Hemodynamic effects of graded exchange of blood by dextran.</th>
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<td>Control</td>
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<td>MAP (mmHg)</td>
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<td>RAP (cmH2O)</td>
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<td>TPR (mmHg min/ml)</td>
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<td>LVdP/dt max (mmHg/s)</td>
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Values are mean±SEM. *p<0.05. MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; CO, cardiac output; SV, stroke volume; RAP, right atrial pressure; TPR, total peripheral resistance.

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blood volume), the actual volume of blood replaced by dextran would be approximately 50% or less and not 60% because after the 1st exchange in the subsequent withdrawal of blood the 20% of volume contained blood mixed with previously infused dextran.

On induction of acute normovolemic hemodilution by this process, Ht dropped from the basal level of 40.1±2.3 to 14.0±20% and hemoglobin (Hb) dropped from a basal level of 14.1±0.4 to 4.0±0.2 g/dl after the 3rd exchange of blood (Table 1). MAP did not show any significant (p > 0.05) change following exchanges of blood (Table 2). With the fall in Ht, there was a corresponding significant increase in HR and CO and TPR decreased significantly (p < 0.05, Table 2). Hemodilution did not produce any significant (p > 0.05) effect on LV dP/dt max (Table 2) or on left ventricular end diastolic pressure. Arterial blood gas tension and pH did not show any significant (p > 0.05) change with the fall in the Ht level (Table 1). The mixed venous blood drawn from right atrium showed a decrease in P O₂ from 38±3 to 33±4 mmHg, P CO₂ did not show any significant change on hemodilution.

Response to adrenaline

Intravenous administration of 10 μg/kg adrenaline at control Ht produced a rapid rise in arterial blood pressure with simultaneous increase in LV dP/dt max (Figs. 1, 2), and no change in RAP. The percent increase in MAP by the drug was significantly (p < 0.05) reduced after the 2nd exchange of blood with dextran, however, after the 3rd exchange the percentage increase in MAP was not signifi-

Fig. 1. Representative tracings demonstrating changes in arterial pressure (AP), right atrial pressure (RAP), heart rate (HR), LV dP/dt max and left ventricular pressure (LVP) to bolus injection of adrenaline (10 μg/kg) at different Ht levels following induction of graded hemodilution. Arrows (↓) indicate drug injection.

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Fig. 2. Percent change in heart rate (HR), LVdP/dt$_{max}$, left ventricular systolic pressure (LVSP), and mean arterial pressure (MAP) following intravenous administration of adrenaline (10 µg/kg) at control (C) and after the first (I), second (II), and third (III) exchanges of blood with dextran. The graphs demonstrate that the maximum increase in cardiovascular parameters after injection of adrenaline occurred at control hematocrit (C). The effect of adrenaline was attenuated with the degree of hemodilution (I–III).

significantly different (Fig. 2). The percentage increases in LVSP, LVdP/dt$_{max}$, and HR were significantly ($p<0.05$) reduced after induction of acute normovolemic hemodilution (Fig. 2).

Response to acetylcholine

Intravenous administration of 10 µg/kg ACh in the control condition produced an instant fall in arterial blood pressure accompanied by a fall in HR and LV dP/dt$_{max}$, and there was no significant ($p>0.05$) change in RAP (Fig. 3).

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<td>L.V.P (mmHg)</td>
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Fig. 3. Representative tracings demonstrating changes in A.P, R.A.P, HR, LVdP/dt max, and L.V.P to bolus injection of acetylcholine (10 μg/kg) at different Ht levels following induction of graded hemodilution. All abbreviations are as in Fig. 1.

The percentage fall in LVSP, LVdP/dt max, HR, and MAP by ACh was significantly (p < 0.05) less after hemodilution except for the percentage fall in HR after the 1st exchange (Fig. 4).

Responses to sodium nitroprusside

Intravenous injection of 20 μg/kg SNP produced a fall in arterial blood pressure and LVdP/dt max accompanied by reflex tachycardia (Fig. 5). Following normovolemic hemodilution, the percentage fall in MAP, LVdP/dt max, and LVSP by SNP (20 μg/kg) was significantly (p < 0.05) attenuated (Fig. 6). Tachycardia in response to SNP was also significantly (p < 0.05) attenuated following hemodilution (Figs. 5, 6).

DISCUSSION

Acute normovolemic hemodilution produced an increase in CO, which is in agreement with earlier findings in anesthetized [14, 18, 19] and conscious animals [20]. The increase in CO during hemodilution could be due to various factors such as change in peripheral resistance, increase in central venous pressure (CVP) and stimulation of chemoreceptors [21]. In our study, we kept arterial Po2, Pco2, and pH in the normal range to eliminate the influence of arterial chemoreceptors. However, it can be argued that reduced oxygen content of the blood might stimulate chemoreceptors [21], although this is still controversial. We found no significant variations in Po2, Pco2, or pH during the experiment, hence we assumed that there was no change in chemoreceptor activity.

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Fig. 4. Percent change in HR, LVdP/dt max, LVSP, and MAP following intravenous administration of acetylcholine (10 μg/kg) at control (C) and after the first (I), second (II), and third (III) exchanges of blood with dextran. The graphs demonstrate that the maximum fall in LVSP, LVdP/dt max, and MAP and maximum rise in HR due to acetylcholine occurred at control hematocrit (C). The effect of acetylcholine was attenuated with the fall in hematocrit (I–III). All abbreviations are as in Fig. 2.

We selected high molecular weight dextran (150,000) for replacing blood because, if the dextran infused is of low molecular weight, molecules of small size are excreted by the kidney and the molecules of high molecular weight pass through capillary walls very slowly and are slowly oxidized over a period of a few weeks. The persistence of high molecular weight dextran in the circulatory system, its slow metabolic disposal and the fact that its colloidal osmotic pressure is similar to that of plasma [15] are desirable features. It is conceded, however, that the possible
Fig. 5. Representative tracings demonstrating changes in AP, RAP, HR, LVdP/dt, and LVP to bolus injection of sodium-nitroprusside (20 μg/kg) at different Ht levels following induction of graded hemodilution. All abbreviations are as in Fig. 1.

change in blood viscosity with use of high molecular weight dextran may also have contributed to the fall in TPR (Tables 1, 2), as suggested by Fan et al. [7].

The other possible mechanism involved in hemodilution-induced peripheral vasodilation could be through the autonomic nervous system. It has been demonstrated that sympathetic innervation of the heart is necessary to achieve and/or maintain the usual CO response during acute anemia [6]. However, Fahim and Singh [14] have suggested that the cardioacceleration during acute normovolemic hemodilution is mediated primarily through the parasympathetic efferents, and the sympathetic efferents do not play any major role in the reflex tachycardia response to hemodilution. Increases in SV and HR may be the main contributing factors to the increase in CO as observed by others [14, 18, 22]. Another important cause of the discrepancies in the results of various workers could be the different anesthetic agents used. Since anesthetics are known to inhibit and even abolish vagal tone [23], we chose the anesthetic mixture of chloralose and urethane because arterial
Fig. 6. Percentage change in HR, \( \text{LVdP/dt}_{\text{max}} \), LVSP, and MAP following intravenous administration of sodium nitroprusside (SNP) (20 \( \mu \text{g/kg} \)) at control (C) and after the first (I), second (II), and third (III) exchanges of blood with dextran. The graphs demonstrate that, on injection of SNP, the maximum fall in cardiovascular parameters occurred at control hematocrit (C). The effect of SNP was reduced with the degree of hemodilution (I–III).

blood pressure is normal and the heart rate is maintained within a small range [23, 24]. HR of our anesthetized cats was slightly higher than in conscious animals. This could partially explain why the increase in HR in response to hemodilution is small. The increase in CO was due to an increase in SV also. Whereas, in an earlier study in anesthetized dogs an increase in CO in response to hemodilution was largely due to an increase in HR [14].

In our study, the cardiovascular parameters showed a reduction in the responsiveness to the same dose of adrenaline as well as ACh and SNP with a progressive increase in hemodilution, suggesting that the cardiovascular responsiveness to drugs in general was reduced with the fall in Ht. Studies in anesthetized
dogs have demonstrated that hemodilution decreases the chronotropic response to selective β-adrenergic receptor stimulation with isoproterenol [9] and the pressor response to selective α-adrenergic stimulation with phenylephrine [10]. The inotropic response of isoproterenol was not altered by hemodilution whereas, peak acceleration of aortic blood flow by isoproterenol was enhanced in anemia as compared to the control condition [9]. Our observations support the findings of Mahdi et al. [10] that hemodilution attenuates the effects of pressor agents. In addition, we found that the cardiodepressor responses of ACh and SNP were also attenuated by hemodilution. The mechanism for the altered vascular responsiveness of these drugs during hemodilution is uncertain. However, Biro et al. [9] suggested that diminished cardiovascular responsiveness to isoproterenol could be due to inadequate myocardial oxygen delivery to meet the increased oxygen demand associated with catecholamines, whereas Mahdi et al. [10] related reduced cardiovascular responsiveness to phenylephrine during hemodilution to antagonism by the local vasodilating effects of anemic hypoxia. The present observation of inhibition of the vasodepressor effects of ACh and SNP suggests that either of the above mechanisms alone is not responsible for the reduced responsiveness of the cardiovascular system to vasoactive agents under acute anemic conditions.

An increase in myocardial blood flow during hemodilution has been attributed to decreased coronary vascular resistance; reduction in viscosity is believed to be less important [25]. However, the influence of change in shear stress due to reduced coronary perfusion pressure is possible. Oxygen uptake during hemodilution is maintained due to increased blood flow without any augmentation in O₂ extraction or any change in O₂ delivery to the myocardium [25]. Whereas, in the case of systemic circulation, O₂ extraction is increased, O₂ delivery is reduced, and O₂ uptake remains unaltered with a fall in venous P₀₂ reflecting a decrease in average P₀₂ in the body tissue [25]. A similar fall in venous P₀₂ following hemodilution observed in the present study might have made the tissue hypoxic and more vulnerable to added stress as suggested by Crystal and Salem [2].

Besides neurohormonal and metabolic factors, endothelium-derived factors, e.g. endothelium-derived relaxing factor (EDRF), prostacyclin and endothelium-derived contracting factor (EDCF) are known to be effective regulators of vascular tone, blood pressure and tissue perfusion [26–29]. However, our observations with SNP, a vasodilator acting directly on the vascular smooth muscle, did not indicate a major role of endothelium-derived factors in the reduced cardiovascular responsiveness to adrenaline and ACh during acute hemodilution.

In ischemic conditions, production of EDRF and prostacyclin is reduced [27], suggesting that oxygen is required for the production and/or release of EDRF [28, 30–32]. On the other hand, the fall in hemoglobin augments release of endothelium-derived factors [33] due to changes in shear stress on the vessel wall as one of the possible mechanisms. The endothelium-dependent control of the arterial vessel lumen by flow-related shear stress is considered to be a potent regulatory mechanism and an increase in shear stress due to increased flow is believed to
induce the endothelium to trigger relaxation of the subjacent smooth muscle through the release of EDRF/EDCF [34].

It has been suggested that hemodilution per se augments cardiac sympathetic activity reflexly which shifts the resting levels of the cardiovascular parameters upwards, thus reducing the reserve available for further pharmacological agents [9, 10]. Our results do not support this concept. Hemodilution did not produce any significant change in MAP or LVdP/dt_max in our cats. HR and CO showed a significant increases with the fall in hematocrit. Reduced cardiovascular responsiveness to both pressor and depressor agents in the anemic state clearly demonstrated that it was neither due to an upward shift in resting levels of the cardiovascular parameters nor due to any reduction in the available reserve. The possibility of a modulatory role of an endothelium-dependent mechanism and altered arterial baroreceptor-mediated reflex regulatory responses during hemodilution also exists. Pressure flow autoregulation within the brain and spinal cord is well known [35], and a fall in cerebral vasodilator reserve is reported to occur during hemodilution [36]. Therefore, the role of the central nervous system in the reduced responsiveness to vasoactive agents during acute hemodilution is also possible. Involvement of other mechanisms such as release of endogenous vasoactive agent(s) on induction of hemodilution and/or administration of drugs, local tonic adjustments of blood vessels, and impaired arterial oxygenation, can not be ruled out [2]. In summary, our results indicated that in the anesthetized cats the cardiovascular responsiveness to adrenaline as well as acetylcholine is significantly reduced during acute hemodilution, possibly due to reduced O_2 supply to the myocardial and vascular systems due to a sharp drop in the O_2-carrying capacity of the blood.

We are thankful to Mr. Maman Singh for technical assistance, Dr. C. K. Gupta for statistical analysis of the data, Mr. Manish Vaid for laboratory assistance and typing the manuscript, S. Mazumdar for photography and Eric Harrison for the preparation of figures. Ms. Talwar worked as JRF under a research grant No. 3-149/87 (SR-II/RBB-I) from the University Grants Commission, New Delhi to Dr. M. Fahim. One of the author (M. E. Hussain) acknowledges Council of Scientific and Industrial Research, New Delhi for financial assistance.

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