The Influence of Circulating Catecholamines and Prostaglandins on Canine Renal Hemodynamics during Hemorrhage

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SUMMARY The relationship between circulating catecholamines and prostaglandins and the independent contribution of circulating catecholamines to renal vasoconstriction during hemorrhage is unknown. The renal hemodynamic effects of a 30% decrease in blood pressure by hemorrhage were therefore studied in three groups of anesthetized dogs which had undergone prior bilateral renal denervation. A constant unilateral infusion of the catecholamine antagonist phenoxybenzamine (POB, 0.2 µg/kg per min) into the renal artery during hemorrhage was also performed. In the control (C) dogs (n = 6), hemorrhage was not associated with significant changes in glomerular filtration rate (GFR) or renal blood flow (RBF) in either POB-infused and denervated or noninfused, denervated kidneys. In the second group of dogs (n = 8), pretreated with the prostaglandin inhibitor indomethacin (IN, 10 mg/kg, iv), POB-infused and denervated kidneys had a significantly higher GFR (30 vs. 23 ml/min, P < 0.05) and RBF (180 vs. 130 ml/min, P < 0.05) than contralateral denervated kidneys during the hemorrhage period. Similar results were observed in the third group of dogs (n = 6) pretreated with the chemically dissimilar prostaglandin inhibitor meclofenamate (M). Circulating plasma catecholamines increased to a similar degree in C (116 to 530 pg/ml, P < 0.005), IN (116 to 488 pg/ml, P < 0.005), and M (75 to 315 pg/ml, P < 0.01) groups; the major part of this increase was due to an increase in plasma norepinephrine (NE). These results indicate that, in this model of hemorrhage, plasma NE exerts a moderate but significant renal vasoconstrictor effect which is unmasked by prostaglandin inhibition.


THE mechanisms by which hypotensive hemorrhage causes a decline in renal blood flow and glomerular filtration rate remain a subject of continuing physiological interest and importance. The role of the sympathetic nervous system in control of renal hemodynamics has been clarified recently by studies that implicate renal nerves as critical renal vasoconstrictor factors during a 30% decrease in blood pressure by hemorrhage (Henrich et al., 1978a). Similarly, a recent investigation using a specific angiotensin antagonist in the 30% hemorrhage model further demonstrated that the renin-angiotensin system also plays a major renal vasoconstrictive role (Henrich et al., 1978b). However, the renal ischemic contribution and physiological importance of circulating catecholamines in this model of moderate hemorrhage remain speculative.

There are several potential reasons for implicating circulating catecholamines as a factor contributing to renal ischemia during hemorrhage. First, hemorrhage is known to result in a prompt increase in concentrations of both epinephrine and norepinephrine in the blood (Watts and Westfall, 1964). Further, detailed histochemical studies show that the kidney is richly endowed with sympathetic adrenergic nerves, particularly in the renal cortex (Barajas, 1978). The functional importance of these nerves has been examined in several studies which show that stimulation of renal nerve fibers results in an increase in both renin secretion (Loeffler et al., 1972) and renal tubular sodium reabsorption (Bello-Reuss et al., 1976). The increase in renin secretion is mediated through a β-adrenergic receptor (Taher et al., 1976), whereas the adrenergic effect on sodium reabsorption is mediated primarily via α-adrenergic receptors (Zambraski et al., 1976). A renal hemodynamic role for these α-adrenergic receptors was suggested initially by Grandchamp et al. (1971) in studies in which α-adrenergic blockade with phenoxybenzamine (POB) reversed the development of the typical, patchy cortical hypoperfusion seen in severe hemorrhagic hypotension. However, since POB infusion counteracts the vasoconstriction mediated by the renal nerves and by circulating catecholamines, the relative contribution of each component to renal ischemia is unknown.

The intrarenal infusion of catecholamines in-
duces a dramatic decrease in renal blood flow and glomerular filtration rate and an increase in the release of renal prostaglandins, an effect which would theoretically oppose vasoconstriction (McGiff et al., 1970; Needleman et al., 1974). The relationship between catecholamines and renal prostaglandins is complicated further by the fact that prostaglandins have been shown to inhibit norepinephrine release by adrenergic nerve stimulation, thereby interrupting sympathoadrenergic pathways and decreasing adrenergic tone (Stjärne, 1973).

Thus, the purpose of the present investigation was to determine the renal hemodynamic importance of circulating catecholamines during a 30% reduction in blood pressure by hemorrhage, and to separate any vasoconstrictor effect of catecholamines from renal nerves. Last, these studies examine the in vivo relationship between circulating catecholamines and renal prostaglandins during hemorrhage.

**Methods**

Twenty mongrel dogs weighing between 20 and 30 kg were used. Food was withheld 20 hours prior to the study, but the animals were allowed free access to water. On the morning of the study, the dogs were anesthetized with pentobarbital (25–30 mg/kg), intubated, and ventilated with a Harvard respirator. Supplemental small doses of pentobarbital were administered as needed during the study to maintain an even state of anesthesia. Polyethylene catheters were inserted into both ureters and renal veins through bilateral flank incisions and with a retroperitoneal approach. A 25-gauge needle was inserted into one renal artery of all of the dogs for infusion of phenoxybenzamine (POB); an equal intrarenal dose of the drug, several preliminary studies were performed in eight different dogs. In the first series of studies, an intrarenal injection of the a-adrenergic agonist phenylephrine (20 V, 1.5 msec, 2.5 mg) was begun into one renal artery at a dose of 0.005 /ig/kg per min (Zambraski et al., 1976). To verify that this amount of POB represented an effective exclusion period.

Baseline clearance measurements.

**Period 2 (Infusion of POB)**

Fifteen minutes prior to the beginning of this period, an infusion of the a-adrenergic antagonist POB was begun into one renal artery at a rate of 0.2 /ug/kg per min (Zambraski et al., 1976). To verify that this amount of POB represented an effective intrarenal dose of the drug, several preliminary studies were performed in eight different dogs. In the first series of studies, an intrarenal injection of the a-adrenergic agonist phenylephrine (20 /ug) acutely decreased RBF (measured with a non-occlusive 10-mm electromagnetic flow probe (Carolina Instruments) from 208 ± 20 to 70 ± 17 ml/min, P < 0.001. Fifteen minutes after the injection, RBF...
had returned to baseline levels (205 ± 19 ml/min). When the phenylephrine injection was repeated with the POB infusion running (0.2 µg/kg per min), RBF did not decrease (199 ± 13 to 196 ± 10 ml/min, NS). A final phenylephrine injection into the ipsilateral kidney was performed while POB was being infused into the contralateral kidney. RBF again decreased (169 ± 14 to 47 ± 17 ml/min, P < 0.001). Thus, the amount of POB infused in these studies represented an intrarenal rather than systemic dose of the drug.

Period 3 (Prostaglandin Inhibitor)

Fifteen minutes prior to the beginning of this period, group I animals received a bicarbonate solution equal in volume to the prostaglandin inhibitor solutions; group II dogs received the prostaglandin inhibitor indomethacin (10 mg/kg iv) in a dose previously associated with prostaglandin inhibition (Henrich et al., 1978a); and group III dogs received the dissimilar prostaglandin inhibitor meclofenamate (5 mg/kg, iv) in a dose previously associated with prostaglandin inhibition (Blasingham and Nasjletti, 1979). Thus, whereas all animals had bilateral renal denervation and unilateral POB infusion, group I animals had intact prostaglandin synthesis whereas groups II and III received prostaglandin inhibitors.

Period 4 (Hemorrhage)

All three groups of animals were hemorrhaged via the arterial catheter to a stable mean arterial blood pressure which was 25–30% less than mean blood pressure in period 2. The hemorrhage was performed over 10 minutes and 2–3 urine collection periods lasting 20 minutes each were then performed.

Period 5 (Post Control)

Thirty to 40 minutes after reinfusion of the shed blood, a post-control collection was made. Since the POB infusion (period 2) did not alter renal or systemic hemodynamics, periods 2 and 3 are consolidated and reported as a “post-infusion” period in the Results section.

Statistical analysis was performed by Scheffe's analysis of variance procedure (Scheffe, 1959) when making comparisons between periods or groups. Student's paired t-test was used to compare POB-infused with uninfused kidneys. All data are expressed as the mean ± SEM and a P value of < 0.05 was considered significant.

Results

Effects of Hemorrhage on Plasma Catecholamines (Fig. 1)

Prior to hemorrhage, comparable levels of plasma catecholamines were observed in each of the three groups of dogs. Following hemorrhage, a prompt and significant increase in circulating catecholamine concentrations was observed in control (116 to 530 pg/ml, P < 0.005), indomethacin-treated (116 to 488 pg/ml, P < 0.005), and meclofenemate-treated (75 to 315 pg/ml, P < 0.01) groups which was reversible upon reinfusion of the shed blood. Plasma norepinephrine accounted for the largest component of circulating catecholamines during hemorrhage (80 ± 7% in group I, 70 ± 11% in group II, and 78 ± 7% in group III). Whereas the increases in plasma epinephrine were significant, these changes were less consistent than those observed for plasma norepinephrine. The percentage increases in plasma catecholamines induced by 30% hemorrhage (356 ± 41% increase in group I, 320 ± 69% in group II, and 320 ± 29% in group III) also were not different between groups. A similar increase in plasma renin activity was also observed in group I (1.28 ± 0.25 to 6.71 ± 7.2 ng Al/ml per hr, P < 0.001), group II (1.03 ± 0.5 to 4.27 ± 1.01 ng Al/ml per hr, P < 0.01), and group III (1.24 ± 0.7 to 4.81 ± 0.4 ng Al/ml per hr, P < 0.001) dogs.

Effects of Hemorrhage on Systemic Hemodynamics (Table 1)

An equivalent volume of blood was removed from each group of animals to cause the decrease in blood pressure. The decrease in blood pressure was similar (group I, 26 ± 2%, group II, 29 ± 2%, group III, 27 ± 2%) in each group and blood pressure returned to pre-hemorrhage values when the shed blood was reinfused. The decrease in cardiac output was also comparable and reversible in each of the three groups. Thus, systemic hemodynamics were similar for each group of dogs before, during, and after hemorrhage.
Effects of Hemorrhage on GFR and RBF (Table 2)

In the group I (control) animals, hemorrhage induced a mild and similar decline in GFR and RBF in both the denervated and POB-infused and denervated kidneys, but in neither case did the change reach the level of statistical significance. Thus, in dogs which had not been given prostaglandin inhibitors, the presence of the catecholamine antagonist infusion did not significantly increase either GFR or RBF in these denervated kidneys. This result is similar to findings previously obtained in dogs treated with prostaglandin inhibitors and undergoing hemorrhage of this amount (Henrich et al., 1978a, 1978b).

In contrast, in group II and III animals subjected to prostaglandin inhibition prior to hemorrhage, several important differences were observed between POB-infused and denervated vs. uninfused, denervated kidneys. In group II indomethacin-treated dogs, hemorrhage was associated with a moderate but significant decrease in GFR (40 ± 3 to 23 ± 4 ml/min, \( P < 0.001 \)) and RBF (241 ± 26 to 130 ± 17 ml/min, \( P < 0.001 \)) in the denervated, uninfused kidneys. The addition of the POB infusion was associated with a further significant decrease in GFR (14 ± 2 to 8 ± 2 ml/min, \( P < 0.001 \)) and RBF (130 ± 17 to 75 ± 14 ml/min, \( P < 0.001 \)) in these denervated kidneys.
sion to denervation was associated with a modestly but significantly greater GFR (30 ± 3 vs. 23 ± 4 ml/min, P < 0.05) and RBF (180 ± 18 vs. 130 ± 17 ml/min, P < 0.05) during the hemorrhage period.

A similar result was observed in group III meclofenamate-treated dogs. Once again moderate decrements in GFR (38 ± 6 to 24 ± 4 ml/min, P < 0.05) and RBF (261 ± 32 to 127 ± 39 ml/min, P < 0.001) occurred in the uninfused kidneys during hemorrhage. The infusion of the catecholamine antagonist POB again raised both GFR (31 ± 5 vs. 24 ± 4, P < 0.05) and RBF (176 ± 27 vs. 137 ± 39, P < 0.05) significantly during the hemorrhage period. Thus, a modest salutory hemodynamic effect was observed in the POB-infused and denervated kidneys only in the presence of prostaglandin inhibition.

Discussion

Hemorrhage and an attendant decrease in renal function remains a common clinical occurrence, thus enhancing the importance of this subject for investigators over the past 20 years. Of particular investigative interest recently has been the importance of the sympathetic nervous system as a determinant of renal vasoconstriction during hemorrhage. Because sympathetic nervous activity is known to increase with hemorrhage and since the cortex of the kidney is richly endowed with an intricate network of renal nerves (DiBona, 1977; Barajas, 1978) the possibility that renal nerves mediate renal vasoconstriction in hemorrhage seemed great. This possibility was verified in earlier studies using a model of modest reversible hemorrhage; however, the ischemic influence of the renal nerves was most dramatic in hemorrhage when the opposing vasodilatory influence of renal prostaglandins was absent (Henrich et al., 1978a). Although further studies have examined in detail the renal vasoconstrictor influence of the renin-angiotensin system in hemorrhage as well as the mediators of renin secretion in hemorrhage (Henrich et al., 1978b, 1979), the precise contribution of circulating catecholamines to renal vasoconstriction in this model of hemorrhage had been unclear.

Earlier detailed studies by Grandchamp (1971) suggested that the usual pattern of redistribution of intrarenal blood flow from cortex to medulla was prevented by POB infusion when mean blood pressure was reduced to 70 mm Hg by hemorrhage. Moreover, in these studies, POB infusion prevented the drop in both GFR and RBF. However, POB an α₁ antagonist (i.e., postsynaptic adrenergic inhibitor) and thus opposes not only the effects of circulating α₁ agonists (norepinephrine, and, to a lesser extent, epinephrine) but also antagonizes the renal vasoconstriction induced by renal nerves (Berthelsen and Pettiger, 1977). Thus, the dissociation of the renal ischemic influence of renal nerves and circulating catecholamines has not been attempted previously. In the previous series of studies in which the renal effects of modest (30%) hemorrhage was examined, the renal nerves were identified as critical renal ischemic forces, particularly in the presence of prostaglandin inhibition (Henrich et al., 1978a). To focus on the independent renal ischemic importance of plasma catecholamines in this same model of hemorrhage, bilateral renal denervation was performed prior to hemorrhage to examine the effect of POB infusion on renal hemodynamics during hemorrhage. This study also examines the in vivo relationship between catecholamines and prostaglandins.

Similar changes in systemic hemodynamics were induced in each group of dogs, and the increase in plasma catecholamines (i.e., plasma norepinephrine and epinephrine) after hemorrhage was also comparable in all groups. This increase represents both adrenergic and sympathetic nerve stimulation and overflow from sympathetic nerve stimulation (Wurtmand and Zigmond, 1968). Moreover, the presence or absence of prostaglandin inhibition did not alter the observed increase in the plasma catecholamines. The increase in catecholamines was accounted for primarily by norepinephrine which has primarily α-agonist properties. The increments in plasma epinephrine were less striking with this amount of hemorrhage, but may be more pronounced when blood pressure is reduced to shock levels (Watts and Westfall, 1964). However, the results are compatible with previous studies (Feurstein and Gutman, 1971; Feurstein et al., 1977) which suggest that modest hemorrhage elicits predominantly norepinephrine secretion of the magnitude observed in the present study. The increase in catecholamines was reversible after reinfusion of the blood volume removed during hemorrhage.

The addition of the α₁ antagonist POB to renal denervation did not detectably improve the renal response to hemorrhage in the presence of an intact renal prostaglandin system (group I). In fact, the reductions in both GFR and RBF occurring in these denervated kidneys (Table 2) did not reach the level of statistical significance. Thus, with the compensatory vasodilatory action of prostaglandins operating, the addition of an α-adrenergic antagonist did not detectably alter renal hemodynamics during hemorrhage. However, in the presence of prostaglandin inhibition with either of two prostaglandin inhibitors (indomethacin in group II and meclofenamate in group III), a modest but significant increase in GFR and RBF was observed in POB-infused and denervated kidneys compared to conterateral uninjured, denervated kidneys (Table 2). This result suggests that, with inhibition of the vasodilatory influence of prostaglandins, the unopposed renal vasoconstrictive forces of hemorrhage are activated, and that the inhibition of one mediator of vasoconstriction (i.e., circulating catecholamines with POB infusion) is rendered detectable. It should be noted, however, that the contribution of circulating catecholamines as assessed by the additive protective effect of POB infusion to renal
denervation in the presence of prostaglandin inhibition is modest, especially when compared to earlier findings which assigned much greater renal vasoconstrictor importance to the renal nerve component of the sympathetic nervous system. Certainly, with greater reductions in blood pressure (e.g., to ≤70 mm Hg) a greater role for plasma catecholamines as vasoconstrictor agents is possible.

These studies reaffirm the critical role the vasodilatory renal prostaglandins play in opposing renal vasoconstriction during hemorrhage. Although prostaglandins have not been demonstrated to have a major influence on the renal circulation in the unanesthetized animal, limited information is available from experiments in the anesthetized state (Swain et al., 1975; Terragno et al., 1977; Zambraski and Dunn 1979), a physiological stress such as hemorrhage markedly increases prostaglandin synthesis and release (Terragno et al., 1977; Johnston and Selkurt 1977; Henrich et al., 1978a) and inhibition of prostaglandin synthesis results in marked enhancement of renal vasoconstrictor in both anesthetized (Leffler and Passmore 1977; Henrich et al., 1978a) and unanesthetized (Swain et al., 1975; Terragno et al., 1977) animals. The similar results in group II and group III dogs suggest that the results are the result of inhibition of prostaglandin synthesis and not secondary to other pharmacological effects of indomethacin.

In summary, plasma norepinephrine accounts for the majority of the circulating plasma catecholamine increment during a 30% reduction in blood pressure by hemorrhage and is uninfluenced by the presence or absence of prostaglandin inhibition. Further, circulating catecholamines may be demonstrated to induce a moderate degree of renal vasoconstriction in the period immediately following hemorrhage of this amount. Finally, this renal vasoconstriction during hemorrhage normally is opposed by the vasodilation caused by renal prostaglandins.

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