SUMMARY The influence of barbiturate anesthesia on renal blood flow was assessed by the xenon washout method in trained dogs with catheters chronically implanted in the renal artery. Anesthesia induced with either thiopental sodium or pentobarbital sodium resulted in a striking reduction in renal blood flow ($4.1 \pm 0.1$ vs. $2.6 \pm 0.2$ ml/g per min; $P < 0.001$) without a change in arterial pressure. The reduction in blood flow was prevented by a high salt intake and partially reversed by agents which interrupt the renin-angiotensin system. These results are consistent with the hypothesis that anesthesia blunts renal vascular responses to angiotensin II.

A major cardiovascular response including reduced renal blood flow is often induced by anesthesia in man and in experimental animals. Although the mechanisms for these are poorly defined, anesthesia-induced activation of the renin-angiotensin system is a possible cause, as the renal vasculature is especially sensitive to angiotensin II. However, there is considerable discrepancy between reports on the sensitivity of the renovascular bed to angiotensin. In man, for example, studies performed without anesthesia demonstrated a threshold response to angiotensin infused into the renal artery at doses generally below 10 ng/min, and the renal vessels were 100-fold more sensitive to angiotensin than to norepinephrine. In anesthetized animals, on the other hand, a number of studies have revealed a requirement for much higher doses of angiotensin, and it has been suggested that the renal vessels are more sensitive to norepinephrine than to angiotensin. These discrepant results may be due to the fact that anesthesia can blunt renal vascular responses to angiotensin II, and perhaps reflect the frequent association of activation of the renin-angiotensin system and a reduction in vascular responsiveness to angiotensin II. It therefore seemed important to assess the role of renin-angiotensin system activation in the renal vascular response to anesthesia. We have performed such a study in dogs with catheters chronically implanted in the renal artery. Using this model, we assessed the effects of barbiturate anesthesia and of sodium intake on renal perfusion and responsiveness to vasoconstrictor agents and appropriate blocking drugs.

Methods

Surgical Preparation

Studies were performed on 25 healthy, mongrel dogs, weighing 21–35 kg and selected for gentleness. In experiments in which surgery was performed, or when the effects of anesthesia per se were to be studied, anesthesia was induced with an initial intravenous dose of sodium pentobarbital (30 mg/kg) and maintained with periodic additional doses of 3–5 mg/kg of the same agent, to prevent spontaneous movement. At the time of preparative surgery a period of at least 60 minutes was allowed after the completion of procedures before vascular studies were performed; a 30-minute period was allowed between the last supplemental dose of anesthesia and the hemodynamic study. On days during which surgery was not performed, the hemodynamic studies were performed 30 minutes after induction of anesthesia.

Several days prior to the surgical procedure an aortogram was obtained to define the renal vascular anatomy. On the day of preparative surgery the renal pedicle was approached through a flank incision by retroperitoneal dissection. The artery was exposed and its lateral margin cleaned suf-
cantly to allow catheterization with a polyvinyl chloride catheter (outside diameter, 0.040 inches; inside diameter, 0.025 inches; length, 50–60 cm; dead space volume about 0.7 ml) with a tapered tip as described in detail by Rudolph et al. To monitor arterial pressure and obtain blood samples, a second, large (o.d., 0.70; i.d., 0.40) polyvinyl chloride catheter was placed in the aorta below the renal artery via a femoral or lumbar artery. A similar catheter was placed in a femoral or lumbar vein. All catheters were exteriorized through a lateral incision in the flank. After completion of the surgical procedure the catheters were flushed with a heparin solution (10 mg/ml), sealed, and protected for chronic use in the pocket of a specially prepared jacket (A. Chatham, Inc., Los Angeles).

TECHNIQUES

Arterial pressure was recorded with a Statham P23DC transducer on a Grass polygraph. During anesthesia respiration was controlled by means of a cuffed endotracheal tube and a Harvard respiratory pump, and rectal temperature was monitored with a Yellow Springs rectal probe and maintained at 36–37°C.

Blood flow was measured in all dogs with radioxenon, as described in detail in earlier publications, and in three dogs with tagged microspheres. Xenon with a specific activity of 1–2 mCi/ml was used. Blood flow was measured by injecting 100–400 μCi in a volume of 0.1–0.4 ml as a bolus into the kidney through the catheter, followed by a 0.7-ml flush. The washout of the radioactive gas was monitored externally by a scintillation probe with a thallium-activated sodium iodide crystal (2 × 2 inches) connected to a pulse height analyzer with a window setting of 70–100 keV, a digital rate meter and recorder (Baird-Atomic, Inc., Cambridge, Mass.). This system was linear to 800,000 counts/min in the range of 70–100 keV. The peak counts were generally in the range of 80,000–400,000 counts/min. Background prior to the first blood flow determination on any study day ranged from 400 to 2,000 counts/min and rose progressively with serial determinations on any day to a maximum of 40,000. The ratio of the peak count to background ranged from 20 to 50.

Two methods of curve analysis were used. Mean blood flow was determined from the initial slope. Compartmental flow rates and distribution were determined by graphical analysis according to the method of Thorburn et al., modified so that a smoothed 3-minute value was used to approximate the two slowest flow components. To assess the fidelity of the graphical analysis the initial slope was also assessed by an unweighted least squares program in 28 dogs with tagged microspheres. Xenon with a specific activity of 1–2 mCi/ml was used. Blood flow was measured by injecting 100–400 μCi in a volume of 0.1–0.4 ml as a bolus into the kidney through the catheter, followed by a 0.7-ml flush. The washout of the radioactive gas was monitored externally by a scintillation probe with a thallium-activated sodium iodide crystal (2 × 2 inches) connected to a pulse height analyzer with a window setting of 70–100 keV, a digital rate meter and recorder (Baird-Atomic, Inc., Cambridge, Mass.). This system was linear to 800,000 counts/min in the range of 70–100 keV. The peak counts were generally in the range of 80,000–400,000 counts/min. Background prior to the first blood flow determination on any study day ranged from 400 to 2,000 counts/min and rose progressively with serial determinations on any day to a maximum of 40,000. The ratio of the peak count to background ranged from 20 to 50.

For every dog, the training period involved the systematic assessment of blood flow variability during any 60-minute period. Other experimental maneuvers were not used. Then a series of pharmacological studies was performed in a random sequence at intervals of 3–4 days. We assessed the influence of barbiturate anesthesia on renal hemodynamics by infusing either pentobarbital or thiopental sodium through the venous catheter. To evaluate the influence of the anesthetic agents on renal vascular reactivity to angiotensin II and norepinephrine we measured responses to graded doses of either agent infused into the renal artery with the dogs awake or anesthetized. A similar approach was used to assess the role of activation of the sympathetic nervous system and renin-angiotensin system by use of the specific pharmacological antagonists: the α-adrenergic blocking agents phentolamine and phenoxybenzamine; the competitive antagonist to angiotensin II, 1-Sar,8-Ala-angiotensin II; BPF 9β, an agent which blocks the conversion of angiotensin I to II; and propranolol, an agent which blocks renin release. All drug doses were calculated as the base. Except in studies performed to assess the efficacy of the doses of antagonists, only a single agent was administered on any day and all studies were performed in both awake and anesthetized dogs.

The relationship between the dose of angiotensin II (Hypertensin, CIBA) infused intra-arterially (i.a.) into the renal artery and the resulting change in blood flow was determined by infusing log-dose increments ranging from 3 to 100 ng/min with a motor-driven syringe in a volume of 0.3–1.0 ml/min. The agent was infused for 3 minutes at each dose level for each measurement of blood flow. Similar dose-response curves were constructed for norepinephrine bitartrate (Levophed, Winthrop), 0.3–3.0 μg/min, i.a. α-Adrenergic blockade was induced with either phentolamine mesylate (Regitine, CIBA), 0.1–1.0 mg/min, or phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline and French), 1.0 mg/kg, infused i.a. for 10–30 minutes before
assessing the renal vascular response or the adequacy of blockade. Similarly l-Sar,8-Ala-angiotensin II (P113, Saralasin, Norwich), 10–300 ng/kg per min, and BPF 9a (Schwarz/Mann), 10–100 ng/kg per min, were infused i.a. for 3–5 minutes before assessing the vascular response or adequacy of blockade. Propranolol hydrochloride (Ayerst), 1.5–2.0 mg/kg, was infused intravenously 20 minutes before the determination of renal blood flow.

The highest dose of phentolamine, phenoxybenzamine, and P113 used was adequate to shift the dose-response relationship for the relevant agonist by more than one order of magnitude. For example, phentolamine in four dogs obliterated the response to an infusion of norepinephrine of 3.0 µg/min (−2.2 ± 0.3 vs. +0.21 ± 0.6 ml/g per min; P < 0.01). These doses of phentolamine did not influence either the renal vascular response to angiotensin or the blood pressure. Because of limited supplies of BPF 9a it was possible to assess its influence on responses to angiotensin I in only one dog. A dose of angiotensin I of 500 ng/min reduced renal flow from 3.2 to 1.3 ml/g per min in the absence of BPF 9a and failed to induce a response during the infusion of BPF 9a at 30 ng/kg per min. In this animal BPF 9a at 100 ng/kg per min did not modify the renal vascular responses to angiotensin II or norepinephrine.

In five dogs balance was achieved on a low salt diet (Nutritional Biochemicals), as evidenced by a drop in weight of approximately 1 kg and a urine sodium concentration of less than 15 mEq/liter, or on a high salt diet which provided at least 40 g (684 mEq) of sodium chloride daily. Tap water was replaced with distilled water for the dogs on a low salt diet. The dogs in the balance study were housed in a metabolism cage which allowed 24-hour urine collection. The other dogs were allowed an intake of standard laboratory chow and tap water ad libitum; this generally provided a sodium intake of about 40 mEq/day.

Group means have been presented with the standard error of the mean as the index of dispersion. Statistical probability was evaluated, where appropriate, with Student's t-test or the paired data test, or by analysis of variance. Otherwise the Wilcoxon rank sum test (WRST) or Fisher exact test (FET) for nonparametric data was used. The null hypothesis was rejected when the P value was less than 0.05.

Results

During the first several weeks after surgery, renal blood flow in the unanesthetized dogs tended to vary from day to day. During this interval the average of 101 determinations was 3.7 ± 0.1 ml/g per min, significantly less than the average value for the same dogs when successful training had been accomplished (4.1 ± 0.1 ml/g per min; n = 91; P < 0.001). The variability is evident in the coefficient of variation, which, in the early period, averaged 28 ± 3% and was significantly greater than the coefficient of variation once stability had been achieved (13 ± 1%; P < 0.005). Typical day-to-day variability for one dog after training had been accomplished is shown in Figure 1.

The effect of induction of anesthesia, without surgery, on renal blood flow in the same dog is also shown in Figure 1. In each animal, induction of anesthesia with either pentobarbital sodium (4.1 ± 0.1 to 2.6 ± 0.2 ml/g per min; n = 91; P < 0.001) or thiopental sodium (4.5 ± 0.4 to 3.2 ± 0.5; n = 8; P < 0.01) resulted in a striking reduction in mean renal blood flow. Mean arterial pressure in the unanesthetized dogs averaged 93.8 ± 2.0 mm Hg, and heart rate was 64.5 ± 1.4 beats/min. In dogs anesthetized with pentobarbital sodium but not exposed to surgical stress or environmental stimuli, arterial pressure was unchanged (91.8 ± 1.3 mm Hg), but there was a striking tachycardia (140 ± 2.4 beats/min). Surgical trauma induced a variable, but generally very large, increase in arterial pressure to levels which often exceeded 140 mm Hg.

The reduction in mean renal blood flow was associated with a significant decrease in both the rapid component flow rate (component I) and the percentage of flow entering this compartment. Components II, III, and IV represent compartments with slower flow rates, probably located in the renal medulla and in fat.
TABLE 1  Reversal of the Effect of Pentobarbital on the Rapid Component of Renal Blood Flow with PI 13

<table>
<thead>
<tr>
<th></th>
<th>Rapid component of renal blood flow</th>
<th>(ml/g per min)</th>
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<tbody>
<tr>
<td></td>
<td>(% of total renal blood flow)</td>
<td></td>
</tr>
<tr>
<td>(1) Unanesthetized</td>
<td>82.7 ± 1.6</td>
<td>6.4 ± 0.3</td>
</tr>
<tr>
<td>(2) Anesthetized</td>
<td>62.6 ± 3.6</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>(3) Anesthetized plus PI 13*</td>
<td>82.4 ± 2.1</td>
<td>6.9 ± 0.3</td>
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</table>

*The PI 13 dose was that in any study which induced the largest increase in mean renal blood flow.

more striking response in the anesthetized dog on a sodium-restricted intake (FET, P = 0.045). Renal vascular responses in unanesthetized dogs on a low salt diet were assessed only with PI 13, because the supply of BPF 90 was limited. The increase in blood flow induced after PI 13 was significantly less than the response in dogs in which anesthesia was added to the regimen of sodium restriction (FET, P = 0.05). Both classes of agents increased both the percentage of flow (P < 0.01) and the flow rate in the most rapid component (P < 0.05) when mean blood flow was increased (Table 1).

Similarly, renal blood flow under pentobarbital anesthesia was increased after administration of propranolol (3.2 ± 0.9 ml/g per min; P < 0.005), although it still was reduced with respect to the control state without anesthesia (P < 0.05).

To assess the role of changing responsiveness to the endogenous vasoconstrictor agents, norepinephrine and angiotensin, we constructed dose-response curves for both agents in animals awake and under the influence of pentobarbital anesthesia. The responses to a dose of angiotensin (10 ng/min) and norepinephrine (1,000 ng/min) which

FIGURE 3 Absence of a renal vascular response to α-adrenergic blockade in the anesthetized dog. Both "spontaneous variation," defined without pharmacological intervention under anesthesia, and flow changes during infusion of phenoxybenzamine and phentolamine were randomly distributed. MBF = mean renal blood flow.

FIGURE 4 The effect of a high salt intake for 7 days prior to induction of anesthesia on the renal vascular response to pentobarbital sodium.

The renal vascular response to anesthesia in six animals treated with very high salt intake for at least a week before induction of anesthesia is shown in Figure 4. In contrast to the striking reduction in blood flow induced by anesthesia in animals on an unrestricted diet, renal blood flow did not fall in the dogs on a high salt intake. Renal blood flow in the unanesthetized animal averaged 4.7 ± 0.3 ml/g per min and was significantly greater than in the animals when intake was unrestricted (P < 0.025). After induction of anesthesia, flow averaged 4.9 ± 0.1 ml/g per min and was not significantly greater than the preinduction value (P < 0.20).

The influence on renal blood flow of the two classes of agents which interrupt the renin-angiotensin system in the three states is summarized in Figure 5. In dogs which were awake and on an unrestricted intake, PI 13 induced a dose-related reduction in mean blood flow (P < 0.01). Conversely, when the same animals were anesthetized with either pentobarbital or thiopental, PI 13 induced a dose-related increase in renal blood flow (chi square = 26.5; P < 0.005). The addition of a second stimulus to activation of the renin-angiotensin system, i.e., sodium restriction, produced a small but statistically significant potentiation in the renal vascular response (FET, P = 0.033). BPF 90 did not increase renal blood flow in the unanesthetized dog on an unrestricted sodium intake, but did increase renal blood flow in the anesthetized dog (WRST, P < 0.001), and induced a

(9.8 ± 1.2% to 26.0 ± 4.0%; P < 0.001). These findings are summarized in Figure 2 and Table 1.

In Figure 3 the renal vascular responses of anesthetized dogs to α-adrenergic blockade with phenoxybenzamine or phentolamine are contrasted to the spontaneous variability of blood flow under anesthesia. Sequential flow determinations in the absence of a pharmacological agent during the 60 minutes after the induction of anesthesia revealed a random change, 11 of 21 second determinations exceeding the first. Neither a-blocker induced a systematic increase in blood flow under anesthesia (7 of 13 increased), despite large doses which were more than adequate to induce a highly significant degree of α-adrenergic blockade. Neither agent induced systemic hypotension when infused into the renal artery in the doses used.

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FIGURE 5 The influence of a converting enzyme antagonist (BPF 9a) and a competitive antagonist to angiotensin II (P113) on renal blood flow in the awake and anesthetized dog. Doses listed in the inset are in ng/kg per min. Note that both agents increase renal blood flow in the anesthetized animal, and that the addition of sodium restriction results in only a small additional vascular response.

induced approximately similar reductions in blood flow in the unanesthetized state (1.54 ± 0.2 vs. 1.04 ± 0.5 ml/g per min; \(t = 0.67; P < 0.50\)) are shown in Figure 6. After induction of anesthesia the response to angiotensin was significantly reduced (\(P < 0.01\)) (Figs. 6 and 7). Conversely, the response to norepinephrine was enhanced so that responses to doses that had been equieffective when the dogs were awake were significantly different during anesthesia (\(-2.8 ± 0.15\ vs. -0.71 ± 0.28; t = 7.45; p < 0.001\)). The entire dose-response curve for angiotensin in both states is presented in Figure 7. Anesthesia reduced the vascular response to angiotensin over the entire dose-response range (chi square = 12.6; \(P < 0.005\)). Over a wide range of flows induced by varying sodium intake and with or without anesthesia there was excellent correlation between the control mean blood flow (x), and the renal vascular response to angiotensin infused into the renal artery at 100 ng/min (\(y = 0.7 - 0.78x; r = -0.91; P < 0.01\)) in 53 determinations. A relationship between resting flow and response to any given dose of norepinephrine was not established. In view of the fact that the reduction in blood flow consequent to anesthesia was associated with a potentiated response to norepinephrine, this was not surprising.

Discussion

Barbiturate anesthesia consistently reduced renal blood flow in this study; this finding confirms many reports on the effects of anesthesia.\(^1\)\(^{11}\) Since arterial pressure did not fall, the increase in renal vascular resistance must have reflected active vasoconstriction. The most likely candidates as mediator of the renal vascular response were the endogenous vasoconstrictor agents, norepinephrine and angiotensin. The failure of the renal vasculature to respond to very large doses of two \(\alpha\)-adrenergic blocking agents rules out a direct effect of norepinephrine, especially in view of the evidence in this study which implicates angiotensin as the mediator. A high salt intake, the most active means of suppressing endogenous renin and angiotensin, prevented the reduction in blood flow. Moreover, three classes of pharmacological agents which interrupt the renin-angiotensin axis increased renal blood flow. It always is possible that the response to a single agent is not related to its primary action, but rather may reflect some nonspecific influence.\(^{26}\) For example, BPF 9a may increase the circulating concentration of bradykinin, an active vasodilator. It is very unlikely, however, that a similar response to these three unrelated classes of agents is nonspecific in view of their dissimilar structures and mechanisms of action.\(^{26}\) P113, an analogue of angiotensin II, acts as a competitive antagonist. BPF 9a, which is unrelated structurally, acts by blocking the conversion of angiotensin I to angiotensin II. Propranolol has an unrelated structure and acts by preventing renin release. Unfortunately it, too, has intrinsic activity and thereby reduces renal blood flow.\(^{26}a\) Because P113 and propranolol both reduce renal blood flow directly, it is difficult to use the magnitude of the flow response to assess the magnitude of the reduction in
siveness to the two agents, with blunting of the response to angiotensin II.

Note the reciprocal effect of anesthesia on renal vascular responsiveness to norepinephrine and angiotensin II infused into the renal artery. The reduction in responsiveness to angiotensin during anesthesia rules out a major role of arteriolar geometry. The change in vascular responsiveness to angiotensin II may have been due to activation of prostaglandin synthetase, mediated by anesthesia-induced renin release. Angiotensin increases renal prostaglandin release, and prostaglandins interfere with responsiveness to angiotensin II. Enhanced responsiveness to norepinephrine during anesthesia rules out a major role of arteriolar geometry. The change in vascular responsiveness to angiotensin II may have been due to activation of prostaglandin synthetase, mediated by anesthesia-induced renin release. Angiotensin increases renal prostaglandin release, and prostaglandins interfere with responsiveness to angiotensin II. Moreover, prostaglandin synthetase inhibitors enhance renal vascular responses to angiotensin II. Indeed, consistent with this possibility is the finding that synthetase inhibitors have less effect on renal blood flow in the unanesthetized than the anesthetized animal.

The renin-angiotensin system is activated and renal blood flow is reduced without major surgery required to expose the kidney; this additional stimulus would be anticipated to enhance the response. In virtually every study on the detailed physiology of the kidney has been performed under the influence of anesthetic agents. Certainly the interpretation of these experiments must take into account a background which includes a reduction in renal blood flow due to biologically significant activation of the renin-angiotensin system. The influence of prior sodium intake on this response not only provides an explanation for discrepancies in earlier reports, but also provides an approach to avoiding this problem. The amount of sodium required to prevent the response, however, must be defined for each species, each anesthetic regimen, and each surgical preparation.

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