Autoregulation of renal blood flow in the puppy

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The ability of the immature kidney to autoregulate blood flow was investigated. Renal blood flow was measured by electromagnetic flowmeter. In six puppies, selective blockade of the intrarenal effects of angiotensin II (AII) by \([1\text{-sarcosine, 8-alanine}]\text{angiotensin II (anti-AII)}\) administered into the renal artery did not change renal blood flow. During selective renal AII blockade, intravenous AII raised perfusion pressure from 76 ± 2 to 100 ± 6 mmHg. Renal blood flow increased from 1.59 ± 0.29 to 1.98 ± 0.59 ml/g per min, but returned to control levels within 40 s in spite of persistent arterial pressure elevation. In another group of seven puppies, renal blood flow remained constant despite reduction of renal perfusion pressure by aortic constriction to 60 mmHg. In two of these seven puppies intrarenal AII did not abolish autoregulation. Autoregulation of renal blood flow occurs in the puppy and is not influenced by inhibition of angiotensin. The renin-angiotensin system does not appear to be involved in the normal regulation of renal blood flow in the puppy.

1) Competitive antagonism of angiotensin II with anti-AII. The initial experiments were performed to confirm that anti-AII inhibits angiotensin II activity in the puppy as has been shown in adult dogs (12).

Six mongrel sucking puppies, 14–25 days old were anesthetized with pentobarbital (23 μg/kg iv or ip) after a 1-h fast. A tracheostomy was performed and the femoral vessels were cannulated for drug administration and blood pressure monitoring. Arterial blood pressure was monitored by a Statham transducer connected to a Sanborn recorder. The puppies were kept inside an Isolette and body temperature was maintained between 36.5 and 37.5°C.

The effects on arterial blood pressure of angiotensin II (Hypertensin-Ciba) given intravenously in bolus doses of 50, 100, 1,000, 5,000, and 10,000 ng were observed. Anti-AII was then infused intravenously at a rate of 6–15 μg/kg per min, and the effects of bolus doses of angiotensin II were observed. The infusion containing anti-AII was then stopped, and 20 min later the effects of varied concentrations of angiotensin II on blood pressure were again observed.

2) Response of renal blood flow to increases in perfusion pressure. To examine autoregulation, the effect of increasing renal perfusion pressure on renal blood flow was studied when renal vascular effects of angiotensin II were demonstrably blocked. Another group of six puppies 28–37 days old were prepared in a manner similar to that described in protocol A1. In addition, the left kidney was exposed by flank incision. The renal artery was dissected gently and fitted with a non-cannulating electromagnetic flow probe (Biotronex Laboratory, Inc.). Probes ranged in size from 0.85 to 2.0 mm in diameter. Individual flow probes were calibrated prior to and after the experiment. Saline and blood were perfused...
through an arterial segment at known flow rates in vitro before the experiment. After the experiment, confirmation was obtained by perfusion at known rates through an arterial segment in situ. The determinations were repeated at least twice for the same flow rate. At least nine flow rates were recorded, ranging from 5 to 60 ml/min. Linear regression was then determined by the least-squares method. The correlation coefficient between recorded and actual flow rates was 0.994. Since very low flow rates were expected in the puppy, the sensitivity of the machine was adjusted so that a 1-mm deflection was equivalent to 0.8–1.0 ml/min according to the size of the flow probe. Distal to the flow probe, a pneumatic cuff was placed around the renal artery so that base-line zero flow could be obtained by remote and complete occlusion of the renal artery. Distal to the flow probe but proximal to the pneumatic cuff, the renal artery was punctured with a 30-gauge lymphangiography needle and an infusion of 5% dextrose solution was started at 0.1 ml/min. After stable blood pressure and flow were obtained for at least 30 min, the intrarenal arterial infusion was changed to 5% dextrose solution containing anti-AII in a concentration calculated to deliver the antagonist at the rate of 2 µg/min. The infusion rate was maintained at 0.1 ml/min. Twenty minutes after the infusion of the antagonist was established, bolus doses of angiotensin II (1,000 ng) were injected into the renal artery to determine adequacy of angiotensin II blockade. Delivery of the antagonist at 2 µg/min proved to be adequate to completely block the intrarenal vascular effects of bolus doses of 1,000 ng of angiotensin II into the renal artery in all six puppies. (This same dose of angiotensin II given intravenously elicited a maximum rise in systemic pressure.) Subsequently, the arterial blood pressure was raised by the intravenous injection of a bolus of 1,000 ng angiotensin II. This protocol enabled us to study the pure effects on renal blood flow of increasing the perfusion pressure while any direct effect of angiotensin II on the renal vasculature was avoided.

In five of these puppies, after the effects of angiotensin II were dissipated, renal blood flow was measured after bolus doses of 2,000 and 5,000 ng of angiotensin II were administered via the renal artery to determine the dose range of inhibition by the antagonist. In these five puppies, the intrarenal infusion was changed to 5% dextrose in water without the anti-AII. Twenty minutes later, bolus doses of angiotensin II (1,000, 2,000, 5,000 ng) into the renal artery were repeated and the effects on renal blood flow were observed.

B) Renal Blood Flow after Decreases in Perfusion Pressure

The effect of decreasing renal perfusion pressure on renal blood flow was studied in another group of seven puppies 9–63 days of age. The puppies were prepared as in protocol A2 except that the occluding cuff was fitted around the aorta above the origin of the renal vessels. For each level of blood pressure reduction, renal artery perfusion pressure was diminished by gradual inflation of the occluding aortic cuff over 25–30 s. Blood pressure was measured below the aortic constriction. The renal artery was punctured in two animals (age 56 and 63 days) with a 30-gauge lymphangiography needle as in protocol A2. In these two animals the effect of decreasing arterial pressure on renal blood flow was observed before and during infusion of 2 µg/min of anti-AII into the renal artery.

Significance was tested by group- and paired-t test. Values are means ± SE.

![Figure 1](http://ajplegacy.physiology.org/)

**FIG. 1.** Effects on mean arterial pressure of intravenous angiotensin II alone (○—○) and after stopping infusion of the angiotensin II antagonist, [1-sarcosine, 8-alanine]angiotensin II (Δ—Δ). MAP, mean arterial pressure (n = 6). Values are means ± SE.
RESULTS

A) Response of Kidney to Increased Perfusion Pressure

1) Competitive antagonism of angiotensin II with anti-AII. The effect of intravenous angiotensin II on mean arterial pressure in six puppies before, during, and 20 min after stopping the intravenous infusion of anti-AII is shown in Fig. 1. Increasing doses of angiotensin II up to a dose of 1,000 ng caused a progressive rise in mean arterial pressure. The administration of larger doses of angiotensin II produced no additional rise in arterial pressure. After the pressor effect of the last dose of angiotensin II had dissipated, the intravenous infusion of anti-AII was started and produced no change in mean arterial pressure (65 ± 5 to 64 ± 5 mmHg). During the intravenous infusion of anti-AII, the pressor effects of up to 1,000 ng of angiotensin II were blocked, and the effects of higher doses were markedly blunted. Twenty minutes after the infusion of anti-AII was stopped, the vasoconstrictor effect of intravenous angiotensin was again observed, with an increase in sensitivity noted in the lower dose ranges compared with control (P < 0.05, paired-t test).

2) Response of renal blood flow to increased perfusion pressure. Figure 2 depicts the effects of intrarenal arterial injection of angiotensin II with and without a simultaneous infusion of anti-AII. Administration of 1,000 ng angiotensin II alone caused a drastic fall in renal blood flow (from 1.64 ± 0.26 to 0.51 ± 0.18 ml/g kidney per min; P < .05, paired-t test), and 2,000 ng reduced blood flow close to zero. In the presence of anti-AII, doses of angiotensin II up to 2,000 ng produced no significant change in renal blood flow. However, 5,000 ng of angiotensin II even in the presence of anti-AII caused a significant fall in renal blood flow (0.94 ± 0.21 ml/g kidney per min; P < .05, paired-t test).

As previously mentioned (in MATERIALS AND METHODS), these results permitted us subsequently to employ a dose of 2 µg/min of the antagonist with confidence that this dose would block any direct effect on the renal vasculature of 1,000 ng of angiotensin II administered intravenously.

Figure 3 shows the effects of intravenous angiotensin II on blood pressure and renal blood flow at a time when anti-AII was being infused into the renal artery in a 38-day-old puppy. Administration of 1,000 ng of angiotensin II caused a prompt rise in arterial pressure (from 105/55 to 115/70 mmHg) and renal blood flow (15.8 to 22.0 ml/min). However, 32 s after the injection of angiotensin II, while blood pressure remained elevated (115/70 mmHg), renal blood flow had already returned to preinjection levels (15.8 ml/min), indicating autoregulation. Blood pressure remained elevated for at least 2.5 min. The results of similar studies in all six puppies are shown in Fig. 4. The intravenous administration of angiotensin II caused a rise in mean arterial pressure from 76 ± 2 to 95 ± 8 mmHg. This was accompanied by an insignificantly increase in renal blood flow from 1.59 ± 0.29 to 1.98 ± 0.59 ml/g per min. Twenty seconds after the injection of angiotensin II injection, while blood pressure remained elevated (98 ± 5 mmHg), renal blood flow remained close to control levels (1.73 ± 0.35 ml/g per min). Forty seconds after the injection of angiotensin II, while arterial pressure remained elevated at 100 ± 6 mmHg, the mean renal blood flow in all the puppies had returned to control values (1.53 ± 0.3 ml/g per min.).
B) Renal Blood Flow after Decreases in Perfusion Pressure

Slowly decreasing mean renal perfusion pressure by aortic constriction to 60 mmHg from control values of 75–125 mmHg produced no change in renal blood flow in six of seven puppies (Fig. 5). In four of these puppies no change was noted down to 50 mmHg. In the oldest puppy (63 days old), with the highest control blood pressure (125 mmHg), autoregulation was demonstrated only to a level of 75 mmHg. In the two puppies (56 and 63 days old) tested during infusion of anti-AII into the renal artery the antagonist had no effect on the autoregulatory phenomenon.

DISCUSSION

The high renal resistance in the young has been attributed to several factors including, but not limited to, the renin-angiotensin system and the sympathoadrenal system. Metcalf (19) has suggested that the high resistance in the neonatal kidney may be due to high interstitial pressure. Possible support for this theory may be found in the report by Horster and Valtin (11) that the water content of renal tissue in puppies less than 30 days old is higher than that in older age groups. These same investigators, however, also showed that even after renal water content approximated adult values, renal blood flow continued to increase for a considerable time, suggesting that some factor other than interstitial pressure is involved. Previous studies in our laboratory (5, 14) and others (11) have indicated that renal blood flow per gram kidney achieves adult levels only after 10–12 wk.

Several other studies have indicated that the rise in renal blood flow with age is mainly due to a fall in vascular resistance (5, 9). Moreover, micropuncture studies in the guinea pig indicated that the increase in filtration pressure noted with maturation was due mainly to a decrease in afferent arteriolar resistance (23). Jose et al. (15) demonstrated in dogs that there is enhanced sensitivity of the neonatal renal vasculature to intrarenal arterial infusion of epinephrine. A declining sensitivity to catecholamines and/or a decrease in sympathoadrenal discharge might then explain the decrease in renal resistance with maturation. The gradual rise in blood pressure seen with maturation may also contribute to an increase in renal blood flow in older puppies.

The effects of catecholamines on renal blood flow in the young may be mediated directly via alpha-adrenergic vasoconstrictor effects or indirectly via beta-adrenergic activity leading to increased renin release. Circulating renin levels and renal renin content are elevated in puppies 2–3 wk old (5, 8) and decline gradually to adult values thereafter. The intrarenal infusion of angiotensin II antagonist did not result in any change in renal blood flow when administered at a dose sufficient to block the effect of 2,000 ng of
angiotensin II. This is sixfold the renin secretory rate per minute per kidney in puppies at 1 mo of age (unpublished observations). Assuming that sufficient and uniform concentrations of the antagonist reached the receptor sites, the inability of the angiotensin II antagonist to increase renal blood flow suggests that the renin-angiotensin system does not play a role in the regulation of resting blood flow in the puppy after the 4th wk of life. Although there may be some question about the completeness of mixing and uniformity of distribution during renal artery infusion of a substance, larger doses of the antagonist (6–15 µg/kg per min) given alone intravenously also failed to alter renal blood flow, yet they were sufficient to block the vasoconstrictor effects of larger doses of exogenous angiotensin II (unpublished observations). Similar results were also observed in the puppy younger than 4 wk and in the fetal lamb (20), indicating that even in younger animals angiotensin probably is not involved in maintenance of renal resistance (13). It is possible, however, that exogenous antagonist does not have prompt access to sites where intrarenally generated angiotensin II exerts its effects. This issue cannot be resolved in the present study.

The current experiments indicate that the phenomenon of autoregulation is present in the puppy long before flow rates similar to those noted in adult kidneys are achieved. The youngest puppy studied was 9 days old. It appears that in the puppies 28 days and younger, autoregulation persisted at a lower perfusion pressure when compared with previously reported data in adult dogs; however, since there were only two puppies in the older age group, statistical comparison with the younger puppies or adult dogs was not possible. Microsphere studies have shown a relatively high inner cortical flow in puppies less than 3 wk of age (5, 18, 21), and the puppy up to 6 wk old has a relatively high outer medullary flow as measured by the xenon-washout method (14). Since the present report demonstrates the ability of the puppy to autoregulate at as early an age as 9 days of life, when inner cortical and outer medullary blood flow may be as high as 50% of total renal blood flow, it appears likely that the medullary circulation participates in this autoregulation.

The mechanism for autoregulation of renal blood flow has been ascribed to myogenic as well as hormonal factors. Of the various hormonal mediators of autoregulation, a role for the renin-angiotensin system has been both advocated and denied (2, 3, 6, 22, 24). If the renin-angiotensin system is involved in the autoregulation of renal blood flow, a decrease in the formation or the activity of angiotensin II should result in impairment of autoregulation. When renin secretion was decreased by saline loading or by the intrarenal infusion of the beta-adrenergic blocker, propranolol, autoregulation persisted (1, 6). Similar results were obtained when the intrarenal conversion of angiotensin I to angiotensin II was blocked (7, 10). However, Kaloyanides et al. (16) showed that the ability of the renin-depleted, isolated, perfused kidney to autoregulate was impaired. In the present report, the role of angiotensin II was studied by the intrarenal infusion of the angiotensin II competitive antagonist, [1-sarcosine,ß-alanine]angiotensin II. When the vascular effects of angiotensin II in the kidney were demonstrably blocked renal blood flow autoregulation remained intact. This applied in all six puppies in which blood pressure was raised and in the two cases in which it was tested during aortic constriction. Similar results have been obtained recently by Anderson et al. (1) in the adult dog and by Kaloyanides and DiBona (17) in the isolated perfused kidney. While these current studies appear to indicate that angiotensin II is not essential in the autoregulatory phenomenon, as mentioned above, appropriate concentrations of the antagonist may not have been uniformly achieved at each receptor site to inhibit completely the vascular effects of locally generated angiotensin II. The dose of anti-AII used, however, could effectively block renin secreted into both renal vein and lymphatics (23).

Recently, Burke et al. (4) have raised the possibility that, “some function of distal calcium delivery could serve as the major mediator (of a distal tubular feedback phenomenon) and that additional factors are not required.” Available micropuncture studies in the puppy have not been addressed to this thesis so it is not possible to draw any further conclusions concerning this mechanism in the immature animal. It should be noted, however, that the fractional tubular reabsorption at the end-proximal tubule is the same in the puppy as in the mature dog (11), making it less likely that distal tubular load in the puppy is greatly dissimilar to that of the adult dog. At this time, the exact mechanism(s) of the autoregulatory process in the immature kidney, as in the adult kidney, remains to be defined.

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