Action of Angiotensin II Upon Renal Vascular Resistance

By WILLIAM E. HAGEMAN* and JOSEPH P. BUCKLEY

The effects of angiotensin II, vasopressin, *l*-norepinephrine, and elevated perfusion pressure were investigated on the peripheral resistance of the vascular beds in isolated rabbit kidneys in which the rate of flow was controlled by a Sigmamotor pump. Angiotensin II produced marked rhythmic fluctuations in perfusion pressure, having a mean amplitude of 52 mm. H_2O (n = 124); and when flow was decreased so that perfusion pressure was equal to control levels, the mean amplitude of rhythmic fluctuations was still 40 mm. H_2O. This effect of angiotensin II appeared to be affected by both age and sex with peak activity occurring at 2 to 4 months of age and the kidneys from female rabbits being significantly more responsive than those of male rabbits. Vasopressin also significantly increased the amplitude of the rhythmic fluctuations in perfusion pressure but to a much lesser degree than angiotensin II. The effect of *l*-norepinephrine was not significantly different from that induced by simple elevation of perfusion pressure. These data suggest that angiotensin II may exert a direct effect upon renal vascular smooth muscle of the "single-unit" type by increasing the number of spontaneous, local depolarizations through an increased influx of Ca^{2+} into the muscle cells. Such an action might also involve angiotensin II in the autoregulation of renal blood flow.

THE INVOLVEMENT of the kidney as a possible cause of, or contributor to, arterial hypertension was suspected in the latter part of the 19th century. Tigerstedt and Bergman (1) demonstrated that crude saline kidney extracts produced a pressor response when injected into anesthetized rabbits. The active principle(s) of the extract was postulated to have a direct vasoconstrictor action; and although it was impure and unidentified, they called it renin. This work lay dormant for over a quarter of a century before interest was renewed when Goldblatt *et al.* (2) demonstrated a reproducible hypertension of renal origin in dogs by the production of renal ischemia, and Volhard (3) introduced his theory of "pale hypertension" and its relationship to nephrosclerosis.

Since Goldblatt's discovery, the vasoactive principles, renin and angiotensin II, have been isolated, identified, and extensively investigated. These findings have stimulated profuse pharmacological research in the area of renal hypertension. However, the true physiological mechanism of the hypertensive state and the relationship of the kidney, angiotensin, and renin in arterial hypertension are not yet clearly defined. Considerable controversy and conflicting experimental evidence exist, and angiotensin is a focal point of these controversies.

Studies involving the role of the kidney in cardiovascular changes or disorders must take into consideration all of the factors involved in the complex relationships of the renal and cardiovascular systems. One such factor is the kidney's remarkable capacity to rapidly readjust in the wake of changing systemic arterial pressure so that renal blood flow remains fairly constant as the arterial pressure is altered. Waugh (4) reported that, within a normal range of arterial blood pressure, a 50% elevation of arterial pressure resulted in only a 5% increase in renal blood flow. Investigators have been cognizant of this phenomenon for many years, and the mechanisms of such "autoregulation" have been the subject of extensive research. However, there is still much discussion over the role of autoregulation and the factors involved in its mechanisms.

Regardless of investigators' disagreement on the mechanisms involved in the initiation of this phenomenon, it is almost universally accepted that vascular components play a major role in autoregulatory processes. In light of this fact, it might well be pertinent to ask what happens in a pathological state such as that produced by hypertension. The classic experiments of Goldblatt *et al.* (2) indicated that pathological changes of the renal arterial vasculature in dogs can produce a sustained arterial hypertension which is hemodynamically similar to essential hypertension in man. Byrom (5) reported uniform constriction interrupted by sharply localized dilatations in intestinal and cerebral arteries of renal hypertensive rats. Giese (6) infused synthetic angiotensin into nephrectomized rats to produce acute hypertensive vascular disease. He was able to not only demonstrate a very characteristic pattern of "alternating constrictions and dilatations" along the course of intestinal arteries, but,

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TABLE I—EFFECT OF PRESSURE AND ANGIOTENSIN INFUSION UPON THE PERFUSION PRESSURE OF ISOLATED PERFUSED[a] KIDNEYS OF RABBITS

Conditions	N	Mean Perfusion Pressure, mm. Hg ± S.E.[b]	Rhythmic Activity Amplitude, mm. H_2O ± S.E.[b]	Rhythmic Activity Rate/min. ± S.E.[b]
Pre-Angiotensin II Infusion				
Perfusion pressure	151	75.31 ± 1.76	9.74 ± 0.89	2.79 ± 0.16
Elevated perfusion pressure[c]	144	108.80 ± 2.05	19.60 ± 1.46	3.68 ± 1.25
Angiotensin II Infusion				
Perfusion pressure	124	109.43 ± 2.32	52.42 ± 3.60	3.27 ± 0.11
Decreased perfusion pressure[d]	44	64.23 ± 6.38	40.14 ± 3.99	2.58 ± 0.22
Post-Angiotensin II Infusion				
Perfusion pressure	123	76.87 ± 2.40	14.41 ± 1.18	2.89 ± 0.20
Elevated perfusion pressure[c]	89	111.24 ± 2.94	29.72 ± 3.07	3.49 ± 0.15

[a] Perfused with Tyrode's solution. [b] S.E. = $\sqrt{\frac{\Sigma(x - \bar{x})^2}{n(n-1)}}$. [c] Perfusion pressure increased by adjusting Sigmamotor pump to approximate pressure induced by angiotensin II. [d] Perfusion pressure decreased by adjusting Sigmamotor pump.

using vascular labeling with colloidal carbon particles, he was able to show increased vascular permeability which was restricted to the dilated segments of the vessels.

Several investigators have reported similar changes in vascular elements subsequent to the infusion of angiotensin. Benelli *et al.* (7) reported a spontaneous rhythmic motility of the parenchymal as well as the vascular smooth muscle apparatus of the isolated cat spleen during perfusion with an angiotensin solution. Buckley (8) reported an induced rhythmicity in isolated rabbit kidneys upon perfusion with angiotensin, which seemed to be diminished with the increasing age of the animal.

Spontaneous rhythmic changes in renal vascular resistance have also been noted. Waugh (9) perfused isolated kidneys of dogs with PVP-Locke solution and observed "an unusually high degree of brief rhythmic changes in renal flow and prevenous resistance," concomitant with autoregulation of flow and subsequent to elevation of perfusion pressure, which were not affected by procaine anesthetization of intrarenal neural elements, but which were abolished by higher doses of procaine which were sufficient to depress the reactivity of the renal vascular elements to barium chloride or epinephrine injections. In view of these aforementioned results, the intent of the present investigation was to investigate further the nature of the rhythmic alterations in the renal vasculature and to determine its relation to angiotensin and other factors.

METHODS

Effect of Angiotensin II Upon Isolated Perfused Rabbit Kidneys and the Production of Rhythmic Alterations in Perfusion Pressure—New Zealand albino rabbits were selected at random and anesthetized with 30 mg./Kg. of sodium pentobarbital *via* a marginal ear vein. Each kidney was carefully isolated, the renal artery and vein were distally ligated, and the renal artery cannulated. A polyethylene cannula, made by building polyethylene tubing up to a $^3/_{16}$-in. bore starting with size PE 60 tubing, was used. Each layer of tubing was treated with epoxy cement to assure a tight, leak-proof seal. The ureter and renal vein were severed and the kidney removed from the animal and quickly suspended in a perfusion system which maintained the temperature of the internal and external environment of the kidney at 37°. The cannula was connected to one orifice of a small T tube which was also connected to a transducer, recording the perfusion pressure onto a Grass polygraph calibrated at two different sensitivities to allow the perfusion pressure to be monitored in both mm. Hg and mm. H_2O.

The kidney was perfused with Tyrode's solution mol. wt. 73,000, containing 1% dextran[1] (Pharmachem Corp., Bethlehem, Pa.) in such a manner the perfusion pressure ranged approximately between 75–100 mm. Hg and the flow between 12 and 20 ml./min. A Sigmamotor pump was used to maintain a constant flow from two 32-oz. reservoir bottles, one containing Tyrode's solution and the other containing Tyrode's solution plus angiotensin II, 0.01 mcg./ml. The reservoirs were kept in a constant-temperature bath at 37° and aerated with 95% oxygen and 5% carbon dioxide *via* fritted-glass gas dispersion tubes in order to assure adequate and balanced aeration of the solutions. The right kidney was prepared first, followed immediately by the left; and both were suspended in similar perfusion systems. Approximately 15–30 min. were allowed for the venous flow to stabilize to between 12–20 ml./min., after which time each kidney was treated according to the following procedure.

(*a*) The perfusion pressure was increased by adjusting the flow of the Sigmamotor pump to a level of pressure similar to that expected to be achieved by the infusion of angiotensin II, as

[1] Stock solution *1:* NaCl, 200 Gm.; KCl, 5 Gm.; $CaCl_2$ (anhydrous), 5 Gm.; $MgCl_2 \cdot H_2O$, 5 Gm.; glass-distilled water *q.s. ad.* 1000 ml. Stock solution *2:* $NaHCO_3$, 50 Gm.; $NaH_2PO_4 \cdot H_2O$, 2.5 Gm.; glass-distilled water *q.s. ad.* 1000 ml. Stock solution *3:* Dextran (mol. wt. 73,000), 100 Gm.; dextrose (anhydrous), 25 Gm.; glass-distilled water *q.s. ad.* 1000 ml. Mix 80 ml. of stock solution *1*, 40 ml. of stock solution *2*, add 200 ml. of stock solution *3*, and make up to a total volume of 2 L. with glass-distilled water.

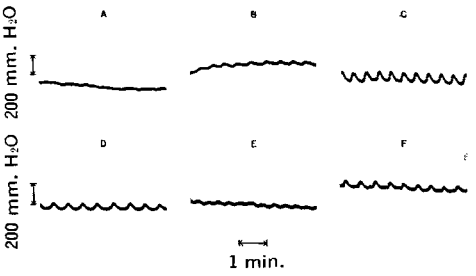

Fig. 1—Typical responses due to angiotensin II infusion and perfusion pressure changes in isolated perfused rabbit kidney. Key: A, *pre-angiotensin II infusion at normal perfusion pressure;* B, *pre-angiotensin II infusion at elevated perfusion pressure;* C, *angiotensin II infusion;* D, *angiotensin II infusion at decreased perfusion pressure level;* E, *post-angiotensin II infusion at normal perfusion pressure;* F, *post-angiotensin II infusion at elevated perfusion pressure.*

determined from preliminary experiments. (*b*) The perfusion pressure was returned to original levels and allowed to stabilize for 5–10 min. (*c*) Angiotensin II was infused until the maximum effect was obtained. If rhythmic fluctuations in perfusion pressure occurred, the perfusion pressure was readjusted to pre-angiotensin II infusion pressure levels by reducing flow *via* the Sigmamotor pump. (*d*) Flow was returned to control levels *via* the Sigmamotor pump, and angiotensin II infusion stopped. (*e*) Steps (*a*), (*b*), and (*c*) were repeated several times until maximal rhythmic alterations were observed. (*f*) Perfusion pressure levels were monitored in mm. Hg, and amplitude changes of the rhythmic alterations were measured in mm. of water. Records were made at a chart speed of 0.25 mm./sec.

Effect of Other Vasopressor Agents on the Rhythmic Fluctuations in the Perfusion Pressure of Isolated Perfused Rabbit Kidneys—The procedure described above was followed utilizing either *l*-norepinephrine,[2] 0.25 mcg./ml., or vasopressin,[3] 0.01 units/ml., in place of angiotensin II in one of the perfusion reservoirs.

Effect of Age and Sex upon the Rhythmic Alterations in Perfusion Pressure Induced by Angiotensin II Infusion into Isolated Perfused Rabbit Kidneys—New Zealand albino rabbits of both sexes were obtained at certain selected ages. Females were used at ages of 1, 2, 4, 8, and 12 months; and males were used up to the age of 8 months since 12-month-old male rabbits were not available during the course of the experiment. The previously described procedure for isolation, removal, perfusion, and treatment was followed.

Analysis of Data—All data were punched onto IBM cards and analyzed by either the BMD 02V, BMD 05V, or BMD 07V analysis of variance programs on the IBM 7090 computer.

RESULTS

Effect of Angiotensin II Infusion Upon Rhythmic Fluctuations in Perfusion Pressure of Isolated Perfused Rabbit Kidneys—The data obtained in these experiments are summarized in Table I. Angiotensin II,[4] when infused at a concentration of 0.01 mcg./ml. and at a rate of 12–20 ml./min., produced rhythmic alterations in perfusion pressure up to 250 mm. H_2O (Fig. 1) with a mean amplitude of 52.4 ± 3.6 mm. H_2O. Even when the perfusion pressure was decreased to pre-angiotensin II levels by decreasing the rate of perfusion, there was only a 15–20% decrease in the amplitude of rhythmic fluctuations to 40.1 ± 4 mm. H_2O. Although simply increasing the perfusion pressure did produce rhythmic pressure alterations, the response to angiotensin II was over 2.5 times that achieved by pressure alone. These data indicated that pressure *per se* cannot fully account for the rhythmic changes in perfusion pressure produced by the infusion of minute doses of angiotensin II, and that this polypeptide plays a direct role in inducing these rhythmic alterations in perfusion pressure. Although the rhythmicity produced by angiotensin II is significantly greater ($p = <0.01$) than that present either prior to or following the infusion of angiotensin II, the post-angiotensin II responsiveness is also significantly greater both at normal and increased perfusion pressure ($p = <0.01$). Angiotensin II did not significantly alter the rate of the rhythmic changes.

Effect of Other Vasopressor Agents on the Rhythmic Fluctuations in the Perfusion Pressure of Isolated Perfused Rabbit Kidneys—The data obtained in these experiments are summarized in Tables II and III. *l*-Norepinephrine at a concentration of 0.25 mcg./ml. and at a rate of 12–20 ml./min. produced rhythmic activity approximately equivalent to that induced by the elevation of perfusion pressure alone. When compared with the effects of angiotensin II infusion, *l*-norepinephrine produced approximately 1/4 the increase in amplitude of rhythmic activity induced by angiotensin II ($p = <0.01$), the mean amplitude of the rhythmic alterations in perfusion pressure induced by *l*-norepinephrine being 12.1 ± 2.9 mm. H_2O compared to a mean amplitude of 52.4 ± 3.6 mm. H_2O produced by angiotensin II infusion.

Vasopressin, 0.01 units/ml., 12–20 ml./min., significantly greater rhythmic fluctuations in perfusion pressure than that produced by simple elevation of perfusion pressure ($p = < 0.05$). However, the 24.6 ± 1.5 mm. H_2O amplitude of rhythmic alterations in perfusion pressure produced by vasopressin was less than 50% of that produced by angiotensin II infusion. Vasopressin did not seem to produce a sensitization as did angiotensin II since there was no significant difference between the pre- and post-infusion period responses at either normal or elevated perfusion pressure. The effect of vasopressin upon the amplitude of the perfusion pressure alterations was 133% of the effect produced by simple elevation of perfusion pressure, but the response to vasopressin infusion was over 200% greater than the response to *l*-norepinephrine infusion ($p = <0.05$).

Effect of Sex Upon Rhythmic Alterations in Perfusion Pressure of Isolated Perfused Rabbit Kidneys—In order to analyze these data for the over-all effect of sex, the kidneys from female rabbits were grouped together, ignoring age, and compared with the kidneys from male rabbits at

[2] Levophed Bitartrate, Winthrop Laboratories, New York, N. Y.
[3] Pitressin, Parke, Davis & Co., Detroit, Mich.
[4] Hypertensin, Ciba Pharmaceutical Co., Summit, N. J.

TABLE II—EFFECT OF PRESSURE AND VASOPRESSIN INFUSION UPON THE PERFUSION PRESSURE OF ISOLATED PERFUSED[a] KIDNEYS OF RABBITS

Conditions	N	Mean Perfusion Pressure, mm. Hg ± S.E.[b]	Rhythmic Activity Amplitude, mm. H_2O ± S.E.[b]	Rhythmic Activity Rate/ min. ± S.E.[b]
Pre-Vasopressin Infusion				
Perfusion pressure	151	75.31 ± 1.76	9.74 ± 0.98	2.79 ± 0.16
Elevated perfusion pressure[c]	144	108.80 ± 2.05	19.60 ± 1.46	3.68 ± 1.25
Vasopressin Infusion				
Perfusion pressure	15	102.47 ± 8.50	24.60 ± 5.18	3.00 ± 0.22
Decreased perfusion pressure[d]	11	69.36 ± 6.50	15.45 ± 4.01	2.18 ± 0.42
Post-Vasopressin Infusion				
Perfusion pressure	15	65.00 ± 6.69	11.67 ± 2.43	2.83 ± 0.33
Elevated perfusion pressure[c]	12	100.83 ± 7.17	21.00 ± 4.27	3.15 ± 0.49

[a] Perfused with Tyrode's solution. [b] S.E. = $\sqrt{\frac{\Sigma(x - \bar{x})^2}{n(n-1)}}$. [c] Perfusion pressure elevated by adjusting Sigmamotor pump to approximate the pressure induced by vasopressin. [d] Perfusion pressure decreased by adjusting the Sigmamotor pump.

TABLE III—EFFECT OF PRESSURE AND *l*-NOREPINEPHRINE INFUSION UPON THE PERFUSION PRESSURE OF ISOLATED PERFUSED[a] KIDNEYS OF RABBITS

Conditions	N	Mean Perfusion Pressure, mm. Hg ± S.E.[b]	Rhythmic Activity Amplitude, mm. H_2O ± S.E.[b]	Rhythmic Activity Rate/ min. ± S.E.[b]
Pre-*l*-Norepinephrine Infusion				
Perfusion pressure	151	75.31 ± 1.76	9.74 ± 0.98	2.79 ± 0.16
Elevated perfusion pressure[c]	144	108.80 ± 2.05	19.60 ± 1.46	3.68 ± 1.25
***l*-Norepinephrine Infusion**				
Perfusion pressure	14	108.57 ± 7.23	12.14 ± 2.90	3.90 ± 0.73
Decreased perfusion pressure[d]	5	81.00 ± 5.79	7.80 ± 3.38	5.40 ± 1.16
Post-*l*-Norepinephrine Infusion				
Perfusion pressure	14	77.50 ± 4.82	4.68 ± 2.15	2.86 ± 0.75
Elevated perfusion pressure[c]	8	112.50 ± 6.94	9.00 ± 3.42	3.75 ± 1.03

[a] Perfused with Tyrode's solution. [b] S.E. = $\sqrt{\frac{\Sigma(x - \bar{x})^2}{n(n-1)}}$. [c] Perfusion pressure elevated by adjusting Sigmamotor pump to approximate pressure induced by *l*-norepinephrine. [d] Perfusion pressure decreased by adjusting Sigmamotor pump.

each of the five parameters studied: pre-angiotensin II infusion at both normal and elevated perfusion pressures, during angiotensin II infusion, and post-angiotensin II infusion at normal and elevated perfusion pressures.

The kidneys from female rabbits were statistically more responsive to angiotensin II than those kidneys obtained from male rabbits ($p = <0.05$), the mean amplitude of rhythmic fluctuations of perfusion pressure of kidneys from females being 56.9 ± 5.4 mm. H_2O compared to a mean amplitude of 42.2 ± 4.1 mm. H_2O in the males' kidneys.

Effect of Age Upon Rhythmic Alterations in Perfusion Pressure of Isolated Perfused Rabbit Kidneys—The contribution of age to the induction of rhythmic changes in perfusion pressure is graphically presented in Figs. 2 and 3. The amplitude of the rhythmic alterations in perfusion pressure was compared at different age levels in kidneys from male and female rabbits separately. The amplitude of the rhythmic changes in perfusion pressure in kidneys from female rabbits was significantly affected by age at all five parameters studied ($p = <0.05$). In kidneys from male rabbits, there was only a significant effect of age during perfusion at normal perfusion pressures prior to and after angiotensin II infusion ($p = <0.05$). The rhythmic fluctuations were greatest between the ages of 1 to 4 months in all five parameters studied in both male and female rabbits' kidneys. In both sexes at normal perfusion pressure prior to angiotensin II infusion, the most sensitive age was 1 month. Under all other parameters the peak response was at 4 months in both sexes except during angiotensin II infusion, where the peak sensitivity was at 2 months of age in kidneys from female rabbits where the mean amplitude of rhythmic changes was 77.5 ± 8.2 mm. H_2O. The peak response produced by angiotensin II in the kidneys of male rabbits was at 4 months of age where the mean amplitude was 53.3 ± 11.2 mm. H_2O.

There was a marked decrease in the amplitude of rhythmic alterations in perfusion pressure in kidneys of 8-month-old rabbits of both sexes, the mean amplitude in the kidneys of females being 45.0 ± 11.3 mm. H_2O and that of kidneys from males being 29.5 ± 6.11 mm. H_2O. Angiotensin II produced rhythmic alterations in perfusion pressure having a mean of 36.3 ± 12.8 mm. H_2O in the kidneys of 12-month-old female rabbits.

DISCUSSION

The relatively slight changes in renal blood flow from normal levels under various physiological conditions have become a well accepted fact. Sympathetic vasoconstrictor control of the renal vessel under normal conditions has been ruled out

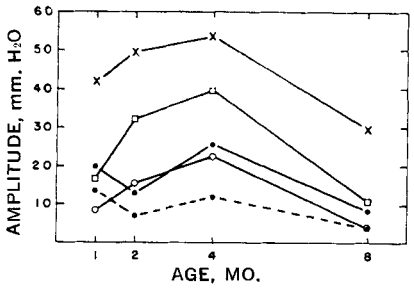

Fig. 2—The effect of age on rhythmic fluctuations in perfusion pressure of isolated kidneys from male rabbits. Key: ×, angiotensin II infusion; □, post-angiotensin II ↑ pressure; ○, post-angiotensin II normal pressure; ●——●, pre-angiotensin II ↑ pressure; ●- - - - -●, pre-angiotensin II normal pressure.

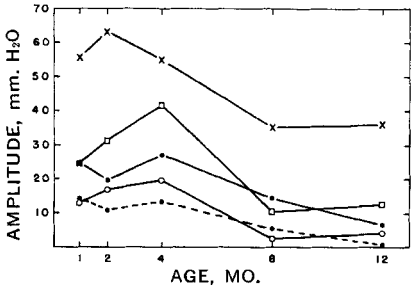

Fig. 3—The effect of age on rhythmic fluctuations in perfusion pressure of isolated kidneys from female rabbits. Key: ×, angiotensin II infusion; □, post-angiotensin II ↑ pressure; ○, post-angiotensin II normal pressure; ●——●, pre-angiotensin II ↑ pressure; ●- - - - -●, pre-angiotensin II normal pressure.

because surgical and pharmacological denervation do not elevate the renal blood flow. Such changes in renal resistance to blood flow as a consequence of changes in blood pressure have inherited the term autoregulation of renal blood flow, because no extrarenal, nervous, or humoral factors are believed to be involved. There is experimental evidence that an elevated tension in the arteriolar wall stimulates the vascular smooth muscle to contract. Little doubt exists that during normal kidney function, resistance changes are caused by smooth muscle reactions. There does exist, however, a basic question of whether smooth muscle contraction during autoregulation is initiated only by the tension change in the arteriolar wall or whether it is a muscle response to either a change in renal metabolism or an intrinsic humoral factor, both of which might be related to arterial perfusion pressure.

There has been a considerable amount of evidence supporting the proponents of the myogenic theory for autoregulation by vascular smooth muscle. Many conclusions have been drawn based upon the electrophysiological studies performed by Bozler (10) and Bulbring (11) on other types of smooth muscles. Funaki (12) confirmed this work on vascular smooth muscle. Evidence from these experiments indicated that there are two types of smooth muscle cells, some with a higher, more stable membrane potential (multi-unit) and some with lower, less stable membrane potentials (single-unit). The multi-unit type smooth muscle is dominant in the larger vessels, and the single-unit type dominates in the smaller vessels. A slight continuous distension of the wall of a blood vessel due to elevated blood pressure might then affect the smooth muscle cell by increasing the rate of the spontaneously generated local depolarizations in proportion to the extent of distension which, in turn, initiates depolarization followed by excitation–contraction.

There is also experimental evidence in dogs suggesting that renin release occurs subsequent to pressure changes within the kidney (13). Anatomically, the individual nephron is ideally arranged so that changes in the dynamic or chemical pattern of the intratubular fluid can be sensed by the vasa afferens at the initial portion of the distal convoluted tubule, the area in which the juxtaglomerular apparatus is located. It has been suggested that the juxtaglomerular apparatus is involved in a mechanism to prevent a further increase in renal blood flow, glomerular filtration rate (GFR), and intrarenal pressure when the renal arterial blood pressure increases above 90 mm. Hg (14). Such a mechanism would narrow autoregulation down to a problem of adjusting the physical factors, which are responsible for the tubular load, to the tubular reabsorption capacity, which has to handle the filtered load. Both physical and metabolic factors are involved in the formation of urine; and since the physical factors occur at the initial phase of urine formation, the most likely candidate for achieving a glomerular–tubular balance might be maintenance of a stable physical component (effective filtration pressure). Also it is possible to regulate the effective filtration pressure by a single mechanism, *i.e.*, tonus of afferent glomerular vessels, whereas regulation of tubular activity would be far more difficult and complex. The possibility then exists that the renin–angiotensin system, which seems to have its origin at the juxtaglomerular apparatus, being sensitive to both changes in electrolytes (15) and effective in altering the vascular resistance in the kidney, may be involved in renal autoregulation of blood flow as a result of the maintenance of glomerular tubular balance. A sudden elevation of renal arterial blood pressure initially increases renal blood flow and thereby increases GFR which causes increased tubular load and urine flow velocity. Such changes could change the dynamics of flow of the tubular fluid and increase the osmolarity at the macula densa within seconds. The increased osmolarity could then stimulate the juxtaglomerular apparatus to release a vasoactive substance which would thereby decrease the GFR *via* afferent vasoconstriction.

The results of the present study indicate that angiotensin II may have an effect upon the vasculature of the kidney other than that of direct vasoconstriction. This effect expressed itself as a rhythmic fluctuation in the perfusion pressure of the isolated perfused rabbit kidney. Pressure elevation was also capable of inducing a similar type of fluctuation, as would be expected according to the myogenic theory; but angiotensin II was significantly more effective in inducing such alterations. Such a reaction could possibly be interpreted on an electrophysiological basis using the results of

Funaki's experiments as guide lines. It is possible that angiotensin II exerts its effect upon vascular smooth muscle, at least in part by decreasing the resting membrane potential by increasing the influx of Ca^{2+} ions. This theory is supported by recent evidence that isolated renal arteries constrict in response to angiotensin II only in the presence of the calcium ion (16). Tobian and Chesley (17) have also demonstrated that small arteries and arterioles from renal hypertensive rats have a significantly greater Ca^{2+} content than those of normotensive control animals. The decreased membrane potential increases the number of spontaneous local depolarizations, thereby reaching more muscle fibers and exaggerating the vasomotion of the small vessels. The frequency of firing, however, did not seem to be influenced in that there was no significant difference in the rate of occurrence of the rhythmic fluctuations between control conditions and during angiotensin II infusion. In other words, the effect of elevated pressure initiates rhythmic fluctuations probably by the myogenic mechanism described by Funaki (12); however, the influence of a hormone closely related to the kidney may be capable of greatly accentuating the effect of pressure upon the activity of vascular smooth muscle and might possibly be the direct-acting intrinsic hormonal factor concerned with renal autoregulation of blood flow.

The effect of angiotensin II in producing the rhythmic changes in perfusion pressure cannot be fully explained by the myogenic theory as described by Bayliss (18), since the rhythmic fluctuations were only slightly decreased when the perfusion pressure was reduced to control levels during angiotensin II infusion. Also, the effect of angiotensin II is significantly greater than the effects produced by elevation of perfusion pressure or the effect produced by the two other vasoconstrictor agents, *l*-norepinephrine and vasopressin. The effect of *l*-norepinephrine was only slightly greater than that of pressure elevation alone, whereas vasopressin, another polypeptide, did have some effect other than the influence of pressure; but it was not nearly so pronounced as that of angiotensin II.

This study also indicated that these effects of angiotensin II upon the vasculature of the kidney are both age and sex dependent. The pattern which is seen when the age of the animal is varied is interesting in that there seemed to be an increasing reactivity up to the age of 4 months, and then a drastic decline in responsiveness of the rabbit kidney occurred. It might be speculated that when the animal is very young, the kidney's vasculature is not completely developed; and the majority of the vessels are still relatively small. It is also possible that during this time these small vessels are dominated by the single-unit type of muscle cell described by Bozler and therefore possess an inherently lower resting membrane potential, thereby being less stable and more prone to depolarization. As the animal matures, the vessels become larger; and the dominance shifts to that of the multi-unit muscle cell which possesses a more stable membrane potential and lacks automaticity. The occurrence of such events seems logical in the light of Funaki's report that the vasculature of the larger vessels is dominated by multi-unit smooth muscle cells and the smaller vessels by single-unit smooth muscle cells, even though both types of cells can be found in both large and small vessels. This is by no means a suggestion that an older animal has lost the ability of autoregulation. The mature animal still possesses an adequate supply of small preglomerular arterioles which are dominated by the single-unit type of muscle cells, but the major vessels are almost completely composed of the multi-unit smooth muscle cells. It would perhaps be more correct to say that the young animal is better able to regulate renal blood flow in the face of changing cardiovascular patterns, so that during its young formative period the rabbit is better able to assure the constancy of its internal environment.

There is also the question of the effects of sex upon the responsiveness of the renal vasculature, since the female rabbit's kidneys were more responsive at almost every age tested. The explanation for this phenomenon probably lies in the difference in the hormonal nature of internal environment of the two sexes; and perhaps the female endocrine system, particularly estrogens, potentiates the responsiveness of the vascular smooth muscle.

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