Agmatine, a Bioactive Metabolite of Arginine
Production, Degradation, and Functional Effects in the Kidney of the Rat

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Abstract

Until recently, conversion of arginine to agmatine by arginine decarboxylase (ADC) was considered important only in plants and bacteria. In the following, we demonstrate ADC activity in the membrane-enriched fraction of brain, liver, and kidney cortex and medulla by radiochemical assay. Diamine oxidase, an enzyme shown here to metabolize agmatine, was localized by immunohistochemistry in kidney glomeruli and other nonrenal cells. Production of labeled agmatine, citrulline, and ornithine from [3H]arginine was demonstrated and endogenous agmatine levels (10^{-6} M) in plasma ultrafiltrate and kidney were measured by HPLC. Microperfusion of agmatine into renal interstitium and into the urinary space of surface glomeruli of Wistar-Frömter rats produced reversible increases in nephron filtration rate (SNGFR) and absolute proximal reabsorption (APR). Renal denervation did not alter SNGFR effects but prevented APR changes. Yohimbine (an α₂ antagonist) microperfusion into the urinary space produced opposite effects to that of agmatine. Microperfusion of urinary space with BU-224 (μM), a synthetic imidazoline₂ (I₂) agonist, duplicated agmatine effects on SNGFR but not APR whereas an I₁ agonist had no effect. Agmatine effects on SNGFR and APR are not only dissociable but appear to be mediated by different mechanisms. The production and degradation of this biologically active substance derived from arginine constitutes a novel endogenous regulatory system in the kidney. (J. Clin. Invest. 1996. 97:413–420.) Key words: agmatine • arginine decarboxylase • diamine oxidase • kidney • glomerular filtration • tubular reabsorption

Introduction

Arginine is an amino acid critical to normal growth and multiple physiologic processes. Arginine concentration in extracellular fluid is maintained at 100–200 μM and is regulated by (a) GI absorption, (b) conversion to ornithine by the urea cycle, and (c) synthesis from citrulline in the kidney (1, 2). The metabolic fate of this amino acid in cells is less well defined, but products derived from arginine are known to be important bioactive compounds. An enormous interest has focused upon the physiologic and pathophysiologic roles of nitric oxide (NO) generated from arginine by NO synthase (1–3). It is now evident that other bioactive compounds are generated from arginine (4, 5). The enzyme arginine decarboxylase (ADC) was previously considered important primarily in bacteria and plants (6–8). However, recent publications have suggested that ADC is present in mammalian systems (9–12).

Earlier work with plants has shown that agmatine is avidly degraded by the enzyme, diamine oxidase (DAO) which converts agmatine to guanidino-butylaldehyde (Fig. 1) (13). Novotny et al. isolated DAO and found that the amino acid sequence was identical to that of “amiloride binding protein” (14). Amiloride and amiloride analogues significantly inhibit DAO by binding at the active site of the enzyme. It is of interest that aminoguanidine, a structurally simplified version of agmatine known to inhibit inducible NOS (15–16), is also a potent inhibitor of DAO (17).

Investigators have pursued identification of the endogenous clonidine displacing substance (CDS) from brain and lung extract. Clonidine and other centrally acting hypotensive agents such as guanabenz are known to exert physiologic effects via α₂-adrenergic and/or imidazole type receptors (18). Imidazoline receptors have been defined (I₁ and I₂) in the central nervous system and other tissues such as the basolateral aspect of proximal tubules (19). Li et al. demonstrated that a significant concentration of agmatine (0.2–0.4 μg/gram) is present in brain and that this compound was a ligand for both imidazoline and α₂ adrenergic receptors (1).

Others have shown that agmatine infusion into the cerebral ventricles increases blood pressure while systemic infusion decreases blood pressure (20). Agmatine is purported to stimulate release of norepinephrine in the central nervous system and in chromaffin cells, a system which does not express α₂ adrenergic receptors. Other investigators have not observed agmatine induced norepinephrine release in the chromaffin cell system but have suggested that agmatine is a nicotinic antagonist in chromaffin cells (unpublished observations and reference 21).

Agmatine is a biologically active substance, but the mode and sites of action have not been fully defined. The current studies provide evidence that this alternate arginine metabolic pathway is of physiological importance to renal function. The

1. Abbreviations used in this paper: ADC, arginine decarboxylase; APR, absolute proximal reabsorption; DAO, diamine oxidase; I₁, imidazoline; SNGFR, single nephron glomerular filtration rate.
aims are as follows: (a) Demonstrate ADC and DAO in various tissues. (b) Demonstrate agmatine production and measure endogenous levels in renal tissue. (c) Determine physiological effects of the ADC/agmatine system on glomerular and proximal tubule function. (d) Characterize receptor types involved in the renal effects of agmatine using selective adrenergic and/or imidazoline agonists and antagonists.

Methods

ADC activity and location in mammalian tissue. Three female Wistar rats (300 grams) were anesthetized and exsanguinated before collecting the brain, liver, heart, spleen, kidneys, and lungs. The kidneys were decapsulated and dissected in order to separate the cortex and medulla. The organs of each animal were individually weighed (after weighing the whole liver a portion was sectioned and weighed for as

enuous tissues. (c) to isolate a mitochondria en-

Vic reaction, 25 ml of standard (100 

Rapid and reproducible derivatization for HPLC obtained by carefully mixing 5 
l of the mixture was injected manually.

Elution was performed using a Waters reverse phase column maintained at 30°C (Novapack, C8, 3.9 x 300) at a flow rate of 1 ml/min. The ratio of solvents A:B:C:D (A = potassium dihydrogen phosphate buffer, pH 5.96; B = acetonitrile; C = water; D = methanol) used was as follows: 80:8:6:6 isocratic for 5 min followed by a gradient change to 74:10:8:8 over 5 min. At 20 min a gradient change to 0:40: 30:30 occurs over 15 min. After 15 min of isocratic elution, initial conditions were restored in a gradient over 10 min. Elution fractions were collected at 0.5-min intervals and prepared for scintillation counting. Absorbance of derivatized compounds was measured at 340 nm, enabling identification of eluate fractions.

Endogenous agmatine levels. We measured endogenous levels of agmatine in plasma and renal cortex tissue using HPLC separation of extracts from control and agmatine treated rats. Lower aorta and vena cava of anesthetized rats (250-gram sibling Frömter-Wistar) cultured cells, and post heparin plasma, and is able to neutralize > 99% of the diamine oxidase activity in placental extract at dilutions of 1/100.

Nonmalignant human tissues from autopsy specimens, and adult mouse tissues were embedded in methyl methacrylate, snap frozen, and stored at —70°C. 10-μm frozen sections were cut, mounted, air dried, and stained immediately. Slides were first blocked with 0.1% goat serum in phosphate buffered saline (PBS), and then incubated with either pre-immune rabbit IgG or anti-diamine oxidase IgG. The secondary antibody used was an immunoperoxidase linked anti-rabbit IgG, and color was developed using amino ethylicarbazole and 0.03% H2O2 (22). The slides were then counterstained with hematoxylin, mounted, and examined.

Agmatine metabolism by DAO — effects of aminoguanidine. Pig kidney DAO was purified as described previously (13). H2O2 generated from agmatine by DAO was measured by fluorometric assay using horseradish peroxidase (HPO Type 2; Sigma Chemical Co., St. Louis, MO) and fluorogene-3-(p-hydroxyphenyl) propionic acid (HPPA; Aldrich Chemical Co. Milwaukee, WI).

Reaction mixture consisted of 650 μl buffer (0.02 M Tris, 0.07 M NaCl, pH 8.9), 100 μl HPPA (7.5 mM), 50 μl HPO (2 U/ml), 100 μl purified DAO (diluted 1:10 in 0.05 M Tris, 0.022 Azide, 0.2 mg/ml BSA) and 100 μl of buffer containing arginine (0–100 μM final concentration). A standard curve for H2O2 (0–50 μM final concentration) was established concurrently. After 60 min at 37°C, reaction was terminated with the addition of 100 μl aminoguanidine (0.1 M: Eastman Kodak Co., Rochester, NY) a competitive inhibitor for DAO. We have previously established a KI of 0.5 μM for aminoguanidine against putrescine in an identical preparation. Also addition of ami

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Figure 1. The major metabolic pathways for arginine are depicted and involve primarily the conversion of arginine to citrulline by NOS, conversion of arginine to ornithine by arginase and, as described in the current study, conversion of arginine to agmatine by arginine decarboxylase (ADC). Downstream products are also depicted.

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were exposed by ventral incision. Aminoguanidine (0.5 cc, 10 mM in sterile 0.9% saline) or vehicle was administered as a bolus via the vena cava and allowed to circulate for 5 min. The animals were exsanguinated via the aorta using a syringe containing 0.4 ml heparin and 0.1 ml aminoguanidine (1000 U/ml and 10 mM, respectively). Kidneys were rapidly excised, decapsulated and dissected to remove inner and outer medulla. Cortex tissue was then transferred to high speed centrifuge tubes containing cold aminoguanidine (1 mM, 1 ml per kidney cortex) in 10% TCA for homogenization and centrifugation (1 h, 14,000 g). Albumin and large proteins were removed from plasma using 0.5 μm and 10,000 mol wt filters (Millipore Corp., Bedford, MA). A partial purification of kidney supernatants and plasma ultrafiltrate fused with warm isotonic NaCl/NaHCO₃ kidney surface exposed. The kidney surface was continuously superfused and sealed with dilute agar leaving the micropuncture ion. The kidney was placed into a lucite cup and the left ureter cannulated from the adrenal gland and perirenal fat via a left subcostal incision. The animals derived from our colony. These animals express at least 20 glomeruli of 1.5 ml/h isotonic saline containing 250 μM COOH maintained for 15 min. At 85 min a 10 min gradient change to 82:18:0 isocratic elution a gradient change to 86:14:0 is complete at 70 min and a gradient change to 94:6:0 occurs over 28 min. After 12 min of isocratic elution used was as follows; 100:0:0 isocratic for 1 min before a step change to 99:1:0 followed by a gradient change to 97:3:0 over 16 min. At 17 min a gradient change to 94:6:0 occurs over 28 min. After 12 min of isocratic elution a gradient change to 86:14:0 is complete at 70 min and maintained for 15 min. At 85 min a 10 min gradient change to 82:18:0 commences. A wash cycle consisting of a gradient change to 060:40 and a return to 100:0:0 over 30 min followed each run. Fluorescence of derivatized compounds in samples and standards was measured at 250 nm exc 395 nm xem, enabling quantification of eluted components.

Micropuncture studies. Studies were performed in Wistar-Frömter rats derived from our colony. These animals express at least 20 glomeruli on the exposed micropuncture surface. Animals were anesthetized (Inactin; 100 mg/kg) and prepared for surgery as previously described with catheters in the jugular vein and femoral artery and bladder (25–27). After preliminary surgery the kidney was dissected free from the adrenal gland and perirenal fat via a left subcostal incision. The kidney was placed into a lucite cup and the left ureter cannulated with a PE-50 catheter. The area around the left kidney was then packed and sealed with dilute agar leaving the micropuncture kidney surface exposed. The kidney surface was continuously superfused with warm isotonic NaCl/NaHCO₃ solution. A sustaining infusion of 1.5 ml/h isotonic saline containing 14C H inulin was supplied to obtain ~ 60–80 μCi/ml for single nephron glomerular filtration rate (SNFGF) and APR measurements. Animals also received littermate plasma infusion (1% TBW in 60 min followed by 0.15%TBW/h) Certain studies were performed in rats which were previously submitted to left unilateral renal denervation as previously described (26, 27), 5–7 d before micropuncture studies. 

(a) Intracortical infusion of substances. After the animal was prepared for micropuncture, a PE-10 catheter was passed through the capsule of the kidney and the tip located at the cortico-medullary junction for the infusion of a NaCl-NaHCO₃ vehicle infused at a constant rate of 0.6 ml/h throughout an initial control and experimental periods. In the test group, agmatine (20 nM/h) was added to the vehicle during the experimental period. 

(b) Urinary space infusion of substances. In these experiments we collected late proximal fluid before, during and after the infusion of substances directly into the glomerular capsule. A 5–7 μm tip pipette connected to a Hample nanoliter microperfusion pump was inserted into the urinary space of a superficial glomerulus (28). After localization of the late proximal segment, an oil block was inserted in the last segment and a timed collection was obtained for the assessment of control SNFGF and APR. The pump was then turned on to infuse either vehicle or test substances at a flow of 5 nl/min in the urinary space (normal SNFGF is ~ 40 nl/min). After 10 min of infusion a second late proximal tubule sample was collected. Subsequently, the pump was turned off and a final assessment of SNFGF and APR was obtained from a late proximal collection after a 10 min recovery period.

Agmatine was infused at concentrations sufficient to establish ~ 6 μM, 0.8 μM and 0.1 μM concentrations in the urinary space. In order to evaluate the role of α₂ receptors, the α₂ antagonist yohimbine (6 μM) was infused either alone or concurrently with agmatine. We also used the selective Iₐ receptor agonist BU-224 (Tocris Cookson, Bristol, UK; 1 μM) and the Iₐ agonist moximodine (Solvay, Hannover, FRG; 1 μM).

Clearance and hemodynamic parameters were calculated as previously described (29). Inulin clearance (UV/P) and volumetric measurement of fluid collected from late proximal tubules were employed to calculate SNFGF and APR. Single nephron plasma flow (SNPF) was also determined (25–27) in interstitial infusion studies whereby a micro-adaptation of the Lowry technique (30) was employed to determine the protein concentrations of systemic (Cₐ) and effluent arteriolar (Cₑ) plasma. The determinants of SNPF were; SNPF = (SNFGF × Cₑ/(Cₑ – Cₐ)). To best advantage of the paired nature of the experiments, the effects of treatments were analyzed by one-way ANOVA with design for repeated measures as described previously (27).

**Results**

**ADC activity and location in mammalian tissue.** ADC activity was assessed from the production of 14CO₂ from radiolabeled L-arginine. Table I depicts the decarboxylation of labeled arginine in various organs expressed as CPM 14CO₂ generated in 60 min on a whole organ basis and per gram wet tissue. Significant activity was observed in all organs within the mitochondria enriched fraction only, validating the results of Li et al. (1). Furthermore, we have observed that mammalian ADC is extremely sensitive to freezing and thawing, resulting in a dramatic loss of activity.

**Polycyclic antibody immunostaining for DAO.** The distribution of diamine oxidase as observed by immunohistochemistry was identical in human and mouse tissues. Diamine oxidase was present in the following locations: the basal level of the skin epidermis, glomeruli, the decidual of the placenta, epithelial cells in the small intestine, bone and tissue eosinophils, macrophages, and smooth muscle cells of veins, arteries, bronchioles, stomach wall, and skin. Diamine oxidase was not present in brain, heart, liver, adrenals, ovary, or skeletal musc.
Fig. 2 demonstrates DAO primarily located in glomeruli of rat kidney. DAO enzyme activity — metabolism of agmatine. A linear relation, \( r = 0.98 \), was established between \( \text{H}_2\text{O}_2 \) concentration and fluorescence over the range tested in our preparation. Maximal production of \( \text{H}_2\text{O}_2 \) in 60 min was observed with a \( K_m \) of 5 \( \mu \text{M} \) (Fig. 3). \( K_m \) for other DAO substrates were \( 2.12 \times 10^{-4} \) \( \text{M} \) for putrescine, \( 1 \times 10^{-3} \) \( \text{M} \) for spermine and \( 1.5 \times 10^{-5} \) \( \text{M} \) for histamine.

HPLC identification of arginine metabolites. Fig. 4 is a chromatogram showing the detection and separation of a standard solution containing 50 \( \mu \text{M} \) citrulline, arginine, agmatine and ornithine. The table in Fig. 4 demonstrates the relative change over time in composition of \(^3\text{H}\)arginine and \(^3\text{H}\)-metabolites, generated by renal cortical homogenate at 37°C. In normal kidney, production of labeled agmatine is second only to the conversion of arginine to ornithine and much in excess of the conversion of arginine to citrulline. Only a small amount of other products were present.

HPLC quantification of endogenous agmatine. Using HPLC we have evaluated agmatine levels in kidney and ultrafiltrate of plasma in normal rats and after the in vivo administration of aminoguanidine to acutely block DAO activity. Circulating plasma levels of agmatine were 0.7 \( \mu \text{M} \) and increased twofold to 1.3 \( \mu \text{M} \) after DAO inhibition (Table II). Agmatine levels in kidney homogenates in both control and aminoguanidine treated rats were 1.5 and 1.7 \( \mu \text{M} \), respectively.

Fig. 3. Arginine metabolism by renal DAO generates \( \text{H}_2\text{O}_2 \) that can be accurately measured using a peroxidase based assay that generates a fluorophore. Fluorescence is linearly related to concentration over the range of interest.

Fig. 4. HPLC chromatogram demonstrating separation of OPA derivatized amino acid/polyamine standard (25 ml, 50 \( \mu \text{M} \)). The values in the lower part of the figure demonstrate the relative production of labeled arginine metabolites by kidney homogenates. Conversion of arginine to agmatine is second only to the conversion of arginine to ornithine.
**Table II. Endogenous Agmatine in Plasma and Renal Cortex: Effect of Acute Aminoguanidine Treatment**

<table>
<thead>
<tr>
<th>[μM]</th>
<th>Plasma ultrafiltrate</th>
<th>Kidney cortex extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Aminoguanidine</td>
<td>1.3</td>
<td>1.7</td>
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</tbody>
</table>

**Interstitial infusion of agmatine.** As seen in Fig. 5, the continuous infusion of control vehicle solution resulted in a modest reduction in nephron filtration rate and absolute proximal reabsorption over the 90-min period. The decrease in SNGFR was due to reductions in nephron plasma flow (114±30 to 97±110 nl/min) (*P*<0.05) (*n*=5). Interestingly, agmatine infusion at 20 nM/h (*n*=7) completely prevented this vehicle associated decrease in SNGFR and APR.

**Urinary space infusion of agmatine.** Vehicle alone infused into the urinary space did not alter SNGFR and APR over these short time periods (*n*=8). However, when agmatine (6 μM) is infused into the urinary space (*n*=12), SNGFR and APR increased significantly (*P*<0.01) (Fig. 6). Values returned to baseline after the infusion was discontinued for a period of 10 min, demonstrating that agmatine effects are reversible. SNGFR and APR also increased in response to 0.8 μM agmatine (*n*=8) (*P*<0.05), but 0.1 μM agmatine microperfusion did not significantly increase SNGFR and APR (*n*=13) (Fig. 7). Agmatine at 0.8 μM increased APR from 8.5±1.2 to 12.1±1.2 nl/min (*P*<0.05).

When yohimbine was infused alone (*n*=18) at a concentration far in excess of its *Kₐ* (nM range) there was a negative effect on SNGFR and APR (Fig. 8). This response was not evident during co-infusion with agmatine (*n*=11).

We then examined the effects of intra-urinary space microperfusion with agmatine (6 μM) in previously denervated kidneys in order to assess the role of prejunctional α₂ adrenoceptors and the requirement of renal innervation. Agmatine administration increased SNGFR transiently in a fashion nearly identical to findings in innervated kidneys (*n*=9) (Fig. 9). Interestingly, APR did not increase during agmatine microperfusion in denervated kidneys.

The I₂ receptor agonist BU-224 infused into the urinary space produced a significant (*n*=9, *P*<0.05) increase in SNGFR (Fig. 10), a response of similar magnitude to that achieved by agmatine. However, the recovery phase differed significantly in that elevated SNGFR persisted after termination of BU-224 infusion. In addition, the I₁ agonist did not increase APR, thus glomerulo-tubular balance was not maintained. In contrast, infusion of moxonidine, a selective I₁ agonist (31), did not affect SNGFR or APR (*n*=7).

**Discussion**

Assessment of ADC activity and identification by HPLC of agmatine production in the present study confirms recent reports of an alternate pathway for arginine metabolism in mammals (9–11). The heterogeneous location of DAO suggests that...
certain tissues or organs may have a capacity to regulate local agmatine levels. In this respect the fact that glomeruli are primary renal sites for DAO may be indicative of local regulation of agmatine levels. We have also demonstrated that agmatine elicited functional responses in the kidney that could involve multiple receptor types and/or sites of action.

\(^{14}\text{CO}_2\) generation from labeled arginine revealed significant ADC activity in the mitochondria enriched fraction of all organs tested (highest in brain, liver and kidney). It should be noted that these values do not indicate the relative enzyme activity among organs since there was no control of cold arginine levels in these preparations. Since arginine levels in kidney are undoubtedly high, these values must underestimate actual ADC activity. The transport of agmatine across the cell membrane has not yet been characterized but must occur since we have demonstrated glomerular effects which can be best explained by transport across proximal tubules into the renal interstitium. HPLC studies clearly demonstrated agmatine production. However, since the metabolic conversion of arginine is a dynamic process involving both synthesis and degradation, the relative amounts of labeled products at 45 and 75 min do not permit quantification of the relative activity of the various enzymes involved. This is evident in the nonlinear rates of arginine conversion which may reflect exhaustion of rate limiting factors in our assay. A more detailed time course in the presence of varying amounts of cold arginine and inhibitors of enzymes such as DAO will be necessary to clearly delinete relative production rates and product half life.

DAO purified from kidney exhibited a capacity to metabolize agmatine with a \(K_m\) of 5 \(\mu\text{M}\), a value somewhat higher than the concentrations of agmatine required for demonstrating physiologic effects (0.8 \(\mu\text{M}\)). Endogenous levels of agmatine increased in plasma after aminoguanidine administration suggesting a biologic role for DAO in the metabolism of circulating agmatine (Table II).

Because DAO has been isolated and purified we were able to use immunohistochemistry to describe the distribution of this enzyme. Surprisingly, brain and adrenal tissue contained no diamine oxidase, in spite of the fact that agmatine has been purported to regulate the release of norepinephrine and to act as a nicotinic antagonist (9, 21). DAO in renal tissue appears to be primarily located in glomerular structures. The presence of DAO in glomeruli may explain why the effects of agmatine are rapidly reversible while that of the synthetic imidazoline-receptor active compounds are not. Endogenous agmatine levels were also assessed by HPLC in plasma ultrafiltrate (0.7 \(\mu\text{M}\)) and kidney cortical homogenates (1.5 \(\mu\text{M}\)). Plasma levels increased twofold after DAO inhibition. Questions arise as to the functional role of DAO with respect to normal sites of action of agmatine and potential effects of the metabolite, guanidino-butyraldehyde. Further studies will be required to establish concentration profiles of endogenous agmatine within glomeruli and other renal structures.

We initially chose to deliver agmatine by interstitial infusion because imidazoline type receptors have been localized to the basolateral aspect of proximal tubules (19). This approach succeeded in demonstrating a biological effect since agmatine prevented the decline in SNGFR and APR observed with the infusion of vehicle alone. The effect on tubular reabsorption may be a direct effect of agmatine or indirect via glomerulotubular balance or flow dependent increases in transport rate (28). A direct effect of agmatine on transport rate is supported by recent studies from Morrissey et al. (10) which demonstrated that agmatine stimulates \(\text{Na}^+/\text{K}^+\) ATPase in kidney membrane preparations.

To discern tubulo-glomerular feedback induced SNGFR responses from direct effects (and since the interstitial infusion method does not allow for rapid termination of infusion) we opted to infuse agmatine into the urinary space of closed loop nephrons. Substances infused by this technique move directly into the proximal tubule and may be transported into the peritubular interstitium. Since alterations in the flow of tubular fluid to the macula densa was prevented by an oil block, tubuloglomerular feedback responses are eliminated. Agmatine infused in this fashion caused an increase in SNGFR, which is most likely the result of an effect on the glomerulus or associated vasculature. The concurrent increase in APR could have resulted from a direct effect of agmatine or by flow dependent mechanisms. It is also important to note that these functional effects were acutely reversible, implying a short half-life of agmatine in vivo.

We have evaluated the effects of a variety of agmatine concentrations on SNGFR and APR responses. Both 6 and 0.8 \(\mu\text{M}\) agmatine concentrations in the urinary space increased both SNGFR and APR, but 0.1 \(\mu\text{M}\) agmatine failed to influence nephron function. If agmatine in the microperfusion pipette exerted any direct physiologic effects at the glomerulus, these values may slightly underestimate the effective physiologic concentration. Endogenous levels of agmatine in plasma ultrafiltrate and kidney were in the \(\mu\text{M}\) range, suggesting that there may be either compartmentalization or significant binding of agmatine in tissue or plasma, thereby reducing bioavailability. It is of interest that urinary space infusion of the selective \(\alpha_2\) antagonist yohimbine alone decreases SNGFR and APR. This laboratory has previously shown similar effects of yohimbine in innervated and denervated kidneys (25) suggesting that the endogenous ligands are either blood borne or locally produced. Simultaneous infusion of yohimbine and agmatine produced no change in nephron filtration rate when infused into the urinary space. It is tempting to conclude, based on these data alone, that the effects of agmatine might be exerted via \(\alpha_2\) adrenergic receptors. However, given the similar responses obtained in denervated kidneys, this conclusion appears unlikely. A recent publication failed to show an agonist or an antagonist effect of agmatine on \(\alpha_2\) receptors either in brain or smooth muscle, although curiously the authors did demonstrate competitive binding (18). In light of this observation an alternative explanation is that yohimbine decreases SNGFR via the \(\alpha_2\) adrenergic receptor system while agmatine exerts a positive effect by another mechanism. Based on these data alone, we...
cannot differentiate between potential agmatine action on α2 type receptors and a physiologically opposing effect mediated by other mechanisms.

Studies were also performed in rats submitted to left kidney renal denervation. Although these studies were designed to examine the potential interaction of arginine with renal nerve prejunctional α2 adrenoceptors in eliciting changes in SNGFR and APR, data also allowed us to separate the glomerular and proximal tubular effects of agmatine. Five to seven days after renal denervation, administration of agmatine increased SNGFR by the same degree as in innervated kidneys, precluding an α2 prejunctional site of action. However, APR did not increase in response to agmatine in these conditions. The fact that APR did not increase in denervated kidneys, in spite of similar increases in SNGFR, raises at least two possibilities (a) glomerular and tubular effects of agmatine are mediated by different mechanisms and/or possibly receptor types such that APR is dependent on renal innervation, and (b) removal of prejunctional α2 adrenoceptors disrupts glomerulo-tubular balance thereby eliminating the agmatine associated increase in APR.

The infusion of the I1 receptor agonist BU-224 increased SNGFR by a magnitude similar to that observed with agmatine. However, there were significant and interesting differences between the effects of the I1 agonist and agmatine. Agmatine effects were reversible within 10 min, whereas BU-224 exerted a sustained effect on SNGFR. This finding could be explained by a longer half life of the synthetic compound at its site of action. Another important difference between the effects of urinary space infusion of agmatine and BU-224 was that APR remained unchanged during the infusion period. In light of the fact that imidazole receptors are located on the proximal tubule (19), it is possible that the quantity of I1 agonist utilized (<1 μM) may have exerted partial agonist effects at these receptors and actually prevented a flow dependent increase in APR. Further studies are required which examine the direct effects of agmatine and imidazoline agonists on major transport systems, such as the Na+/H+ antiporter in the proximal tubule.

We cannot discount the possibility that agmatine might also exert effects by non–receptor-dependent mechanisms. Di- and polyamines have been suggested to exert effects on P and L-type calcium channels (32). Effects on the reloading or release of calcium from IP3 sensitive pools could account for alterations in proximal tubular transport by agmatine. Since arginine decarboxylase and nitric oxide synthase use a common substrate, arginine, it is not unreasonable to speculate that agmatine might exert a modulatory effect on nitric oxide production. Biologic amines do inhibit inducible nitric oxide synthase (33), but effects on the constitutive NOS have not been described. Previous studies from our laboratory have suggested that L-NMMA, by inhibiting constitutive nitric oxide synthase in renal tissue, produces a reduction in proximal reabsorption (34).

We have demonstrated arginine decarboxylase activity and the generation of agmatine in renal tissue as well as endogenous agmatine in both plasma and kidney. The enzyme, DAO, metabolizes agmatine and is located in the kidney, primarily in glomeruli. Infusion of exogenous agmatine increases SNGFR and APR. Data suggest that these effects might involve I1 (but not I2) imidazoline receptors. Furthermore, opposing effects of agmatine and yohimbine could suggest a functional role for agmatine as a modulator of α2 adrenergic effects, although it is unclear that agmatine acts directly via α2 receptors. This alternate arginine metabolic pathway located in the kidney may help clarify some of the many renal physiologic effects of arginine. Further studies are required to delineate the cell systems which are influenced by arginine decarboxylase products as well as the mechanisms and transduction pathways whereby these compounds exert biologic effects.

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