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RAPID COMMUNICATION

Partial cloning and characterization of an arginine decarboxylase in the kidney

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Partial cloning and characterization of an arginine decarboxylase in the kidney. Using homology-based polymerase chain reaction (PCR) amplification, we demonstrate the presence of arginine decarboxylase mRNA in tissues involved in arginine metabolism (brain, kidney, gut, adrenal gland, and liver of the rat) but not in organs (lung, heart, and spleen) in which arginine metabolism is low or absent. The polymerase chain reaction product from the kidney had a nucleotide sequence 61% identical to that of the *E. coli* biosynthetic arginine decarboxylase. On a whole tissue basis, kidney homogenates were three times more active than brain homogenates at decarboxylating [1-^{14}C]arginine. Subcellular fractionation localized the arginine decarboxylase activity of the kidney to the mitochondria fraction. Agmatine, one of the products of arginine decarboxylation, was found to inhibit nitric oxide formation by post-mitochondrial supernatants of the brain or kidney. We propose that arginine is metabolized to two structurally different signaling molecules, nitric oxide and agmatine. Furthermore, agmatine can influence the nitric oxide synthase pathway.

L-arginine is essential for the synthesis of urea, creatine and nitric oxide [1], in addition to its role in protein synthesis. A novel metabolic pathway resulting in decarboxylation of L-arginine and formation of agmatine has been described in the brain [2]. Agmatine, a "clonidine-displacing substance," can bind to both α_2 adrenergic and imidazoline-guanidinium receptor sites (IGRS), with a wide variety of biologic effects [2]. Clonidine-displacing substance is present in peripheral tissues and circulation of the rat [3], and is increased in the serum of some patients with pregnancy-induced hypertension [4]. The IGRS of proximal tubule basolateral membranes recognize clonidine displacing substance [5, 6]. Agmatine synthesis depends on the presence of an L-arginine decarboxylase (E.C. 4.1.1.19); however, an mRNA for this enzyme has not been described in mammalian cells [7]. In this investigation, we used homology-based PCR amplification to uncover an arginine decarboxylase mRNA in several tissues of the rat.

Methods

Measurement of arginine decarboxylation

The production of labeled CO_2 from [1-(^{14}C)]arginine was determined essentially as described [2]. Briefly, whole rat kidney or manually dissected kidney cortex, outer and inner stripe of the kidney outer and inner medulla or whole rat brain were homogenized in 3 volumes (wt/vol) of ice-cold 5 mM Tris chloride, pH 8.8, 0.8 mM magnesium sulfate. In some experiments the cortex was separated into glomeruli and tubules by sieving [8] prior to homogenization. The protein content of each homogenate was determined by the method of Bradford [9]. Preliminary studies revealed that [^{14}C]-CO_2 formation was linear with time through at least 90 minutes of incubation. In each case the activity of a boiled (microwaved) enzyme blank was subtracted. This activity usually amounted to about 10 to 15 pmol/hr/mg protein.

Subcellular fractionation of kidney

Rat kidney cortex was homogenized with 6 volumes (wt/vol) of ice-cold 0.25 M sucrose, 25 mM Tris chloride pH 7.4 and 0.1 mM phenyl methyl sulfonylfluoride. The homogenate was filtered through four layers of gauze followed by centrifugation at 500 ×g for 10 minutes. This supernatant was further centrifuged at 30,000 ×g for 20 minutes. In other experiments, the 500 ×g supernatant was first centrifuged at 7,500 or 10,000 ×g for 10 minutes followed by 30,000 ×g for 20 minutes. The different pellets of membrane protein were resuspended in 5 mM Tris chloride pH 8.8, 0.8 mM magnesium sulfate prior to assay. The activity of succinate dehydrogenase in the various fractions of the kidney was measured using triphenyltetrazolium chloride as described previously [10].

Measurement of nitric oxide synthase activity

The production of labeled citrulline from [U(^{14}C)]arginine was determined essentially as described [11, 12]. Briefly, whole rat cerebellum or kidney cortex was homogenized in 0.32 M sucrose, 50 mM Tris chloride, 1 mM EDTA, 1 mM dithiothreitol, 100 μg/ml phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 2 μg/ml aprotinin pH 7 [12]. A post-mitochondrial (7,500 ×g) supernatant was added to a buffer consisting of 50 mM potassium phosphate, 60 mM valine, 120 μM NAPDH, 1 mM L-citrulline, 25 μM L-arginine, 1.2 mM magnesium chloride, 0.25 mM calcium chloride and 0.075 μCi L-[(U)^{14}C]arginine pH 7.2 with or without 1 mM N$^{\omega}$-nitro-L-arginine methylester (L-NAME). The reaction was incubated at 37°C for 10 minutes. The amount of [^{14}C] citrulline formed was determined by dilution of the reaction mix with 4 ml of water and 1 ml of a 1:1 (vol/vol) mix of water with Dowex-50WX8 (200 to 400 mesh) in the sodium form. Two milliliters of the aqueous supernatant were used for liquid scintillation determination of radioactivity [11]. The amount of agmatine added to the reaction

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mix did not influence the binding of labeled arginine to the ion exchange resin (not shown).

Amplification of arginine decarboxylase mRNA

Female Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) were used throughout the study. Rats were fed a standard rat chow (Purina, St. Louis, MO, USA). Single strand oligo dT-primed cDNA libraries were prepared from the kidney, brain, liver, gut, lung, heart, spleen and adrenal gland of the rat as described [8]. Primers for the homology-based amplification of putative arginine decarboxylase mRNA were selected by comparing the sequence of the *E. coli* biosynthetic [13] and the oat *(A. Sativa)* [14] enzymes. The primer pairs consisted of sense (GGG CTG GAG GCA GGC TCC AAG CCA GAG TTG), representing amino acids 125-134, and antisense (GGA GCC GAC GTG GAA GTG CAG CAG), representing amino acids 249-256 of oat arginine decarboxylase [14]. Amplification was performed with cDNA using 35 cycles of denaturation at 94°C for one minute and annealing at 60°C for one minute followed by extension at 72°C for two minutes. The amount of the arginine decarboxylase cDNA determined by PCR was normalized by the amount of glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA also determined by PCR, but using only 28 cycles [8]. This number of cycles allowed for the relative assessment of the amount of GAPDH cDNA and arginine decarboxylase cDNA. The products were separated by means of 1% agarose gels (Fisher Scientific, Fair Lawn, NJ, USA) in 40 M Tris acetate, 2.5 mM ethylene diaminetetraacetic acid buffer pH 8.0 containing ethidium bromide. The product for arginine decarboxylase migrated at 387 bp while the GAPDH product migrates at 515 bp.

Sequence of arginine decarboxylase

The 387 bp PCR product arising from the amplification of kidney cDNA using the primers for arginine decarboxylase was sequenced by two techniques. One utilized a Femtomole sequencing kit (Promega, Madison, WI, USA), while the other required ligation of the product into the vector pCRII (Invitrogen, San Diego, CA, USA). The protocol for dideoxy sequencing with 7-deaza guanosine supplemented reaction mixes was as described previously [8]. The final sequence reported here was a consensus of several sequencing reactions performed on three independently purified arginine decarboxylase DNA containing plasmids.

Results and discussion

To identify the presence of arginine decarboxylase mRNA in different tissues of the rat, we used oligonucleotide primers to conserved regions of the *E. coli* and oat enzymes—which, in turn, flanked conserved regions—and this disclosed a product of expected size. All results were normalized for the relative amount of glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA. The whole kidney contained considerably more mRNA for arginine decarboxylase than other tissues (Fig. 1). Appreciable amounts of arginine decarboxylase cDNA were also found in the brain, liver, gut and adrenal gland (Fig. 1). Undetectable to barely detectable amounts of the arginine decarboxylase mRNA were found in organs with low or absent arginine metabolism (lung, heart and spleen). The amplification of the GAPDH cDNA had been stopped at cycle 28, which, as shown in preliminary studies, is in the quantitative range prior to plateauing of product formation. Similarly, the arginine decarboxylase cDNA amplification was stopped at cycle 35. The consistent finding when tissues from several different animals were analyzed was that the kidney had a higher amount of putative arginine decarboxylase mRNA than found in the other tissues examined under these conditions. At the least this survey identifies tissues that contain arginine decarboxylase mRNA.

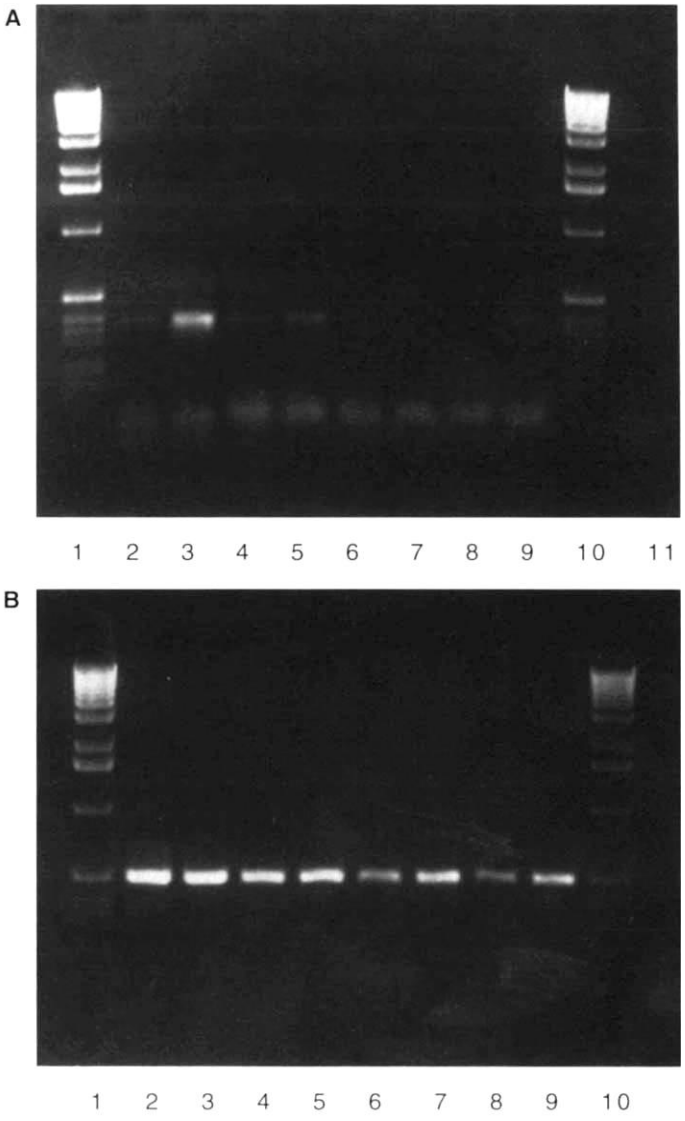

Fig. 1. *Polymerase chain reaction amplification of arginine decarboxylase.* Represented are: brain, lane 2; kidney, lane 3; liver, lane 4; gut, lane 5; lung, lane 6; heart, lane 7; spleen, lane 8; and adrenal gland, lane 9. Single strand cDNA was amplified for arginine decarboxylase (**A**) or GAPDH (**B**). Lanes 1 and 10 represent sizing markers while lane 11 of A represents the arginine decarboxylase amplification mix without added cDNA.

The PCR product from the kidney was cloned and the DNA sequence determined. The nucleotide sequence of this product was 61% and 47% identical to the *E. coli* biosynthetic [13] and oat [14] sequences, respectively (Fig. 2). There was 56% and 49% conservation of the amino acid sequence compared to the *E. coli* biosynthetic and oat enzymes. Our partial sequence is equivalent to amino acids 146-275 of the *E. coli* enzyme [13] and 125-256 of the *A. Sativa* enzyme [14]. The rat kidney arginine decarboxylase

Rat	1	GGG	CTG	GAG	GCA	GGC	TCC	AAG	CCA	GAG	TTG	CTG		GCG	GTC	CTC	ACG	CCT	TGC	CTG	AAG
E. coli	1449	--T	---	--A	--C	--T	---	--A	G-C	---	---	A--		--A	--A	--G	G-A	-A-	GCT	GGC	-T-
Oat	392	---	---	---	---	---	---	---	---	---	---	---	ATT	--C	A-G	AG-	TGC	-TC	AC-	AAA	GCC
Rat	58	GGC	GGC	ACC			ATC	GTC	TGC	AAC	GGC	TAC	AAA	GAC	CGC	GAG	TTC	ATC	CGC	CTG	GCG
E. coli	1506	AC-	C-T	-G-	GTC		---	---	---	---	--T	--T	---	---	---	---	--A	-AT	---	---	--A
Oat	452	AAG	CCT	GGA	GCC	TAC	C-G	--A	---	---	---	---	--G	---	TCG	-CT	-AT	G--	GCG	--C	--C
Rat	112	CTG	ATG	GGC	CAG	AAG	CTG	GGC	CAC	AAC	GTG	TTC	ATC	GTG	ATC	GAG	AAA	GAG	TCC	GAG	GTG
E. coli	1563	T-A	--T	---	G--	---	A--	--G	---	--G	--C	-AT	C-G	--C	--T	---	--G	AT-	--A	--A	A-C
Oat	512	---	GC-	-CG	-GC	GCC	A--	---	-TG	---	--C	A--	---	---	C-G	---	-TG	---	GAG	---	C--
Rat	172	GCG	CTG	GTG	ATC	GAA	GAG	GCC	GCC	GAC	CTG	AAG	GTC	AAG	CCG	CAG	GTC	GGC	CTG	CGT	GTG
E. coli	1623	--C	A-T	---	C-G	--T	--A	--A	-AA	CGT	---	--T	---	GTT	--T	-GT	C-G	---	G--	---	-CA
Oat	572	-AC	A-C	TGC	---	--G	---	AG-	AG-	A-G	--C	GGC	--G	G--	--C	GTC	A--	---	G-C	--C	-CC
Rat	232	CGC	CTG	TCG	TCG	CTG	GCT	TCG	AGC	AAG	TAG	TCC	GAT	ACC	GGT	GGC	GAG	AAG	TCC	AAG	GTC
E. coli	1683	--T	---	CGT	---	-A-	-G-	---	G-T	--A	-G-	CAG	TCC	T--	--C	--G	--A	--A	--G	---	T--
Oat	632	AAG	---	CTC	A-C	AA-	ATA	C--	G--	C-T	-TT	GGG	TCC	--G	-CC	---	A--	C-C	GGT	---	T--
Rat	292	GGC	CTG	TCG	GCT	AGC	CAG	TTG	TTA	TCG	GTG	GTC	CAG	CGC	ATC	CGC	ATA	GCA	AGC	CTG	GAC
E. coli	1743	---	---	G-T	--G	-CT	---	G-A	C-G	CAA	C--	--G	G-A	AC-	C-G	--T	GA-	--C	G-G	-GT	CT-
Oat	692	--G	--A	C--	--G	GAG	A--	A-T	-AT	GA-	---	-C-	A--	AAG	C--	AAG	GCT	CTG	-A-	AA-	CTG
Rat	352	CAG	GGC	ATC	CGC	CTG	CTG	CAC	TTC	CAC	GTC	GGC	TCC								
E. coli	1803	G-C	A--	C-G	-AA	--A	---	---	---	---	C--	--T	--G								
Oat	752	G-T	T-G	C--	AAG	---	---	---	---	---	---	---	---								

Fig. 2. *Nucleotide sequence of rat kidney arginine decarboxylase.* The sequence of the PCR amplified arginine decarboxylase is compared to the nucleotide sequence of the E. coli biosynthetic [13] and the oat *(A. Sativa)* [14] enzymes. The numbers in the left margin refer to the positions within the respective sequence. A hyphen (-) indicates the same nucleotide as in the rat sequence; a space indicates a deletion in the sequence. Underlining indicates the primer regions used in the amplification.

Table 1. Arginine decarboxylase activity of rat brain and kidney

Tissue	Activity *pmol CO_2/hr/mg/protein*
Whole brain	74 ± 3
Whole kidney	245 ± 21, $P < 0.004$
Kidney cortex	199 ± 38
Glomeruli	41 ± 2
Tubules	165 ± 24, $P < 0.02$
Kidney outer medulla	
Outer stripe	240 ± 70
Inner stripe	184 ± 18
Kidney Inner Medulla	Not greater than blank values

Values shown are the mean ± SD of three separate determinations. A heat-inactivated blank "activity" was routinely subtracted from each value. This blank usually amounted to 10 to 15 pmol/hr/mg protein.

Table 2. Subcellular localization of arginine decarboxylase activity in rat kidney

Fraction	Activity *pmol CO_2/hr/mg protein*	Succinate Dehydrogenase *fold enrichment*
Homogenate	206 ± 38	0
30,000 ×g supernatant	83 ± 4	0.07 ± 0.02
500–30,000 ×g pellet	1364 ± 32	3.09 ± 0.42
500–7,500 ×g pellet	1545 ± 105	7.10 ± 0.82
7,500–30,000 ×g pellet	21 ± 4	1.07 ± 0.75

Values shown are the mean ± SD of three separate determinations. The succinate dehydrogenase enrichments were calculated setting the activity of the homogenate at 1.0.

was not found to be homologous to the *E. coli* biodegradative enzyme [15].

We also examined the liberation of [^{14}C]-CO_2 from labeled arginine by tissue homogenates. We found that under identical isolation and assay conditions whole kidney homogenates liberate about threefold more labeled CO_2 from C-1 labeled arginine than whole brain homogenates (Table 1), when normalized for the amount of total protein.

The arginine decarboxylase activity in different regions of the kidney was measured. On a total protein basis both the outer and inner stripe of the outer medulla had greater decarboxylase activity than the cortex (Table 1). The inner medulla had no demonstrable activity above the heat inactivated tissue blank. If the cortex was mechanically separated (sieved) into glomeruli and tubules, the decarboxylase activity of the cortex was present mainly in the tubules, with about 20% of the activity present in the glomeruli (Table 1).

Using differential centrifugation of kidney homogenates, we found that the activity of the arginine decarboxylase was located mainly, if not exclusively, in the membrane fraction of the cell. Within this membrane fraction the activity was located in the 500 to 7,500 ×g fraction, which is enriched in mitochondria based upon the enrichment of the enzyme succinate dehydrogenase (Table 2).

In further studies we determined that agmatine affects the formation of nitric oxide in post-mitochondrial supernatants of cerebellum or kidney cortex. These are sources of constitutive nitric oxide synthase [12]. Agmatine at millimolar concentrations inhibited nitric oxide synthase activity as measured by the conversion of radiolabeled arginine to radiolabeled citrulline (Table 3). The synthase activity measured was found to be calcium-dependent since without the addition of calcium chloride no activity was measured.

Table 3. Inhibition of nitric oxide synthase activity by agmatine

Tissue	Condition	Activity *pmol citrulline/min/mg protein*
Cerebellum	Control	6857 ± 582
	+ 5 mM agmatine	1500 ± 71, $P < 0.01$
Kidney cortex	Control	201 ± 17
	+ 0.5 mM agmatine	188 ± 14
	+ 1.0 mM agmatine	124 ± 13, $P < 0.04$
	+ 5.0 mM agmatine	55 ± 3, $P < 0.01$

The values given represent the mean ± SD of three separate determinations normalized for the amount of protein.

Thus, in the rat kidney arginine can be decarboxylated to produce agmatine. The physiological role of agmatine as a signaling molecule in the different tissues with arginine decarboxylase activity (brain, kidney, liver, gut and adrenal gland) remains to be defined. Metabolism of L-arginine through this pathway may influence the activity of the nitric oxide pathway. Indeed, agmatine inhibits constitutive nitric oxide synthase activity (present investigation) and the presumed inducible enzyme [16]. Nitric oxide inhibits mitochondrial respiratory chain enzymes [17]. Therefore, agmatine production by the mitochondria could serve as a protective mechanism against cytotoxicity from excessive nitric oxide formation. Although this inhibition *in vitro* is in the millimolar range, it is not known what concentrations of agmatine may be achieved *in vivo*.

It is likely that kidney function(s) influenced by α_2-adrenergic or IGRS are modulated by locally produced (renal cells) agmatine. Clonidine displacing substance has a higher affinity (about 24-fold) for the IGRS than the α_2-adrenergic receptor on renal cortical membranes [17]. Of interest is the fact that mitochondrial outer membranes contain a significant number of IGRS which do not appear to influence oxidative phosphorylation, however [18]. The Na^+/H^+ exchange activity of proximal tubule cells is inhibited by compounds that bind to the IGRS [19] but is stimulated through the α_2 adrenergic receptor [20]. Defining the effects of agmatine on kidney function, therefore, awaits further studies.

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References

1. Reyes AA, Karl IE, Klahr S: Role of arginine in health and disease. *Am J Physiol* 267 (*Renal Fluid Electrol Physiol* 36):F331–F346, 1994
2. Li G, Regunathan S, Barrow C, Eshraghi J, Cooper B, Reis D: Agmatine: An endogenous clonidine-displacing substance in the brain. *Science* 263:966–969, 1994
3. Biollaz J, Roch-Ramel F, Kirchertz EJ, Atkinson J, Peters-Haefeli L: The renal effects of clonidine in unanesthetized rats. *Eur J Pharmacol* 58:407–418, 1979
4. Kreisberg GA, Diamant S, Diamant YZ, Atlas D: Raised levels of an endogenous nonadrenergic substance in the serum of pregnancy-induced hypertension patients. *Israel J Med Sci* 23:1194–1197, 1987
5. Coupry I, Atlas D, Podevin R-A, Uzielli I, Parini A: Imidazoline-guanidinium receptive site in renal proximal tubule: Asymetric distribution, regulation by cations and interaction with an endogenous clonidine displacing substance. *J Pharmacol Exp Ther* 252:293–299, 1989
6. Ernsberger P, Feinland G, Meeley MP, Reis DJ: Characterization and visualization of clonidine-sensitive imidazole sites in rat kidney which recognize clonidine-displacing substance. *Am J Hypertens* 3:90–97, 1990
7. Kierszenbaum F, Wirth JJ, McCann PP, Sjoerdsma A: Arginine decarboxylase inhibitors reduce the capacity of *Trypanosoma cruzi* to infect and multiply in mammalian host cells. *Proc Natl Acad Sci USA* 84:4278–4282, 1987
8. Morrissey J, McCracken R, Kaneto H, Vehaskari M, Montani D, Klahr S: Location of an inducible nitric oxide synthase mRNA in the normal kidney. *Kidney Int* 45:998–1005, 1994
9. Bradford M: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein dye binding. *Anal Biochem* 72:248–259, 1976
10. Brunskill NJ, Morrissey JJ, Klahr S: Association and interactions of GTP-binding proteins with the rat medullary H^+-ATPase. *Am J Physiol* 267:F944–F951, 1994
11. Salter M, Knowles RG, Moncada S: Widespread tissue distribution, species distribution and changes in activity of Ca^{2+}-dependent and Ca^{2+}-independent nitric oxide synthases. *FEBS Lett* 291:145–149, 1991
12. Weiner CP, Lizasoain I, Baylis SA, Knowles RG, Charles IG, Moncada S: Induction of calcium-dependent nitric oxide synthases by sex hormones. *Proc Natl Acad Sci USA* 91:5212–5216, 1994
13. Moore RC, Boyle SM: Nucleotide sequence and analysis of the *speA* gene encoding biosynthetic arginine decarboxylase in *Escherichia coli*. *J Bacteriol* 172:4631–4640, 1990
14. Bell E, Malmberg RL: Analysis of a cDNA encoding arginine decarboxylase from oat reveals similarity to *Escherichia coli* arginine decarboxylase and evidence of protein processing. *Mol Gen Genet* 224:431–436, 1990
15. Stim KP, Bennett GN: Nucleotide sequence of the *adi* gene, which encodes the biodegradative acid-induced arginine decarboxylase of *Escherichia coli*. *J Bacteriol* 175:1221–1234, 1993
16. Hibbs JB Jr, Vavrin Z, Taintor RR: L-arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. *J Immunol* 138:550–565, 1987
17. Moncada S, Higgs A: The L-arginine-nitric oxide pathway. *N Engl J Med* 329:2002–2012, 1993
18. Limon I, Coupry I, Lanier SM, Parini A: Purification and characterization of mitochondrial imidazoline-guanidinium receptive site from rabbit kidney. *J Biol Chem* 267:21645–21649, 1992
19. Bidet M, Poujeol P, Parini A: Effect of imidazoline on Na^+ transport and intracellular pH in renal proximal tubule cells. *Biochim Biophys Acta* 1024:173–178, 1990
20. Nord EP, Howard MJ, Hafezi A, Moradeshagi P, Vaystub S, Isel PA: Alpha-2 adrenergic agonists stimulate Na^+-H^+ antiport activity in the rabbit renal proximal tubule. *J Clin Invest* 80:1755–1762, 1987