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Vasodilatory N-Methyl-D-Aspartate Receptors Are Constitutively Expressed in Rat Kidney

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Abstract. N-methyl-D-aspartate receptor (NMDA-R) is an amino acid receptor and membrane calcium channel. NMDA-R is activated by binding of coagonists, L-glutamine and L-glycine. In the brain, calcium entry via NMDA-R activates type I nitric oxide synthase (NOS I). The kidney also contains NOS I and vasodilates in response to L-glycine. In this study, NMDA-R mRNA was demonstrated in rat kidney cortex by reverse transcriptase–PCR and cDNA sequencing. NMDA-R protein was demonstrated in kidney cortex by immunoblotting. To study the functional role of renal NMDA-R, renal hemodynamic effects of NMDA-R inhibition were assessed in rats using a blocker of the NMDA calcium channel (75 mg/kg MK-801 intraperitoneally) or an inhibitor of glycine binding to NMDA-R (30 mg/kg 5,7-dichlorokynurenic acid intraperitoneally). Renal blood flow was measured by perivascular pulse Doppler. GFR was measured by 3H-inulin clearance. Measurements were made before and during glycine infusion. Both NMDA-R antagonists caused renal vasoconstriction and attenuated the renal vasodilatory response to glycine infusion. These effects were not mediated by the renal nerves. The glycine response was not inhibited by aortic snare used to mimic the effects of NMDA-R inhibitors on basal renal blood flow. NMDA-R are expressed in kidney cortex, where they exert a tonic vasodilatory influence and may account for the vasodilatory response to glycine infusion.

The N-methyl-D-aspartate receptor (NMDA-R) is a heterotetrameric amino acid receptor that functions as a membrane calcium channel. The NMDA-R has been studied extensively in neural tissue where binding of L-glycine and L-glutamate leads to channel opening and calcium influx (1). This, in turn, may lead to neuronal cell death from calcium toxicity or to activation of calcium-dependent type I nitric oxide synthase (NOS-I) (2,3). Numerous functions have been ascribed to NO formed in the brain as a result of NMDA-R activation. Among these functions are the suppression of peripheral sympathetic reflex discharges (4,5) and cerebral vasodilatation (6). Little is known about NMDA-R outside of the nervous system, although NMDA-R has been recently reported to stimulate NO release from aortic rings (7).

Systemic infusion of L-glycine causes renal vasodilation, which is prevented by NOS blockade (8). NOS-I colocalizes with NMDA-R in the brain and is prominently expressed in macula densa cells of the kidney (9), where it exerts a tonic vasodilatory effect on the glomerular microvasculature by modulating tubuloglomerular feedback (10). Therefore, we tested for the presence of NMDA-R in the kidney and examined the effects of NMDA-R antagonists on the renal hemodynamic response to glycine infusion.

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Methods

Animal experiments described herein were performed in male Wistar rats in accord with the National Institutes of Health guidelines.

Messenger RNA for NMDA Receptor

Total RNA was isolated from kidney cortex with Qiagen RNeasy Mini kit (Qiagen, Chatsworth, Ca) and one 95-μg was used for cDNA synthesis. Reverse Transcriptase–PCR (RT-PCR) was performed with a kit (10904–018, Life Technologies, Gaithersburg, MD). A primer pair was chosen corresponding to a sequence of the NMDA NR1 subunit, which is common to all known splice variants (exon 12 to exon 17; bp 1715 to 2310; Gene Bank accession no. x63325). The primer sequences: sense, 5'-TTTGGCACACAGGAGCGGGTAAAC-3' and antisense, 5'GCGTAGATGAACTTGTCTGAGGGG-3'.

Sequencing of cDNA

The PCR band was purified with a Qiagen MinElute Gel Extraction Kit. The sequence was read with a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) at the University of California San Diego Cancer Center DNA Sequencing Service.

Immunoblotting for NMDA-R Protein

One milligram of lysate protein from kidney cortex was used for immunoprecipitation as described (11), using a commercial monoclonal antibody against a fusion protein from amino acids 1 to 564 of rat NMDA-R NR1 (Affinity Bioreagents, Golden, CO). One milligram of cerebral cortex protein served as positive control. Immunoprecipitated proteins were separated in a 10% sodium dodecyl sulfate–polyacrylamide gel as described (12). After blotting and blocking with 5% dry milk, the

membrane was incubated in 1:300 antibody for 1 h and then with 1:2000 horseradish peroxidase–labeled anti-mouse IgG (Santa Cruz Biotecnologies, CA), developed with ECL plus (Amersham Pharmacia Biotech, Piscataway, NJ), and digitized with an optical scanner.

Immunostaining for NMDA-R

Kidneys were perfused *in situ* and fixed with 4% paraformaldehyde, and 5-μm slices were cut from paraffin-embedded tissue. After performing heat-induced antigen retrieval, slides were incubated with primary antibody (anti-NMDAR1 monoclonal antibody; Chemicon, Temecula, CA) diluted 1:200 in phosphate-buffered saline (1 h at room temperature). The sections were washed in phosphate-buffered saline and incubated with biotinylated anti-mouse IgG (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) diluted 1:250 for 1 h and then in Vectastain ABC reagent diluted 1:250 for 1 h. Signal was then amplified with the TSA system (NEN, Boston, MA). Tissue-bound peroxidase was visualized by using 0.05% 3,39-diaminobenzidine and 0.003% hydrogen peroxide in 0.1 M Tris-HCl buffer (pH 7.6) for 5 to 10 min under visual control.

NMDA-R Antagonists and Renal Function

Experiments were performed under Inactin anesthesia (Andrew Lockwood Assoc., Ann Arbor, MI) with surgical preparation and monitoring as described (13). Two NMDA-R inhibitors were tested. These included an NMDA ion channel blocker (MK-801, 75 mg/kg intraperitoneally) and a glycine antagonist (5,7-dichlorokynurenic acid, 30 mg/kg intraperitoneally). Inhibitors were administered in dimethyl sulfoxide at the onset of surgery. To control for possible effects mediated through increases in renal nerve activity, prior renal denervation was performed in some experiments (14). Additional experiments were performed with suprarenal aortic snare to control for the effect of baseline renal blood flow (RBF) on the response to glycine.

RBF was measured in left renal artery by pulse Doppler flowmetry (Transonics, Ithaca, NY). GFR was measured by 3H-inulin clearance. Ringers' saline was infused at 2 ml/h throughout equilibration and control periods. Glycine infusion was added (2.66 M at 1.5 ml/h) as described (15). Clearances were based on 30-min urine collections. RBF, systemic arterial BP, and heart rate were averaged over the same 30 min.

Statistical Analyses

Comparisons were by ANOVA with Tukey *post hoc* testing. Basal RBF was also treated as a covariate for effects of drugs on the glycine response.

Results

NMDA Receptor mRNA and Sequencing of RT-PCR Product

A PCR band of the appropriate size was obtained in each of six rats. By sequencing, this product was confirmed as identical to the intended region of the NR1 gene (Figure 1).

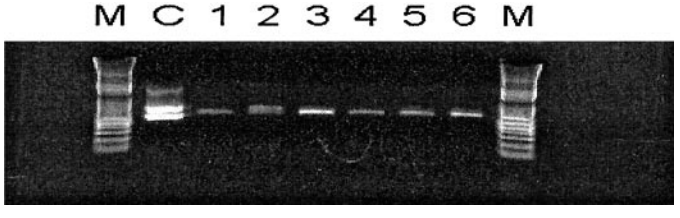

Figure 1. N-methyl-D-aspartate (NMDA) NR1 PCR. M, marker; C, control RNA from the kit. Lanes 1 to 6, RNA from six rats.

Immunoblot Analyses

As expected, Western blot analyses revealed NMDA-R NR1 subunit protein in brain. The protein was also clearly expressed in rat kidney (Figure 2).

Immunostain Analyses

Staining for NMDA-R appeared in the renal cortex, primarily in basolateral proximal tubules. No staining was apparent in the medulla (Figure 3).

Renal Hemodynamics

Neither drug caused systemic hypertension or increased heart rate (Table 1). There was no tendency for renal denervation to alter baseline hemodynamics or the response to NMDA-R inhibitors. Therefore, data from innervated and denervated kidneys were pooled for analysis.

Both NMDA-R inhibitors significantly reduced basal RBF and tended to reduce GFR (Figures 4 to 5). In control animals, RBF increased by 36 $\pm$ 4% during glycine infusion. Each NMDA-R inhibitor significantly attenuated the increase in RBF during glycine. Two approaches were employed to confirm that inhibition of the glycine response occurred independently of the lower baseline RBF. First, NMDA-R inhibition remained a significant predictor of the glycine response when baseline RBF was treated as a statistical covariate. Second, the glycine response remained intact when baseline RBF was adjusted by aortic snare, which required a 36 $\pm$ 1 mmHg reduction in renal perfusion pressure.

In control animals, GFR increased by 24 $\pm$ 12% during glycine. In contrast, there was no tendency for GFR to increase during glycine in rats treated with either NMDA-R inhibitor. By intergroup comparison with Tukey correction for multiple comparisons, GFR during glycine was significantly less among animals receiving either NMDA-R inhibitor than among control animals (Figure 5).

Discussion

To our knowledge, this is the first report of functioning NMDA-R in the kidney. These studies were motivated by the link between NMDA-R and type I NOS in the brain (1–6), the influence of macula densa NOS I on renal hemodynamics (9–10), and the well-recognized, but poorly understood, renal vasodilatory response to systemic infusion of glycine (15). The data document the presence of NMDA-R mRNA and protein in rat kidney cortex. These data also confirm a role for renal NMDA-R in maintaining normal renal function and suggest

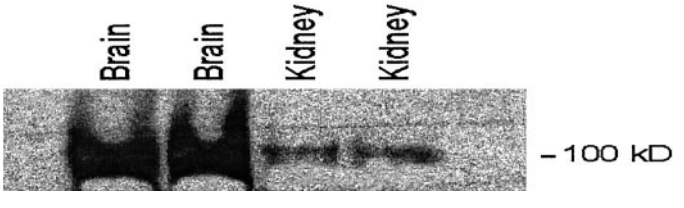

Figure 2. Western blots generated after immunoprecipitation of lysates from rat brain and kidney using a monoclonal antibody against the NR1 subunit of the NMDA-R. Data are from two rats.

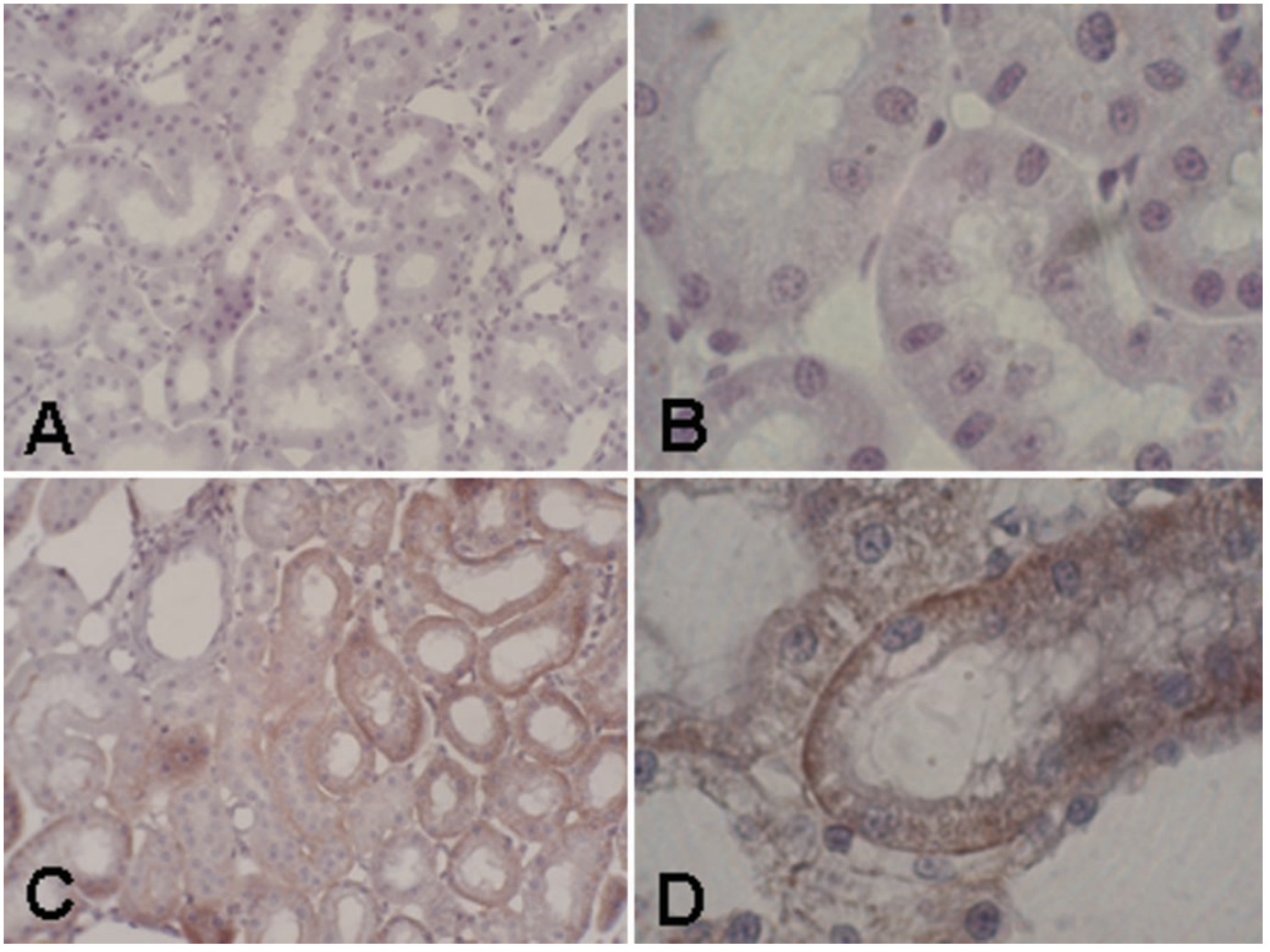

Figure 3. Immunostaining of kidney cortex for NMDA-R NR1 showing staining in proximal tubular epithelium. A and B, control (secondary antibody only). C and D, anti-NMDA receptor antibody.

Table 1. Renal perfusion pressure and heart rate[a]

Group	Renal Perfusion Pressure (mmHg)		Heart Rate (bpm)	
	Baseline	Glycine	Baseline	Glycine
Control ($n = 9$)	110 ± 3	107 ± 4	423 ± 17	420 ± 13
NMDA-R channel blocker ($n = 9$)	95 ± 5[c]	94 ± 5[c]	336 ± 12[c]	342 ± 10[c]
Glycine antagonist ($n = 10$)	112 ± 4	107 ± 4	399 ± 8	408 ± 9
Aortic snare ($n = 4$)	76 ± 2[b]	79 ± 2[b]		

[a] Four experiments in each group, except aortic snare, were studied after renal denervation. Denervation had no effect, and the data are pooled here.
[b] $P < 0.05$ *versus* all other groups.
[c] $P < 0.05$ vs. control and glycine antagonist.

that activation of NMDA-R mediates the renal response to glycine infusion. The co-agonist requirement of the NMDA-R for glycine (1) gives credibility to the latter suggestion as does the ability of systemically administered NMDA-R inhibitors to selectively alter renal hemodynamics. Furthermore, NMDA-R inhibitors attenuated the subsequent glycine response independently of their effects on baseline RBF.

Renal sympathetic nerve activity can cause renal vasocon-

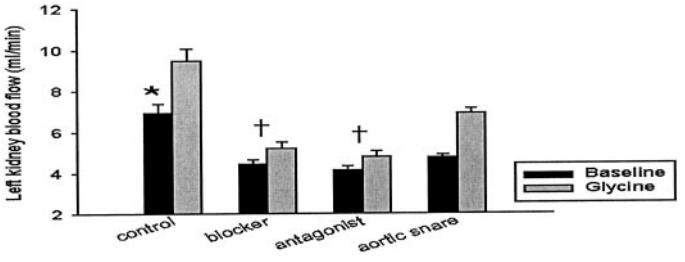

Figure 4. Effects of glycine on left kidney blood flow in rats pretreated with NMDA channel blocker or NMDA competitive glycine antagonist. Aortic snare was employed to mimic the effect of NMDA antagonists on baseline blood flow. *$P < 0.05$ *versus* all other baselines. †$P < 0.05$ *versus* control for group-×-glycine crossterm by two-way ANOVA.

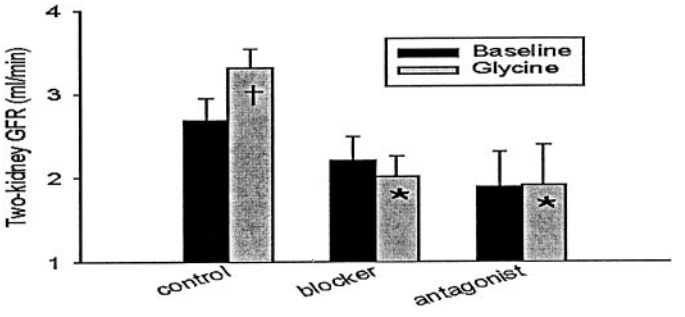

Figure 5. Effects of glycine on two-kidney GFR in rats pretreated with NMDA channel blocker or NMDA competitive glycine antagonist.*$P < 0.05$ *versus* control rat during glycine. †$P < 0.05$ *versus* baseline.

striction (16), and centrally active NMDA antagonists could increase peripheral sympathetic activity. However, available data on the NMDA-R antagonists suggest that these drugs do not cross the blood brain barrier (4), and there was no evidence for generalized sympathetic overactivity in these experiments. Also, renal denervation did not alter the renal response to either NMDA-R inhibitor.

It is clear by immunostaining that NMDA-R reside in proximal tubules, where they are positioned to account for the effects of NMDA-R antagonists on basal RBF and GFR by enhancing tubular reabsorption and reducing the macula densa signal for tubuloglomerular feedback. Recent micropuncture data from our lab suggest that increased tubular reabsorption accounts for about half of the vasodilatory response to glycine infusion (17). However, these preliminary findings do not exclude the existence of NMDA-R in other kidney cells that may be revealed by future detailed study.

Although these experiments were inspired by knowledge that NOS-I is normally expressed in the kidney and affects glomerular hemodynamics, the present experiments were not designed to confirm whether the hemodynamic effects of renal NMDA-R are mediated through NOS. Furthermore, linkage to NOS-I seems unlikely in the arcuate/interlobular artery. Future studies will be required to understand the downstream effects of NMDA-R.

Acknowledgments

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