NMDA Receptors as Potential Therapeutic Targets in Diabetic Nephropathy: Increased Renal NMDA Receptor Subunit Expression in Akita Mice and Reduced Nephropathy Following Sustained Treatment With Memantine or MK-801

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More than 40% of end-stage renal disease in the U.S. can be attributed to diabetes (1). The pathophysiology of diabetic nephropathy is complex (2–5), and the reasons why only a subset of patients with diabetes experience renal complications are not well understood. Current mainstay therapies are based on glycemic and blood pressure control and renoprotective inhibition of renin-angiotensin signaling systems (4,5). Although these therapies can slow the progression of diabetic nephropathy, many patients will nonetheless progress to end-stage renal disease (4,5).

N-methyl-D-aspartate (NMDA) receptors are a class of cation-selective ionotropic receptors with a high intrinsic Ca²⁺ permeability (6,7). These receptors are heterotetrameric and are assembled from multiple subunits (NR1, NR2A, NR2B, NR2C, NR2D, NR3A, and NR3B) encoded by seven different genes. Functional NMDA receptors require two NR1 subunits that contain binding sites for the coagonist glycine. They also require either two NR2 subunits, which bind multiple endogenous diacidic agonists, or one NR2 and one NR3 subunit (8). NMDA receptors were first characterized in the central nervous system (CNS) but are also expressed in peripheral organs, including the kidney. There is evidence that renal NMDA receptors play a role in the regulation of blood flow, glomerular filtration, proximal tubule reabsorption, and urine concentration in the collecting duct (9–14). NMDA receptors can be
activated by several endogenous diacidic molecules, including t-glutamate, t-aspartate, t-homocysteic acid (HCA), t-quinolinic acid, and guanidinosuccinic acid, although t-glutamate and t-aspartate are relatively weak agonists for podocyte NMDA receptors (10,12). In the CNS, sustained activation of neuronal NMDA receptors can induce a form of neurodegeneration known as excitotoxicity (15). Sustained exposure of mouse podocytes to NMDA or HCA evokes a similar phenomenon characterized by increased Ca2+ influx, oxidative stress, altered expression of slit diaphragm proteins, and apoptosis (11,16,17).

Renal NMDA receptor NR1 subunit expression is upregulated in the Akita mouse model of type 1 diabetes (18). In addition, elevated plasma t-homocysteine often occurs in patients with type 1 or type 2 diabetes and is significantly correlated with renal complications (19–21). Moreover, genes regulating t-homocysteine metabolism are possible susceptibility loci for diabetic nephropathy (22–24). t-Homocysteine spontaneously oxidizes to the NMDA agonist HCA, and albuminuria and glomerular damage in mice with elevated plasma homocysteine can be prevented by treatment with NMDA antagonists (16). On the basis of these previous studies, we hypothesized that sustained hyperactivation of NMDA receptors drives some of the nephropathy in type 1 diabetes. Consistent with this hypothesis, we show that sustained treatment with either of two structurally dissimilar NMDA receptor antagonists, MK-801 or memantine, reduced nephropathy in the Akita mouse model of type 1 diabetes. Memantine is widely used clinically for the treatment of Alzheimer disease and is currently undergoing a number of clinical investigations primarily for neurological disorders (25–28).

**RESEARCH DESIGN AND METHODS**

**Cell Culture Protocols**

A mouse podocyte cell line (MPC-5) was propagated and differentiated as described previously (10,11). Primary mesangial cells were prepared using standard methods (29). Briefly, decapsulated glomeruli from 100–150-g Sprague-Dawley rats (Charles River Laboratories) were isolated by using a sieving procedure (30,31). The glomeruli were then maintained in RPMI medium containing 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C for 2 weeks. At that time, cells that had migrated out of the glomeruli were ~80% confluent. The majority of those cells had an elongated spindle shape as expected for mesangial cells, and they expressed the mesangial cell marker α-smooth muscle actin (Supplementary Fig. 1). In high-glucose experiments, cultured cells were exposed for 24 h to RPMI medium supplemented with 16 mmol/L glucose (for a final glucose concentration of 25 mmol/L) or with 16 mmol/L mannitol (a metabolically inert osmotic control).

**RNA Isolation, RT-PCR, and Immunoblot Analysis**

Total RNA from mouse renal cortex, mouse podocytes, or rat mesangial cells was isolated by using the QIAshredder and RNeasy Mini Kit (QIAGEN). Aliquots of total RNA were reverse transcribed by using the ThermoScript RT-PCR System (Invitrogen). The PCR primers were as follows:

**NR1:** forward ACTCCCAAGACGACTTCAC, reverse GTA GACGGGACATCATCTCAA
**NR2A:** forward AGACCTTTAGCAGGCCCTTCTC, reverse CTTCTGCTCTTCCAGACCC
**NR2B:** forward CCGCAGCCTATTGAGCACC, reverse ATCCATGTGTAACCTGATCC
**NR2C:** forward GCAGAACCTCTTGAGCTTGC, reverse CACAGCAACACTCCTAGT
**NR2D:** forward CAGCTCGAGGTCACTTGGTTA, reverse GGAATCCTGCACTGACACTA
**NR3A:** forward CAGAGGATGAGCCAGAGTC, reverse CTTCCACACGGTTCAGGGTTT
**β-actin:** forward AGCCATGTCAGTAGACCAC, reverse CTCTCAGCTGTGGTGGTAGAA

Cycling parameters were 94°C for 5 min; then 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 10 min. Immunoblot (31–33) was carried out by using a panel of primary antibodies selective for various NMDA receptor subunits (NMDA Receptor Antibody Explorer Kit, Alomone Labs).

**Mouse Models of Type 1 Diabetes**

All animal experiments were conducted according to protocols approved by the Institutional Animal Care and Use Committee of the University of Houston. Male DBA/2J mice (strain 000671) and D2.B6-Ins2Akita/MatbJ mice (strain 007562) from Jackson Laboratory were given free access to food and water during the time of the experiments. These Akita mice have been backcrossed onto the DBA/2J background for >12 generations. In a subset of experiments, 10-week-old DBA/2J mice were administered five injections of streptozotocin (STZ) (40 mg/kg i.p. in 0.1 mL of 0.1 mol/L sodium citrate buffer [pH 4.5]) or with sodium citrate vehicle at 24-h intervals. Treatment with NMDA antagonists began 15 weeks after the completion of STZ treatment.

**Drug Delivery**

NMDA antagonists or saline vehicle were delivered continuously using ALZET 1004 osmotic minipumps (Durect Corporation) implanted subcutaneously in the midscapular area under isoflurane anesthesia. These pumps contained memantine HCl, MK-801, or 0.9% saline vehicle. Memantine was delivered at a dose of 0.2 mg/kg/day, whereas MK-801 was delivered at 0.5 mg/kg/day.

**Assessment of Diabetic Nephropathy**

Before initiation of treatment with NMDA antagonists or vehicle, 8-week-old mice were weighed, and mean arterial blood pressure was measured by tail cuff plethysmography for the MK-801 experiments (Kent Scientific). Mice were then placed in metabolic cages for 24 h, and urine was collected and albumin quantified (Albuwell M ELISA kit). Animals were weighed every 7 days. After 28 days of drug or vehicle treatment, mean arterial blood pressure...
was measured again, a final 24-h urine sample was collected for albumin analysis, and animals were killed by CO₂ inhalation. Kidneys were excised, and a portion of renal cortex was reserved for biochemical analysis. The rest of the kidney was used for histological and ultrastructural analysis.

**Histology, Immunohistochemistry, and Electron Microscopy**

To assess surface expression of NMDA receptors, podocytes cultured in normal or high-glucose medium were fixed in 4% paraformaldehyde, rinsed, and blocked. Primary antibodies were rabbit anti-NR1 (AGC-001 1:100; Alomone Labs) or rabbit anti-NR2A (AGC-002 1:100; Alomone Labs) for 24 h at 4°C. These antibodies recognize epitopes on extracellular loops of NMDA receptor subunits. Cells were not permeabilized before exposure to primary antibodies. The secondary antibody was Alexa Fluor 488-conjugated anti-rabbit IgG (Invitrogen). The surface expression of NR1 and NR2A were compared with Alexa Fluor 594-conjugated wheat germ agglutinin (Invitrogen), a plasma membrane marker. Images were collected on an Olympus FV1000 confocal microscope with a Plan Apo N 60×1.42NA objective. Laser intensity and detector sensitivities were held constant. Fluorescence intensity for NR1 and NR2A was quantified using Photoshop software. For in vivo experiments, one of the kidneys was immersion fixed in 4% paraformaldehyde and embedded in paraaffin, and 2.5-μm sections were stained with periodic acid Schiff (PAS). Some paraffin sections were immunostained with antibodies against various NMDA receptor subunits (anti-NR1 from Abcam, anti-NR2A and anti-NR2B from Thermo Fisher Scientific, and anti-NR2C from Alomone Labs). Antigens were unmasked, and slides were quenched, blocked, and incubated with primary antibody overnight at 4°C. Immunostaining was produced by using the VECTASTAIN Elite ABC HRP Kit and the DAB Peroxidase (HRP) Substrate Kit (Vector Laboratories). Mesangial matrix expansion in PAS-stained sections was calculated from fractional volume of the mesangium on all the glomeruli in a section using Adobe Creative Cloud Photoshop CC on PAS-stained slides (34). The percentage of mesangial matrix occupying each glomerulus was rated on the following scale: 0–25% = 0, 26–50% = 1, 51–75% = 2, and 76.0–100% = 3. For each mouse, glomerular matrix expansion was evaluated in a minimum of 15 glomeruli and averaged to obtain a value for that animal. Statistical analysis was carried out on the mean values from each group of animals, with four mice per group.

The other kidney was fixed by immersion in 3% glutaraldehyde/3% paraformaldehyde in 0.1 mol/L sodium cacodylate buffer (pH 7.3) for transmission and scanning electron microscopy (EM). For transmission EM, the samples were postfixed with 1% cacodylate-buffered osmium tetroxide for 30 min and stained with 1% uranyl acetate. The samples were dehydrated, embedded in LX 112 resin, and allowed to polymerize at 60°C for 3 days. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a JEM-1010 transmission electron microscope (JEOL USA, Inc.) at an accelerating voltage of 80 kV. The thickness of the glomerular basement membrane (GBM) was measured using Adobe Creative Cloud Photoshop CC software. For each mouse, three to four different points of GBM from the same glomerulus and from a total of 15 glomeruli were measured to calculate a mean GBM thickness for that animal. We also measured foot process width for every foot process in a capillary loop through multiple glomeruli, with a mean calculated independently for each animal. Statistical analyses of GBM thickness and foot process width was then carried out in groups of four mice each. For scanning EM, fixed samples were washed with 0.1 mol/L sodium cacodylate buffer (pH 7.3), postfixed with 1% cacodylate-buffered osmium tetroxide, and washed in 0.1 mol/L sodium cacodylate buffer and then in distilled water. The samples were then treated sequentially with filtered 1% aqueous tannic acid, distilled water, filtered 1% aqueous uranyl acetate, and distilled water. Samples were dehydrated, transferred through a graded series of hexamethyldisilazane, and then air dried overnight. Samples were coated under vacuum with platinum alloy to a thickness of 25 nm and immediately flash carbon coated under vacuum. Samples were imaged in a JSM-5910 scanning electron microscope (JEOL USA, Inc.) at an accelerating voltage of 5 kV. Images were quantified by measuring the number of foot processes per micron in numerous capillary tufts in each animal. A mean was then calculated independently for each animal. Statistical analyses of the number of foot processes per micron was then carried out in groups of three to four mice each.

**Statistical Analysis**

Data from immunoblot and RT-PCR, based on triplicate measures, are shown graphically as mean ± SD and analyzed using Student unpaired t test, with α = 0.05. Fluorescence intensity in confocal microscopy was also analyzed by unpaired t test. Data from animal experiments are presented graphically as mean ± SEM. Quantitative data on renal phenotypes were analyzed using weighted-measures two-way ANOVA. In these analyses, the two independent variables were genotype (Akita vs. DBA/2J) and drug treatment (NMDA antagonist vs. saline vehicle). A statistically positive result was inferred when F values for the interaction between drug effects and genotype, or for drug effects alone, indicated P < 0.05. All statistical analyses were carried out using the online computational packages found at http://vassarstats.net.

**RESULTS**

**Increased NMDA Receptor Abundance in Glomerular Cells Cultured in High Glucose**

Immortalized podocytes (33,35,36) were cultured for 24 h in a medium containing 9 mmol/L glucose supplemented with 16 mmol/L mannitol or in a medium containing 25 mmol/L glucose. We focused on NR1 and NR2 subunits required to form functional receptors that respond
to NMDA and other diacidic agonists. By using a semi-quantitative RT-PCR procedure, we observed that 24-h exposure to high glucose increased the apparent abundance of NR1, NR2A, NR2B, and NR2C subunit transcripts (Fig. 1A and B). The same pattern was observed using immunoblot analysis (i.e., increased abundance of NR1, NR2A, NR2B, and NR2C subunits but not NR2D subunits) (Fig. 1C and D). Increases in NMDA subunit abundance also occurred in primary rat mesangial cells after 24-h exposure to high glucose. In mesangial cells, we observed increased abundance of transcripts encoding NR1, NR2B, and NR2C but not NR2A or NR2D subunits (Fig. 2A and B). By immunoblot, we observed increased abundance of those same subunits (Fig. 2C and D). Thus, elevated glucose is sufficient to increase the expression of multiple NR1 and NR2 subunits in two different glomerular cell types. Of note, there is a corresponding increase in the expression of NR1 and NR2A subunits at the surface of podocytes cultured in high glucose, indicating that these extra receptors are likely to be functional (Supplementary Fig. 2).

Increased NMDA Receptor Subunit Expression in the Akita Mouse Model of Type 1 Diabetes

We used male Akita mice (D2.B6-Ins2<sup>Akita</sup>/MatbJ) heterozygous for the C96Y mutation in the Ins2 gene (37). The control mice were wild-type DBA/2J. We examined expression of NMDA receptor subunits in renal cortex from 12-week-old animals. By using a semiquantitative RT-PCR procedure, we obtained evidence for an increased abundance of transcripts encoding NR1, NR2A, and NR2C but not NR2B or NR2D subunits (Fig. 3A and B). This pattern was also observed by immunoblot analyses of renal cortex of these 12-week-old animals (Fig. 3C and D). NR1, NR2A, and NR2C subunits are already increased by 7 weeks of age (Supplementary Fig. 3), which is well before the full manifestation of nephropathy in Akita mice. The increase in NMDA receptor subunits is seen in glomeruli and in tubules (Fig. 4). Using immunohistochemistry, we observed marked increases in the abundance of NR1, NR2A, and NR2C subunits in tubules (Fig. 4A). Within glomeruli, we observed increases in NR1, NR2A, and NR2C subunits in Akita mice, but the signal appeared to be intense in only some of the cells (Fig. 4B). Signal intensity is quantified in Fig. 4C, but these should not be interpreted as a comparison of the abundance of different subunits analyzed with different antibodies.

Blockade of NMDA Receptors Reduces Progression of Diabetic Nephropathy in Akita Mice

Sustained activation of NMDA receptors can drive glomerulosclerosis in mice and rats (16,17). Increased numbers of
receptors could lead to excessive NMDA receptor activation on cells even if endogenous ligands are not changed. However, there is evidence that diabetes causes metabolic changes, leading to increased levels of circulating agonists in humans and Akita mice (19–22,38–42). To examine whether sustained NMDA receptor activation contributes to the progression of diabetic nephropathy, we implanted osmotic minipumps containing the NMDA antagonist MK-801 or saline subcutaneously into Akita mice and DBA/2J controls at 8 weeks of age, an age at which there are modest increases in urine albumin excretion. The pumps delivered a dose of 0.5 mg/kg/day of MK-801 continuously for 28 days. We observed that saline- and MK-801–treated Akita mice do not gain weight at the same rate as DBA/2J controls (Fig. 5A). Reduced weight was actually greater in MK-801–treated Akita mice than in the saline group. The reason why sustained MK-801 attenuates normal weight gain is not known. In any case, this pattern suggests that MK-801 does not alleviate the primary metabolic consequences of type 1 diabetes. In addition, 4 weeks of MK-801 treatment had no effect on blood glucose levels (data not shown) or blood pressure (Supplementary Fig. 4).

As with previous reports (37), we observed markedly increased 24-h urine albumin excretion in 12-week-old Akita mice compared with DBA/2J controls (Fig. 5B). This was attenuated in mice treated with MK-801 compared with mice treated with saline. These data were analyzed using two-way ANOVA. We observed a significant effect of genotype (P < 0.0001) and a significant interaction effect between drug treatment and genotype (P < 0.05), indicating that MK-801 reduces renal manifestations of diabetes. MK-801 also reduced mesangial expansion in Akita mice (Fig. 5C and D). Mesangial expansion is not severe in Akita mice on a DBA/2J background (37) but is nonetheless reduced in Akita mice that receive MK-801 compared with saline, and the effect was significant based on two-way ANOVA (P < 0.05). We also observed marked

Figure 2—Exposure to high glucose (HG) increases expression of NMDA receptor subunits in primary cultures of rat mesangial cells. A: Representative results of RT-PCR showing significantly increased abundance of transcripts encoding NR1, NR2B, and NR2C subunits but not of NR2A or NR2D in cells cultured for 24 h in HG medium compared with cells cultured in normal glucose (control [Con]). B: Densitometric analysis of three repetitions of the experiments shown in A. C: Immunoblot analysis showing increased abundance of NMDA receptor subunits in primary cultures of rat mesangial cells cultured in HG. D: Densitometric analysis of three repetitions of the experiments shown in C. Data are mean ± SD. *P < 0.05 by Student unpaired t test.
foot process effacement and thickening of the GBM in Akita mice by 12 weeks of age (Fig. 5E–G). Ultrastructure was improved in Akita mice treated with MK-801 (Fig. 5E–G). There was a significant (P < 0.05) interaction effect between the effects of MK-801 and genotype on GBM thickness and foot process width, again indicating a therapeutic effect of the drug. MK-801 had no discernible effect on glomerular ultrastructure in DBA/2J controls. The effect of MK-801 was also seen with scanning EM (Fig. 6).

Thus, we observed marked foot process flattening and disorganization in Akita mice treated with saline (Fig. 6A) and a reduction in the number of foot processes per micron measured along the long axis of a capillary tuft (Fig. 6B). There were still some abnormalities in Akita mice that received MK-801, but overall structure is closer to normal, and there was a significant (P < 0.05) improvement in the number of foot processes per micron. In general, effects of MK-801 on renal structure and ultrastructure were greater than effects on albumin excretion. This was also seen in the low-dose STZ model of type 1 diabetes in DBA/2J mice (Supplementary Fig. 5).

MK-801 is a strong NMDA receptor antagonist that produces profound cognitive and behavioral effects (25,43). By contrast, memantine is a structurally distinct NMDA receptor antagonist that spares a basal level of NMDA receptor–mediated synaptic transmission while reducing excitotoxicity (25,43) and is in widespread clinical use (26–28). Memantine (0.2 mg/kg/day) or saline were applied using osmotic minipumps starting at 8 weeks of age. As with MK-801, we observed that memantine did not alleviate lower growth rates (Fig. 7A) but reduced 24-h urine albumin excretion in Akita mice (Fig. 7B). The interaction between drug and genotype on albumin excretion was significant (P < 0.05). Memantine also

![Figure 3](image-url)
caused a trend toward reduction in mesangial matrix expansion \((P = 0.0743)\) (Fig. 7C and D) in Akita mice. Memantine improved glomerular ultrastructure in Akita mice (Fig. 7E–G). The reduction in the mean foot process width (effacement) revealed a significant \((P < 0.05)\) interaction effect between memantine treatment and genotype, indicating a therapeutic effect of the drug (Fig. 7G).

**DISCUSSION**

We have tested the hypothesis that renal NMDA receptors contribute to the progression of diabetic nephropathy in the Akita mouse model of type 1 diabetes. We observed that elevated glucose causes marked increases in NMDA receptor abundance in two glomerular cell types implicated in diabetic nephropathy, that there are marked increases in the abundance of NMDA receptor subunits throughout the kidney of Akita mice, and that sustained inhibition of NMDA receptors in vivo using two structurally dissimilar antagonists reduces urine albumin excretion. In addition, MK-801 and memantine improved ultrastructural changes that occur in glomeruli of Akita mice during the early stages of diabetic nephropathy. A surprising observation in this study is that all known NMDA receptor subunits and their transcripts can be detected in an immortalized podocyte cell line (MPC-5) and in primary cultures enriched in rat mesangial cells. High glucose caused robust increases in NR1 subunits in both cell types. These cell types differed in terms of which NR2 subunits were upregulated by high glucose. Nevertheless, the biochemical pattern in both cell types predicts marked increases in functional responses to agonists. The overall pattern in podocytes is similar to that seen in the renal cortex of Akita mice, but of note, the biochemical signal in kidney cortex extracts is almost certainly dominated by increases in the expression of NMDA receptor subunits in tubules. Increased NMDA receptor abundance was already present by 7 weeks of age in Akita mice, a time at which nephropathy cannot be discerned with light microscopic histological methods and urine albumin excretion is only slightly increased. This suggests that elevated renal NMDA receptors drive the glomerular pathology and are not simply a response to it.

Previous studies have shown statistically significant correlations between serum L-homocysteine and the subsequent appearance of microalbuminuria in patients with diabetes (19–22). However, the marked induction of renal NMDA receptors in mouse models of diabetes means that an excessive number of these receptors could be activated even if endogenous agonists (e.g., HCA and L-quinolinic acid) are present at normal levels. Any increase in circulating NMDA agonists, which can occur as renal failure progresses (38–42), would cause even more NMDA receptors to become active.

Although many studies of early-stage diabetic nephropathy have focused on glomerular and vascular elements, diabetes produces effects throughout the kidney as a result of hyperfiltration, proximal tubule hyperreabsorption, alterations in sodium delivery to distal tubules, and marked increases in tubular flow rates (44,45). Indeed, in diabetes, there are sustained changes in overall regulatory set points, resulting in marked changes in Na⁺ dynamics in diabetic tubules (44,45). An intriguing question is whether NMDA receptor activation leads to sustained changes in expression of other transport proteins in a wide range of renal cells in a manner reminiscent of biochemical changes in neurons that occur during NMDA receptor–mediated synaptic plasticity (46).

Excessive activation of CNS NMDA receptors induces Ca²⁺ overload and oxidative stress, leading to neurodegeneration (47). These observations spurred development of several NMDA antagonists, many of which are effective in animal models of neurodegeneration (25,43). MK-801 can cause nearly complete inhibition of NMDA receptors,
Figure 5—The NMDA antagonist MK-801 reduces progression of nephropathy in Akita mice. Continuous drug exposure at 0.5 mg/kg/day or treatment with saline vehicle was carried out using osmotic minipumps starting at 8 weeks of age in Akita mice and DBA/2J controls. A: Reduced weight gain in Akita mice was not alleviated by MK-801. B: Reduced 24-h albumin excretion in Akita mice treated with MK-801 for 28 days compared with saline-treated controls. The effect of genotype ($F = 52.15$, $P < 0.0001$) and an interaction effect between drug treatment and genotype ($F = 5.2$, $P = 0.0283$) were significant by two-way ANOVA with 9–10 mice/group. C: Representative PAS-stained sections of saline-treated and drug-treated Akita mice and DBA/2J controls. D: Mesangial matrix expansion expressed as mesangial score shows a modest increase in 12-week-old Akita mice treated with saline. This increase did not occur in Akita mice treated with MK-801. An interaction effect between drug and genotype on this outcome is significant by two-way ANOVA ($F = 5.33$, $P = 0.0396$, $n = 4$ mice/group). E: Representative transmission EMs of mice treated with MK-801 or saline. Extensive foot process effacement (arrowheads) and GBM thickening (asterisk) in Akita mice treated with saline. The ultrastructure was markedly improved in Akita mice treated with MK-801. An interaction between the effects of MK-801 and genotype ($F = 6.22$, $P = 0.0373$, $n = 4$ mice/group) on GBM thickness was significant by two-way ANOVA. G: The effect of MK-801 on podocyte foot process width at the contact with the GBM, a measure of foot process effacement, was significant based on interaction effect by two-way ANOVA ($F = 21.66$, $P < 0.0016$). Data are mean ± SEM. *$P < 0.05$. 

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resulting in cognitive and behavioral deficits, and therefore strong NMDA antagonists of this type are not used clinically. We observed that continuous MK-801 treatment for 28 days reduced nearly every index of diabetic nephropathy. Of note, a reduction in albumin excretion, foot process effacement were observed with memantine, a second structurally distinct NMDA receptor antagonist. Memantine blocks the NMDA receptor pore, although blockade with this drug is less complete than MK-801. Therefore, synaptic transmission through NMDA receptors is maintained in the presence of memantine, whereas excessive activation is eliminated (25,43). Clinical studies have shown that memantine slows the progression of cognitive decline in Alzheimer disease (26–28). We observed that memantine reduced albumin excretion, foot process effacement, and mesangial matrix expansion in Akita mice, albeit less than MK-801. Memantine was administered at a dose of 0.2 mg/kg/day. This dose was chosen to be comparable to the U.S. Food and Drug Administration–approved regimen of 20 mg daily recommended for the treatment of Alzheimer disease (48). However, higher doses of memantine are well tolerated and may produce additional benefits for patients with Alzheimer disease (48). It is possible that higher doses of memantine might produce greater effects in diabetic nephropathy, as we saw with MK-801.

The mechanisms whereby NMDA antagonists could be beneficial to renal function in diabetes are likely to be complex. NMDA antagonists at the doses used here did not affect systemic mean arterial blood pressure. However, NMDA receptors in renal cortex mediate a tonic vasodilatory response, and it is possible that inhibition of these receptors produces hemodynamic effects that help to preserve renal function in the presence of hyperglycemia. NMDA receptors in collecting ducts are upregulated in response to osmotic challenge (14), and perhaps this is relevant in the context of polyuria that occurs in diabetes. As mentioned previously, sustained application of NMDA to cultured podocytes induces Ca^{2+} influx, oxidative stress, and reduced abundance of slit diaphragm proteins (11). If this occurs in vivo, one would expect glomerulosclerosis and glomerular dysfunction, as is seen in mouse models of hyperhomocysteinemia (16,17). With continuing loss of renal function, circulating NMDA agonists such as HCA, guanidinosuccinate, and L-quinolinic acid may increase, thereby producing a positive feedback loop driving renal pathology.

The major limitation of this study is its reliance on small-molecule pharmacological inhibitors of NMDA receptors, which are not just expressed in the kidneys but also expressed in other peripheral organs, including pancreatic β-cells, and throughout the CNS. Although the two inhibitors studied here are structurally dissimilar, it is certainly possible that they have off-target effects in common. Therefore, it is not possible to pinpoint exactly where NMDA antagonists are acting to reduce renal complications in Akita mice, especially because both agents cross the blood-brain barrier.

On the other hand, this study suggests possible utility of a pharmacological strategy based on well-tolerated drugs that are already in widespread clinical use. The availability of usable drugs to block NMDA receptors, such as memantine, contrasts with the future promise of gene therapies or other as-yet unproven strategies that may be required for some other potential therapeutic targets.

In summary, we observed that hyperglycemia and diabetes cause marked increases in the expression of renal NMDA receptors throughout the kidney and that sustained treatment with NMDA antagonists reduces the progression of nephropathy in a mouse model of type 1 diabetes. One of the agents tested here, memantine, is well tolerated and in widespread clinical use for other conditions. It is possible that other clinically used drugs in this class, such as dextromethorphan, might also be useful. In this regard, the present experiments were carried out in a mouse model.
of type 1 diabetes in which there is quite minimal β-cell function. However, there is evidence that patients with type 2 diabetes have improved insulin secretion and glycemic control after treatment with dextromethorphan partly due to effects of NMDA receptors on pancreatic islets (49). In addition, a large number of NMDA antagonists that bind to sites on NR1 subunits have been discovered (50). Many of those have limited ability to cross the blood-brain barrier. Although that would limit their usefulness for most neurological conditions, it could represent a strategy to strongly inhibit peripheral NMDA receptors without producing cognitive dysfunction.

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