Renal NMDA receptors independently stimulate proximal reabsorption and glomerular filtration

Aihua Deng and Scott C. Thomson

Department of Medicine, University of California School of Medicine and Veterans Affairs San Diego Healthcare System, San Diego, California

Submitted 26 June 2008; accepted in final form 6 March 2009

Deng A, Thomson SC. Renal NMDA receptors independently stimulate proximal reabsorption and glomerular filtration. Am J Physiol Renal Physiol 296: F976–F982, 2009. First published March 11, 2009; doi:10.1152/ajprenal.90391.2008.—N-methyl-D-aspartate receptors (NMDA) are expressed in the kidney, where little is known of their functional role. Several series of micropuncture experiments were performed in hydropenic rats using the NMDA channel blocker, MK801, and the NMDA coagonist, L-glycine, to probe NMDA for effects on single-nephron glomerular filtration rate (SNGFR) and proximal reabsorption (Jfprox). During intravenous infusion of MK801 or L-glycine, Henle’s loop was perfused to manipulate SNGFR via tubuloglomerular feedback (TGF), thereby facilitating analysis of glomerulotubular balance. To confirm local actions on the kidney, MK801 was delivered to the glomerulus by microperfusion past the macula densa and to the proximal tubule by microperfusion into the early S1 segment. By all measures, MK801 acted on the glomerulus to reduce SNGFR, and acted on the proximal tubule to suppress Jfprox, while having no effect on the responsiveness of TGF. L-Glycine raised SNGFR, dampened the TGF response, and could not be proved to independently stimulate proximal reabsorption. NMDA exerts a tonic vasodilatory influence on the glomerulus and a proreabsorptive effect on the proximal tubule. These combined effects allow NMDA to modulate SNGFR with minimal impact on late proximal flow. The full effects of L-glycine infusion on proximal tubule and TGF response do not extrapolate from the response to NMDA blockade.

N-methyl-D-aspartate receptors; L-glycine; tubuloglomerular feedback; glomerulotubular balance; micropuncture

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 two L-glutamate and two L-glutamate molecules leads to channel opening and calcium influx (9). Numerous functions have been ascribed to nitric oxide formed at various locations in the brain as a result of NMDA-R activation, but little is known about NMDA-R outside of the nervous system. We previously identified immunoreactive NMDA-R in the rat kidney cortex. We also observed renal vasoconstriction in response to NMDA-R blockers, implying that NMDA-R subserves a vasodilatory role in the kidney (2). NMDA-Rs were subsequently identified in the subapical proximal tubule (11). The present studies were undertaken to determine whether the renal hemodynamic response to NMDA-R blockade originates in the proximal tubule, which affects glomerular filtration via tubuloglomerular feedback. The alternative would be that NMDA-R blockade causes renal vasoconstriction as a primary vascular event.

Several studies were performed, all involving renal micropuncture in the rat. Results indicate that NMDA-R blockade independently reduces glomerular filtration rate (GFR) and proximal reabsorption (Jfprox).

METHODS

Overview. All animal experiments were conducted in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals with an Institutional Animal Care and Use Committee-approved protocol. Male Wistar rats (Harlan Labs) or Wistar-Froemter rats (locally bred) were given free access to standard rat chow and water up until the time of the experiments. Wistar rats were employed in most experiments. Wistar-Froemter rats, which manifest glomeruli on the kidney surface, were used when it was necessary to access the earliest proximal tubule for microperfusion.

Surgical preparation for micropuncture. Inactin anesthesia (100 mg/kg ip; Research Biochemicals International), surgical preparation, equilibration, and monitoring during micropuncture were performed according to protocols as previously described (1). Hydropenia was maintained by infusing Ringer solution at 2 ml/h. (H-inulin (80 μCi/ml) was added to the Ringer solution for those experiments requiring a marker of GFR. Tubular microperfusion was performed with Hampel nanoliter pumps (Dept. of Pharmacology, University of Tuebingen) and artificial tubular fluid (ATF). ATF for orthograde perfusion of Henle’s loop was of the following composition (in mM): 130 NaCl, 10 NaHCO3, 4 KCl, 2 CaCl2, 8 urea, 0.1% FD&C green, pH 7.4. ATF for the perfusion from the early proximal tubule was of the following composition (in mM): 115 NaCl, 25 NaHCO3, 4 KCl, 2 CaCl2, 4 urea, 5.5 glucose, 0.1% FD&C green, pH 7.4. Studies designed to test for effects of systemically administered drugs included two experimental periods. Control data were obtained during the first period. Then, the drug was begun by bolus or continuous intravenous infusion, 30 min were allowed for reequilibration, and then data gathering was begun for the second period.

Assessing Jfprox by manipulating single-nephron GFR. To test for primary effects on tubular reabsorption, one must control for differences in load delivered to the tubule by glomerular filtration. To accomplish this, we used tubuloglomerular feedback (TGF) as a tool for manipulating single-nephron GFR (SNGFR) so that Jfprox could be determined as a function of SNGFR in each nephron as previously described (12, 26, 28). Late proximal nephrons were localized on the kidney surface and an obstructing wax block was inserted immediately upstream from the most downstream accessible segment. A microperfusion pipette with ATF was inserted downstream from the wax block to perfuse Henle’s loop with the aid of a Hampel nanoliter pump. Controlled perfusion of Henle’s loop was performed to activate TGF, thereby causing SNGFR to change. While perfusing Henle’s loop to manipulate SNGFR, timed collections of tubular fluid were made upstream from the wax block to measure SNGFR and late proximal flow (LVP) rate. Collections were made from each nephron during maximal TGF activation (loop of Henle microperfusion at 40 nl/min) and minimal TGF activation (loop of Henle microperfusion at 0–8 nl/min). Nephrons were vented upstream from the wax block before each collection to prevent pressure from building up in the...
NMDA-R blocker MK801 (Sigma cat. no. M-107) was infused to the proximal tubule, MK801 (2 μM) with 2.66 M L-glycine at 1.4 ml/h as per a standard protocol from multiple prior studies (3–8, 20, 21). In all two-period studies, 30 min four collections were made from each late proximal nephron as the loop, drugs that are reabsorbed across the macula densa can affect each nephron returns to pass by its glomerulus at the end of Henle’s loop, VLP were determined by collecting from the late proximal tubule. Two minutes were allowed for equilibration before a final 3-min timed collection was made from the late proximal tubule.

RESULTS

Effects of systemic infusions. As described under methods, two-period microperfusion experiments were performed with an initial control period followed by a second period during which animals received some agent intravenously. TGF was used as a tool to manipulate SNGFR so that primary effects on tubular reabsorption could be distinguished from the effects of glomerulotubular balance (GTB). Separate sets of experiments were performed to establish the glomerular and proximal tubular effects of the NMDA-R channel blocker, MK801, the NMDA-R coagonist, L-glycine, and the vasodilator, hydralazine. The latter was employed as a hypotensive control after MK801 was found to lower blood pressure.

Response to systemically administered NMDA-R blocker, MK801. Two-period microperfusion experiments were performed in six rats. Late proximal collections were obtained with and without TGF activation in each of 22 control nephrons and 24 nephrons during systemic MK801 infusion. Results are depicted in Fig. 1. By least-squares ANOVA, MK801 reduced SNGFR by 16% (P = 0.006) irrespective of the applied TGF stimulus. The average TGF response was 10 nl/min and was unaffected by MK801.

MK801 also decreased J_prox, by 34% (P < 0.0005). Due to GTB, some decline in J_prox is expected to result from the
ANCOVA, L-glycine appeared to reduce rat. The response to L-glycine was the same in both sets of experiments, baseline SNGFR was typical for this strain of rat. In the second set of experiments that were performed several months apart. In the entire first set, the baseline (preglycine) was unaffected by MK801. As is apparent in Fig. 1, the data are compatible with this assumption.

Fractional proximal reabsorption (FRprox) increased with TGF activation in both groups (P < 0.005). This reflects the nature of FRprox as a stochastic process whereby the probability that a given fluid element will escape reabsorption by a nephron segment decreases with its residence time in that segment (reviewed in Ref. 23). The fact that FRprox varies with SNGFR in a given nephron makes it dicey to infer primary effects on the proximal tubule from simple differences in FRprox. In the present case, the slope of FRprox vs. SNGFR was unaffected by MK801 (Fig. 1, middle), so the primary effect of MK801 was tested by ANCOVA with SNGFR as covariate to reveal a 23% decline in FRprox during MK801 (P < 0.0005), which confirms that MK801 suppresses Jprox, as shown above using Jprox.

Systemic infusion of MK801 also lowered mean arterial blood pressure (BP) by 13 ± 2 mmHg, motivating additional hypotensive control experiments with hydralazine (vide infra).

Response to NMDA-R coagonist, L-glycine. Data were obtained from 60 nephrons in 8 rats. These were divided into two separate sets of experiments that were performed several months apart. In the entire first set, the baseline (preglycine) SNGFR was unusually high for a Wistar rat. In the second set of experiments, baseline SNGFR was typical for this strain of rat. The response to L-glycine was the same in both sets of animals, so the results are pooled for presentation. Results are depicted in Fig. 2. By least-squares ANOVA, L-glycine increased SNGFR by 28% irrespective of the TGF stimulus being applied (P = 0.001). L-Glycine also reduced the maximum range of the TGF response by about half (13 ± 2 vs. 6 ± 2 nl/min, P = 0.025). After controlling for SNGFR by ANCOVA, L-glycine appeared to reduce Jprox by ~30% (P < 0.001) with the caveats that ANCOVA could be deemed unreliable since L-glycine also reduced the efficiency of GTB and SNGFR was distributed differently between the two groups. By least-squares ANOVA applied to the raw data, L-glycine caused VLP to increase by 11 nl/min, or 46%. The relative contributions of SNGFR and primary tubular effects on the overall 11-nl/min increase in VLP were parsed by comparing the least-squares VLP from ANOVA vs. ANCOVA. By this analysis, 75% of the increase in VLP was attributable to GTB and the remainder (2.7 nl/min) was attributable to the primary decrease in Jprox. The tubular component appears modest in Fig. 2, but was statistically significant (P < 0.001).

Effects of BP lowering with hydralazine. Systemic infusion of MK801 lowered BP by 13 ± 2 mmHg. As a hypotensive control, additional experiments were performed using intravenous boluses of hydralazine intended to match or exceed the impact on mean arterial pressure of MK801 (n = 5 rats). Rats wound up receiving 50–100 µg hydralazine, which reduced BP by 27 ± 5 mmHg, actually exceeding the hypotensive effect of MK801. Hence, the necessary impact on BP was achieved. Paired collections (with and without TGF activation) were made from 13 control nephrons and from 24 nephrons during hydralazine. Based on ANOVA applied to the raw data, hydralazine did not affect SNGFR (30.7 vs. 30.4 nl/min), Jprox (10.4 vs. 10.3 nl/min), VLP rate (20.2 vs. 20.2 nl/min), or the range of the TGF response (6.6 vs. 6.6 nl/min). Hence, the baroceptor signal elicited by hydralazine was insufficient to induce a measurable increase in Jprox in these experiments. The preserved TGF response after BP lowering is discrepant with a previously observed decline in stop-flow pressure responses (19). This may owe to the decidedly nonlinear mapping of glomerular capillary pressure onto SNGFR during the TGF response (29).

Effects of localized MK801 delivery via Henle’s loop. As described under METHODS, Henle’s loop was perfused downstream from a wax block placed in the late proximal tubule using ATF ± MK801. This method has been fruitfully employed by us in the past to deliver drugs to the glomerulus by way of the macula densa (22, 25, 27). Nephrons were perfused at both 8 and 40 nl/min without and with addition of MK801 to the perfusate to examine the effects of MK801 over the range of the TGF response. The concentration of MK801 was chosen based on normalizing the systemic dose to the fraction of body volume occupied by a nephron and on the in vitro IC50 for MK801.

Full sets of four collections per nephron were obtained from 16 nephrons in five rats. SNGFR pre- and post-MK801 was highly correlated (r = 0.8). Likewise, the TGF responses pre- and post-MK801 were correlated (r = 0.6). Hence, the experimental design proved fortuitous for making effects of MK801 detectable by repeated-measures analysis. Compared with control perfusions done at the respective flow rates, perfusing Henle’s loop with MK801 reduced SNGFR by ~13% (P < 0.024 for effect of MK801 by repeated-measures ANOVA, averaging the values for the 8- and 40-nl/min perfusion rates). Adding MK801 to the perfusate had no apparent effect on the range of the TGF response (9.5 ± 3.7 vs. 10.0 ± 2.7 nl/min). Results are shown in Fig. 3 where comparison is also made between the effects of systemic MK801 and local delivery of MK801.

Effects of early proximal microperfusion with MK801 in free-flowing nephrons. As described under METHODS, late proximal collections were made before and during addition of 5...
from a wax block in the early S1 segment at 20 nl/min with ATF or ATF + MK801. Data were obtained from 18 nephrons in 5 Wistar Froemter rats. Four collections were made from each nephron, two each for placebo and MK801 perfusions. Results are shown in Fig. 5. MK801 reduced reabsorption by 28% (P < 0.001 by repeated measures). Repeated collections with placebo or MK801 served as time controls and confirmed that reabsorption was insensitive to repeated puncturing of the nephron, per se.

**DISCUSSION**

In the present studies, blocking systemic or renal NMDA-Rs reduced SNGFR and lessened $J_{\text{prox}}$. Furthermore, these effects on glomerular and tubular function occurred independently of each other. In other words, NMDA-R blockade suppressed $J_{\text{prox}}$ independent of the filtered load and reduced SNGFR independent of TGF. Finally, these effects of NMDA-R blockade were observed during systemic administration of the NMDA-R blocker and when the blocker was applied directly to the glomerulus or proximal tubule by microperfusion. Thus, NMDA-Rs in the hydropenic rat kidney cortex tonically affect vasoconstriction and provide a stimulus for TGF. Whether these glomerular and tubular actions stem solely from activation of these receptors known to reside in the proximal tubule or whether there is a separate population of vascular receptors remains to be determined. It is also unknown how the tonic influence of NMDA would compete with other homeostatic mechanisms that impinge on kidney functions under conditions other than hydropenia.

These micropuncture data are consistent with our prior observation that systemic administration of MK801 lessens renal blood flow and GFR at the whole kidney level in the hydropenic rat (2). In those experiments, the impact of NMDA-R blockade on kidney function was confirmed with an alternate NMDA-R antagonist, 5,7-dichlorokynurenic acid, and confirmed to be unaltered by renal denervation (2). Given that NMDA-Rs influence a complex array of brain functions (9) that could conceivably influence the kidney independent of the renal nerves, it is also helpful to know that NMDA-R...
blockade has the same effects when administered directly to single nephrons as when given systemically.

The only other report of NMDA-R and renal hemodynamics was by Yang et al. (31), who cited the role of NMDA-R in excitotoxic death of ischemic neurons to justify a potential role in renal ischemia-reperfusion. In normal kidneys, high-dose agonist NMDA caused a major decline in inulin clearance but had no effect on renal blood flow. Furthermore, major reductions in GFR and salt excretion 24 h after ischemia-reperfusion were completely reversed by infusing D-AP5, which competes for glutamate binding to NMDA-R. The authors concluded that NMDA-Rs exist in the kidney to mediate injury (31). This impression is contrary to our earlier finding that inhibiting NMDA-R with a channel blocker or glycine antagonist causes renal vasoconstriction (2) and to the phenomena presently observed at the single-nephron level. Assuming that everyone’s data are valid, the effects of NMDA-R on the kidney must be dichotomous. There is precedent for this in the central nervous system (14, 16). An oversimplified explanation for its dichotomous role in the brain is that low-level NMDA-R activity provides calcium entry that drives nitric oxide synthase to produce nitric oxide needed for neuronal plasticity or vasodilation, whereas high-level NMDA-R activity floods the cell with toxic amounts of calcium leading to injury and death.

Contrary to the notions of Yang et al. (31) that NMDA-Rs exist in the kidney to cause harm, our first intuition about the functional role of NMDA-Rs in the kidney is that they might mediate the selective renal vasodilatory response to amino acid infusion or protein feeding. It has been known since the 1940s that the healthy kidney hyperfilters in response to dietary protein or glycine infusion (18), yet a full explanation of this phenomenon has been elusive. An amino acid receptor in the kidney seemed an obvious candidate and we initially confirmed that the vasodilatory response to L-glycine was mostly prevented by NMDA-R blockers or antagonists (2). We also observed a forward conditioning of this system whereby prior exposure to dietary protein simultaneously augmented the vasodilatory response to acute infusion of L-glycine and increased the renal NMDA-R protein expression (21). These observations fall short of actual proof that NMDA-R is the final mediator of the puzzle.

We are presently interested in the extent to which the renal hemodynamic effects of NMDA-R are rooted in feedback from the tubule. There is a background to this. First, NMDA-Rs are known to exist in the proximal tubule, but remain to be discovered in the glomerulus. Second, there is a wealth of circumstantial evidence that a normal vasodilatory response to L-glycine requires a cooperative proximal tubule. For example, the vasodilatory response to L-glycine is impaired during nitric oxide synthase blockade (3), two-kidney one-clip Goldblatt hypertension (6), chronic glomerulonephritis (7), and short-term cyclosporin administration (8). In each of these models, a failed GFR response to L-glycine correlates with a decrease in J_prox, which could act as a brake on GFR via TGF (reviewed in Ref. 10). But in all these studies, impressions regarding the effect of L-glycine on the proximal tubule were based on late proximal collections made in the absence of a TGF signal, a method that precludes one from distinguishing GTB from primary effects on J_prox. The present experiments were conducted to mitigate this shortcoming, by using TGF to manipulate GFR, thereby allowing us to quantify the contribution of GTB to a given change in J_prox.

Since we previously found macula densa salt concentration to be stable during L-glycine (20), we anticipated primary decrease in J_prox during NMDA-R blockade and a primary increase during L-glycine. The former expectation was realized but the latter was not. In fact, L-glycine actually exerted a minor negative effect on J_prox while appearing to lessen the efficiency of GTB. It is probable that L-glycine exerts competing influences on the proximal tubule, leading to qualitatively different behavior according to circumstances. To the extent that L-glycine activates NMDA-R, the response to MK801 indicates that L-glycine should be proreabsorptive. Meanwhile, a potential anti-reabsorptive influence of L-glycine is tied to the osmotic burden that it places on the tubule. This standard model of L-glycine infusion previously yielded L-glycine concentrations of 14 and 20 mM in the late proximal tubule and plasma, respectively (4, 5). Combining this with late proximal TF/Pinulin of 2 yields 13 mmol of L-glycine reabsorbed per liter of glomerular filtrate. While this transepithelial gradient would not support a net osmotic diuresis due to L-glycine, this puts L-glycine on comparable footing with bicarbonate, which is the dominant solute for driving for J_prox (13). If the fractional reabsorption of L-glycine is less than bicarbonate, then adding L-glycine would reduce net reabsorption, notwithstanding that L-glycine contributes to reabsorption. Adding L-glycine would also lessen the efficiency of GTB if L-glycine were to operate near its transport maximum. As it turns out, 20 mM plasma L-glycine was required to saturate low-affinity L-glycine reabsorption in the dog (calculated from the original work of Pitts (17)). The foregoing effects can all be deduced from the thermodynamics of solute-solvent coupling. It is also likely that L-glycine affects tubular reabsorption by other biological
mechanisms that we are unaware of, since the proximal tubule also processes glycine into bioactive peptides, such as glutathione, which it holds internally (15).

TGF became less responsive during l-glycine infusion. We had originally wondered whether NMDA-R would exert a tonic influence on TGF via macula densa nitric oxide synthase, but the MK801 results rule that out. Since it is unlikely that l-glycine could elicit an increase in Loop of Henle reabsorption adequate to overcome 40 nl/min microperfusion of Henle’s loop, l-glycine must attenuate feedback either by interfering with signal transmission across the juxtaglomerular apparatus or by a direct effect on glomerular microvessels.

Returning to the response to MK801, we note direct or “primary” effects on both the glomerulus and proximal tubule. In other words, MK801 reduced $J_{\text{prox}}$ independent of GTB and decreased SNGFR independent of TGF. The negative feedback system comprised of GTB and TGF normally operates near the inflection point of the TGF curve (24) and deviates from that point in the course of compensating for an acute disturbance. For example, a primary decline in $J_{\text{prox}}$ will push the operating point toward the elbow of the TGF curve and a primary decline in SNGFR will push it toward the shoulder. A noteworthy feature of the current data is that the combined primary disturbances allow for a change in SNGFR with negligible residual error in VLP, no shift in the operating point with respect to the TGF inflection point, and no resetting of the TGF response. This is illustrated in Fig. 6, which was generated by taking GTB relationships and limits of the TGF response from the present micropuncture data, setting peak open loop gain for the TGF-GTB system at 2 (24), and assuming, for purposes of illustration, that MK801 did not affect alignment of the ambient flow relative to the TGF inflection point. This construct yields a remarkably stable value for ambient VLP and suggests uncanny coordination of the “independent” actions of NMDA-R in the tubule and glomerulus. Overall homeostasis will be more efficient if the mechanisms for compensating disturbances in dietary salt and protein are separable. A system that increases GFR in response to protein while simultaneously preventing the increase in filtered sodium from passing beyond the proximal tubule would accomplish this. Hence, if the body invokes renal NMDA-R signaling in its response to dietary protein (21), the dual actions of NMDA-R on the glomerulus and proximal tubule make sense. If tubular and glomerular NMDA-R signaling are simultaneous, but independent, then how do they manage to appear so well balanced? In fact, we haven’t proven them to be balanced since it is possible that the TGF operating point and inflection point no longer coincide after NMDA-R blockade. But unless NMDA-R blockade interferes with the TGF system per se (and we failed to find evidence for this), internal resetting of the TGF system should restore the operating point to the steep portion of the TGF curve (30). Given that fine tuning occurs through TGF resetting, the only requirement is that the glomerular and tubular effects of MK801 were roughly balanced.

In summary, renal NMDA-Rs exert tonic positive influence on SNGFR and $J_{\text{prox}}$ in the hydropenic rat kidney, with the latter effect exceeding what is expected of GTB.

ACKNOWLEDGMENTS

Technical assistance was provided by S. Khang.

REFERENCES


