Green mamba peptide targets type-2 vasopressin receptor against polycystic kidney disease

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Polycystic kidney diseases (PKDs) are genetic disorders that can cause renal failure and death in children and adults. Lowering cAMP in cystic tissues through the inhibition of the type-2 vasopressin receptor (V2R) constitutes a validated strategy to reduce disease progression. We identified a peptide from green mamba venom that exhibits nanomolar affinity for the V2R without any activity on 155 other G-protein–coupled receptors or on 15 ionic channels. Mambaquaretin-1 is a full antagonist of the V2R activation pathways studied: cAMP production, beta-arrestin interaction, and MAP kinase activity. This peptide adopts the Kunitz fold known to mostly act on potassium channels and serine proteases. Mambaquaretin-1 interacts selectively with the V2R through its first loop, in the same manner that aprotinin inhibits trypsin. Injected in mice, mambaquaretin-1 increases in a dose-dependent manner urine outflow with concomitant reduction of urine osmolality, indicating a purely aquaretic effect associated with the in vivo blockade of V2R. CD1-pcy/pcy mice, a juvenile model of PKD, daily treated with 13 μg of mambaquaretin-1 for 99 d, developed less abundant (by 33%) and smaller (by 47%) cysts than control mice. Neither tachyphylaxis nor apparent toxicity has been noted. Mambaquaretin-1 represents a promising therapeutic agent against PKDs.

polycystic kidney disease | Kunitz peptide | snake toxin

Polycystic kidney diseases (PKDs) are potentially life-threatening genetic disorders that are characterized by the presence of multiple fluid-filled cysts in the kidney. In PKDs, cyst formation and enlargement progressively compromise normal renal parenchyma functions and with time severely distort the entire kidney, leading to end-stage renal failure (1). With an estimated prevalence of 1 in 1,000, autosomal-dominant PKD (ADPKD), caused by mutations in either the polycystin-1 or polycystin-2 gene, is one of the most common monogenetic disorders affecting millions of people worldwide (2, 3). Autosomal-recessive PKD caused by mutations in the fibrocystin gene is 20 times less common than ADPKD and affects children in the first year of life with a high mortality rate. No satisfactory therapy for PKDs has yet been developed despite huge efforts for more than 30 y. All of the strategies, from microtubule stabilization with the antitumor drug paclitaxel to the inhibition of either epidermal growth factor receptor or B-Raf, MAPK/ERK, and mTOR kinases have failed to significantly improve the pathological state (4). A key advance in ADPKD treatment has been the observation that in the polycystic kidneys a pathogenic cAMP accumulation stimulates cell proliferation and chloride-driven fluid secretion into cyst lumen (5, 6). The cAMP level can be reduced in two ways: through Gi-protein activation by the somatostatin SSTR2 receptor or, alternatively, through type-2 vasopressin receptor (V2R) inhibition that decreases Gs-protein signaling. Although both strategies appear to be effective in humans and constitute an efficient therapeutic approach, the use of V2R antagonists has certain advantages (7, 8). Most renal ADPKD cysts develop within the vasopressin-sensitive tubular parts of the nephron, where vasopressin is also the main hormonal regulator of adenylyl cyclase and thus a stimulator of cAMP production. Moreover, increased levels of circulating vasopressin and elevated expression of V2R have been observed in human ADPKD and in animal models. Finally, in contrast to somatostatin receptors, V2R has kidney-specific expression.

Among the orally bio-available vasopressin antagonists, originally licensed for short-term treatment of heart failure and hyponatraemia, tolvaptan has been proved to be beneficial for the long-term treatment of ADPKD in adult patients (9). Tolvaptan is an aquaretic agent that inhibits V2R and reduces cAMP production in principal cells of the collecting tubules (10). In

Significance

Polycystic kidney diseases (PKDs) are genetic disorders in which multiple cysts grow in kidneys, leading to end-stage renal failure. Vasopressin antagonists (vaptans) currently used to treat PKDs have side effects due to liver toxicity. We report the characterization of Mambaquaretin-1, a Kunitz-fold polypeptide isolated from mamba venom that selectively and fully inhibits three major signaling pathways of the vasopressin type-2 receptor. Mambaquaretin-1 induces a purely aquaretic effect on mice and reduces cyst development in a mouse model. We produced mambaquaretin-1 by peptide synthesis and determined its X-ray structure, its binding mode, and functional properties. With high selectivity and without toxic metabolic byproducts associated with its peptidic nature, mambaquaretin-1 could become the preferential treatment for these disorders.

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Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 5M4V).

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a large randomized phase III clinical trial, tolvaptan was found to slow down cyst development and renal insufficiency (8). Since 2014, marketing authorization has been granted in Japan, Canada, and four European countries (France, Germany, the United Kingdom, and Switzerland) and only more recently in the United States, delayed because of concerns regarding its harmful effects on liver function. Indeed, tolvaptan degradation by cytochrome P450 generates hepatotoxic metabolites (9). Additionally, because vaptans bind at a highly conserved orthosteric site, common to all vasopressin-sensitive receptors, they have weak selectivity.

Venoms constitute a reservoir of millions of disulfide-rich peptides, which are biochemically stable with particular pharmacological properties. Due to their high affinity and selectivity, polypeptides derived from venom of different animals are interesting alternatives to small nonpeptidic compounds for drug development (11). Mamba snake venom contains toxins, such as MT7, ρ-Da1b, and ρ-Da1a specific for GPCRs, able to modulate their functions in unique ways. MT7 acts as a negative allosteric modulator of the M1 muscarinic receptor (12), ρ-Da1b is a noncompetitive antagonist of the α2A-adrenoreceptor (13), and ρ-Da1a is able to antagonize in an insurmountable way the α1A-adrenoreceptor (14). Marine cone snails are currently the only species known to secrete toxins active on vasopressin receptors. These toxins mimic vasopressin with weak activity against the V1a and V1b receptors (15, 16). Here we report the identification, 3D structure, and properties of mambaquaretin-1 discovered by screening green mamba venom. Mambaquaretin-1 is a highly selective V2R antagonist able to protect against renal cyst development in vivo. For patients with PKD for which tolvaptan is contraindicated, a peptidic drug may represent an interesting alternative.

Results

Mambaquaretin-1 Selectively Interacts with V2R. Mambaquaretin-1 was purified once from the venom of the green mamba (Fig. 1*A*) by cation exchange and reverse-phase liquid chromatography. The active fraction *F* from the cation exchange step (Fig. 1*B*) was subfractionated on a reverse-phase column (Fig. 1*C*) and fraction *D* active against V2R was isolated. The final purification step allowed separation of the minor active fraction *A* (Fig. 1*D*). The monoisotopic molecular mass of the active polypeptide was 6,367.2 Da and 6,373.2 Da before and after reduction, indicating the presence of three disulfide bridges (Fig. S1). Its sequence of 57 residues was determined by Edman's degradation and mass analysis (Fig. S2 and Table S1). Mambaquaretin-1 was then produced by solid-phase peptide chemical synthesis (Fig. S3 *A* and *E*). The synthetic homologue had the same elution time as the toxin isolated from the venom (Fig. 1*E*) and it was found to bind V2R with the same affinity (data not shown). Synthetic mambaquaretin-1 inhibits tritiated arginine–vasopressin (^{3}H-AVP) binding to V2R with a K_i of 2.81 nM (pK_i 8.55 $\pm$ 0.25, Fig. 1*F*). At concentrations up to 10 μM, no binding could be measured on V1a, V1b, and the oxytocin receptor. At similar high concentrations, mambaquaretin-1 showed moderate activity only for K_v1.1 subtype but not on other potassium, sodium, or calcium channels (tested on nine K_v and on two Na_v oocyte expressed channel subtypes, Fig. S4, and on rat brain N-type calcium channel, data not shown). Mambaquaretin-1 at 1 μM was inactive on nine cardiac ionic channels and on 155 G-protein–coupled receptors expressed on eukaryotic cells (*SI Method 1: Mambaquaretin-1 Identification* and data not shown).

Loop 1 of Mambaquaretin-1 Mediates Binding to V2R. Mambaquaretin-1 shares between 49% and 57% of sequence identity with typical Kunitz-fold proteins (Fig. 2) such as α-dendrotoxin (α-DTX) (17, 21), which blocks the potassium channel K_v1.1 conductivity, and three inhibitors of trypsin activity: aprotinin (22), textilinin-1 (18), and boophilin (20). The molecular strategies used by the α-DTX and the aprotinin to interact with their respective targets are different. α-DTX blocks K_v1.1 mainly with its lysine in position 5 (21) whereas aprotinin to inhibit trypsin activity uses a dyad of residues composed of a lysine and an alanine (KA) located in its first loop (23). These positions are occupied by a serine (position 3 in mambaquaretin-1; Fig. 2*A*, orange circle) and by asparagine and glycine (positions 15 and 16 in mambaquaretin-1; Fig. 2*A*, green circles). Three variants were synthesized to question the strategy used by mambaquaretin-1 to target V2R: S3K, an N-ter truncated version lacking the first four residues, and the N15G16/K15A16 double variant (Fig. S3). The S3K variant and the truncated toxin were found to have the same affinity for V2R (pK_i 8.87 $\pm$ 0.14 and pK_i 8.84 $\pm$ 0.38, respectively) whereas the NG/KA variant had an affinity 1,000-fold lower than WT (pK_i 5.50 $\pm$ 0.46; Fig. 2*B*). Remarkably, the NG/KA variant inhibited trypsin with nanomolar affinity (IC_{50} = 0.77 $\pm$ 0.18 nM vs. WT: IC_{50} = 14.8 $\pm$ 1.1 μM, Fig. 2*C*), the S3K variant was found to be more active on K_v1.1 (IC_{50} = 308 $\pm$ 52 nM vs. WT: IC_{50} = 8.2 μM), and the truncated polypeptide was inactive against K_v1.1 (IC_{50} > 50 μM, Fig. 2*D*). Mambaquaretin-1 uses its first loop to interact with V2R, like aprotinin to inhibit trypsin (Fig. 2 *B*–*D*). On their own, at concentrations up to 10 μM, neither α-DTX nor aprotinin was found to bind V2R (Fig. 2*B*).The X-ray structure of mambaquaretin-1 (variant KA) was obtained from crystals diffracting to 1.06 Å resolution (Fig. 2*E*, *SI Method 2: Crystallization and Structure Determination*, Table S2, and Fig. S5). The structure is highly compact with

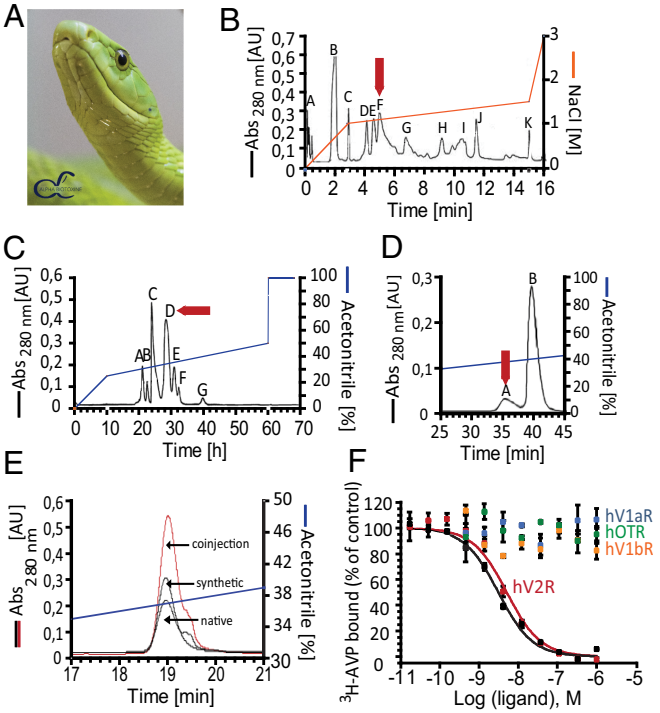

Fig. 1. Identification and characterization of mambaquaretin-1. (*A*) Green mamba, reproduced with permission from C. Vanbellingen, Alphabiotoxine Laboratory sprl, Belgium. (*B*) Fractionation of 1 g of *Dendroaspis angusticeps* crude venom by ion-exchange chromatography. (*C*) Fractionation by reverse-phase chromatography of fraction *F*. (*D*) Fractionation by reverse-phase chromatography of fraction *D*. Arrows indicate active peaks. (*E*) Coelution between fraction A and synthetic mambaquaretin-1. (*F*) Binding of ^{3}H-AVP on V2R, V1aR, V1bR, and Oxytocin R (hOTR) in the presence of mambaquaretin-1 (colored circles) and AVP (black circles). Data represent at least three independent experiments and are presented as mean $\pm$ SD. Abs, absorbance; AU, absorbance unit.

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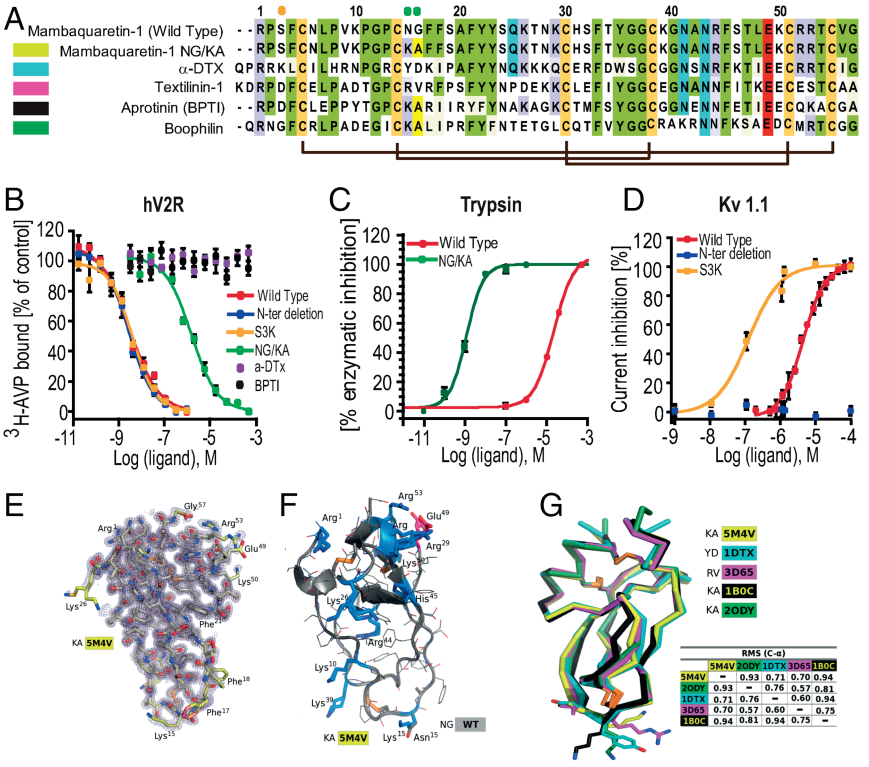

Fig. 2. Mambaquaretin-1 binds V2R with its first loop. (*A*) Sequence comparison between mambaquaretin-1, its KA variant, α-DTX, Australian common brown snake textilinin-1, aprotinin (BPTI), and cattle tick clotting inhibitor boophilin. Cysteines are shaded light green, positively charged residues are shaded blue, negatively charged residues are shaded red, and other conserved residues are shaded gray. Orange and green circles indicate residues critical for α-DTX and BPTI activity, respectively. (*B*) Binding inhibition of ^{3}H-AVP on hV2R by mambaquaretin-1, its three variants, BPTI, and α-DTX. (*C*) Trypsin inhibition by mambaquaretin-1 and its NG/KA variant. (*D*) Current inhibition of Kv1.1 channels obtained by plotting the percentage of blocked current in function of increasing toxin concentrations by mambaquaretin-1, N-ter deletion, and S3K variant. (*E*) X-ray structure of mambaquaretin-1 KA in stick representation colored according to *B* value (light blue to red). (*F*) Cartoon representation of mambaquaretin-1 and the KA variant showing the preponderance of positively charged residues. (*G*) Superposition on C-α of mambaquaretin-1 KA (PDB ID: 5M4V), α-DTX [PDB ID: 1DTX (17)], textilin-1 [PDB ID: 3D65 (18)], BPTI [PDB ID: 1B0C (19)], and boophilin [PDB ID: 2ODY (20)] with rmsd between the structures from PyMOL alignment.

extremely low mobility apart from the first loop including the two adjacent Phe residues in positions 17 and 18. A model of WT mambaquaretin-1 can be built from the variant K15A16 by K15N and A16G substitutions with the selection of the closest Asp rotamer to that used by the Lys (Fig. 2*F*). Mambaquaretin-1 has a large number of positively charged residues distributed throughout the sequence and only two negative ones: the conserved Glu-49 and the C-terminal Gly-57. The backbone of mambaquaretin-1, that of α-DTX, and those of three other serine-protease inhibitors superimpose very well with an rmsd on C-α between 0.57 Å and 0.94 Å (Fig. 2*G*). Mambaquaretin-1 was submitted to the MEROPS database (24). It has been classified as belonging to the I2 family that contains diverse Kunitz peptides found in animals, similar to α-DTX and aprotinin.

Mambaquaretin-1 Competitively Antagonizes V2R-Induced Signaling Pathways. By acting on V2R, vasopressin activates at least three signaling pathways: a Gs-coupled stimulation of adenylyl cyclase that initiates intracellular cAMP production, an arrestin-mediated V2R desensitization, and a stimulation of MAP kinase phosphorylation. Mambaquaretin-1 alone was unable to activate these signaling pathways (Fig. 3 *A*, *C*, and *D*), but could antagonize all of them with a K_i of 12.0 nM (PA2 = 7.92 ± 0.02, $n = 3$) for cAMP production in CHO cells stably expressing V2R, 110 nM (PA2 = 7.0 ± 0.2, $n = 3$) for β-arrestin-1 mobilization in a tsA cell line transiently transfected with β-arrestin-1–YFP and hV2R-Rluc, and 210 nM (PA2 = 6.9 ± 0.2, $n = 7$) for MAP kinase phosphorylation in a tsA cell line transiently transfected with hV2R (Fig. 3 *A*, *C*, and *D*). Increasing concentrations of mambaquaretin-1 induced parallel rightward shifts of the activation curves. Arunlakshana–Schild plots show a purely competitive behavior between vasopressin and mambaquaretin-1 on the three different activation pathways because the IC_{50} regression can be linearized with unitary slope (Fig. 3 *B*, *D*, and *F*). Mambaquaretin-1 was tested on immortalized KC3AC1 renal cells that naturally express V2R. These cells, isolated from the cortical collecting ducts of a transgenic mouse, display an epithelial polarized phenotype when cultivated on filters (25). ^{3}H-AVP was found to bind to KC3AC1 cells with an affinity between 4 nM and 6 nM and a B_{max} of 21,000 receptors per cell. 1-Desamino-8-D-arginine vasopressin (dDAVP) induced cAMP production in a dose-dependent manner with an EC_{50} of 0.3 ± 0.2 nM, whereas in the presence of 1 μM mambaquaretin-1, its EC_{50} was 60-fold higher (18 ± 1.2 nM; Fig. 3*G*). Moreover, mambaquaretin-1 antagonized the effect of 0.8 nM dDAVP (EC_{80}) with an IC_{50} of 37 ± 15 nM. No change in cAMP production was observed with mambaquaretin-1 alone (Fig. 3*H*).

Aquaretic Effect of Mambaquaretin-1. The CD1-pcy/pcy mouse strain suffers from type 3 nephronophthisis, similar in many aspects to ADPKD, caused by a spontaneous missense mutation (T1841G) in the gene orthologous to human NPHP3 (26). This strain is a pertinent model for in vivo evaluation of renoprotective efficacy of novel drug candidates (6). Ten-week-old C57BL/6 female mice were given 1 μmol/kg by body weight (BW) mambaquaretin-1 via daily intraperitoneal (i.p.) injections for 6 d

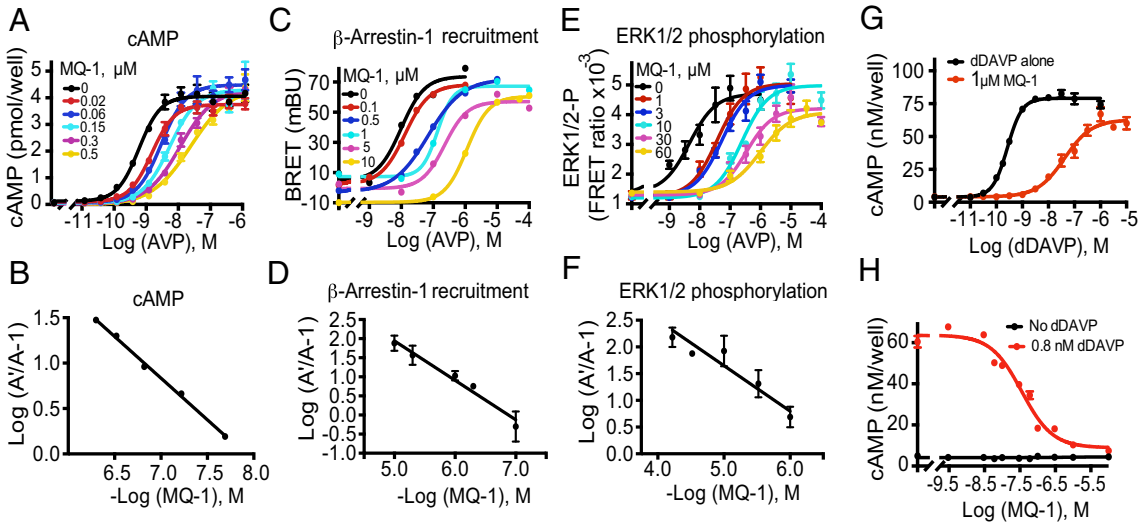

Fig. 3. V2R antagonist activity of mambaquaretin-1 (MQ-1). (*A*, *C*, and *E*) Competitive inhibition of AVP-induced (*A*) cAMP production in stable CHO-hV2R cell line, (*C*) β-arrestin-1 recruitment by BRET-β-arrestin-1–YFP and hV2R-Rluc tsA transfected cell line, and (*E*) MAP kinase phosphorylation on hV2R tsA transfected cell line. (*B*, *D*, and *F*) Corresponding Arunlakshana–Schild plots. (*G*) Antagonistic effect of mambaquaretin-1 on cAMP production in dDAVP-stimulated renal KC3AC1 cells. (*H*) Effect of increasing concentrations of mambaquaretin-1 on cAMP production in the absence or in the presence of 0.8 nM of dDAVP in renal KC3AC1 cell line. All panels are representative of at least three independent experiments and Schild representations are plotted as mean ± SEM. mBU, milliBRET unit.

whereas female CD1-pcy/pcy mice received three different doses of mambaquaretin-1: 0.01 > µmol/kg BW, 0.1 > µmol/kg BW, and 1 µmol/kg BW. We used female mice to maximize the likelihood to observe a therapeutic effect of mambaquaretin-1. Urine output increased and urine osmolality decreased dramatically under mambaquaretin-1 treatment without differences between the two mouse strains (Fig. 4 *A* and *B*). For the two largest doses of mambaquaretin-1, a marked diuretic effect was observed within 1 d after the first injection. The effect reached a maximum with urine volumes around 4 mL/d. For the 0.01-µmol/kg BW dose of mambaquaretin-1, four injections were necessary to provoke an increase in diuresis with a stabilized urinary volume around 2 mL/d. The diuresis caused by mambaquaretin-1 was paralleled by a decrease in urine osmolality consistent with an aquaretic effect in vivo. Intraperitoneal and subcutaneous injections gave identical biological results in terms of urine volume and osmolality (Fig. S6). The i.p. route was found to be more practical. Starting at 10 wk of age, CD1-pcy/pcy female mice received 0.1 µmol/kg BW ($n = 6$) mambaquaretin-1 intraperitoneally for 99 d, whereas control mice received saline solution ($n = 7$). Mambaquaretin-1 was well tolerated as judged by the equivalent body weight (21.1 ± 2.2 g vs. 21.3 ± 0.8 g in controls, $P = 0.41$, Fig. S7) and by their normal social behavior during the course of the experiment. In addition, we did not notice any difference in the locomotive behavior, in the exterior aspect, or in the amount of food or water ingested between the two groups. Constant increase in diuresis and decrease in urine osmolality were observed at each time point until the end of the trial (Fig. 4 *C* and *D*). Protein and electrolyte excretions in the urine samples were similar in the treated and control groups (Fig. 4 *E* and *F*). No differences were found in cation and anion levels (Fig. S8).

Blood creatinine and urea, two important parameters related to renal function, were measured at the end of the trial (Fig. S9 *A* and *B*). No difference in urea concentration was noted between the two groups (73.1 ± 6.6 mg/dL vs. 75.9 ± 11.9 mg/dL in controls, $P = 0.3$). However, a threefold decrease of the creatinine levels was measured in the mambaquaretin-1–treated group (0.59 ± 0.11 mg/dL vs. 1.86 ± 0.73 mg/dL in controls, $P = 0.002$). Urea and creatinine levels were consistent with the mambaquaretin-1–treated mice having better renal function.

Mambaquaretin-1 Inhibits Cyst Progression in pcy Mice. By the end of the treatment, mice were perfusion fixed and organs were removed for analysis. Heart weights were found to be equivalent between treated and nontreated female mice (113 ± 10 mg vs. 114 ± 10 mg in controls, $n = 6–7$, $P = 0.45$; Fig. S9*C*). Kidneys from mambaquaretin-1–treated mice appeared smaller relative to those from control mice (kidney to body ratio: 0.029 ± 0.005 vs. 0.043 ± 0.018 in controls, $P = 0.06$; Fig. 5*A*). The renoprotective effect of mambaquaretin-1 was clearly discernible in quantitative analysis of imaged kidney sections. The ratio of

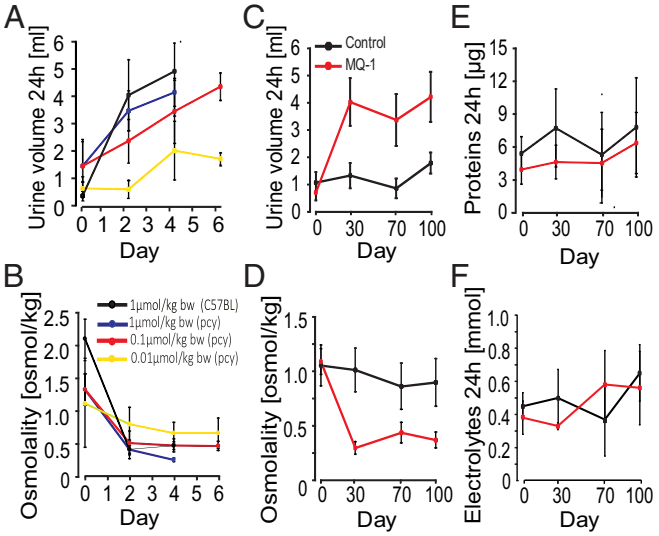

Fig. 4. Aquaretic effect of mambaquaretin-1. (*A* and *B*) Increase in urinary volume (*A*) and decrease in urine osmolality (*B*) after daily administration of mambaquaretin-1 at three doses to pcy mice and to C57BL/6 mice (violet curve, one dose) for 6 d. (*C*–*F*) Monitoring of (*C*) diuresis, (*D*) urine osmolality, (*E*) excretion of proteins, and (*F*) electrolytes during the 99-d treatment with 0.1 µmol/kg BW of mambaquaretin-1. Data are presented as mean ± SD, $n = 6–7$.

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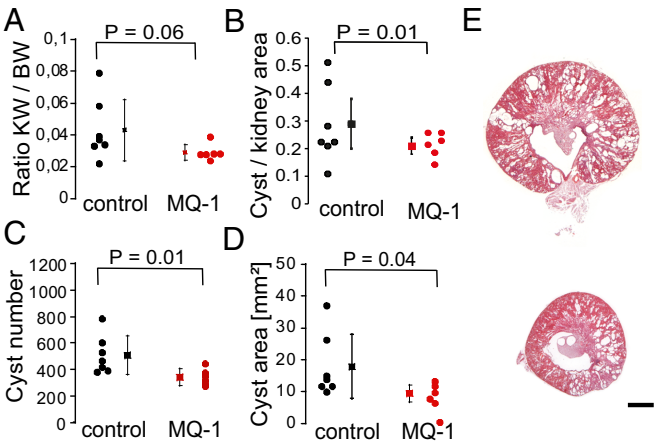

Fig. 5. Beneficial effect of treatment with mambaquaretin-1 (MQ-1) in pcy mice. Ratio of kidney weight to body weight (KW/BW) (*A*), ratio of cyst area to kidney area (*B*), cyst number (*C*), cyst area (*D*), and representative H&E-stained sections from kidneys of nontreated pcy mice (*E*, *Top*) and treated with mambaquaretin-1 (*E*, *Bottom*). *P* values were calculated with a *t* test by comparing treated and control animals, $n = 6–7$. (Scale bar, 2 mm.)

cyst to kidney area was significantly smaller after treatment with mambaquaretin-1 (0.21 ± 0.03 vs. 0.29 ± 0.09 in controls, decrease by 28%, $P = 0.03$; Fig. 5*B*). The average number of renal cysts was reduced by 33% (from 509 ± 134 in control animals to 342 ± 57 in those treated with mambaquaretin-1, $P = 0.01$; Fig. 5*C*). The total area of renal cysts was decreased by 47% (from 17.9 ± 9.3 mm^2 in controls to 9.4 ± 2.4 mm^2 in the treated mice, $P = 0.04$; Fig. 5*D*). Finally, analysis of representative kidney sections (Fig. 5*E*) showed a globally beneficial effect of mambaquaretin-1. The tests carried out on a limited cohort of mice demonstrated that a low dose of mambaquaretin-1 (0.1 μmol/kg BW) was effective.

Discussion

Currently V2R inhibition represents the most promising pharmaceutical strategy against PKD (27). We report the identification of mambaquaretin-1, a venom peptide that strongly antagonizes V2R with a therapeutic potential against renal polycystic disease. Venom components target principally enzymes and ionic channels involved in the control of the hemostatic, nervous, and cardiovascular systems (28). Components acting on the GPCR superfamily are rare and represent less than 1% of the characterized toxins. Mamba snakes (*Dendroaspis* sp.) are among the most dangerous snakes in Africa because their toxins act on potassium channels and acetylcholinesterase. Currently, their venom is also the richest source of GPCR ligands. These ligands are all three-finger–fold toxins active on amine-sensitive receptors (29–33). Mambaquaretin-1 is unique because it is a Kunitz-fold toxin active on a peptide-sensitive receptor. Mambaquaretin-1 is a minor component and its role as part of the venom has not been determined. Kunitz-type toxins were first identified in the early 1970s in black mamba (*Dendroaspis polylepis*) venom (34). Kunitz peptides are also common in other snakes, scorpions, spiders, ticks, and sea anemones and are known to interact with diverse targets. These include calcium channels (35), TRPV1 (36), cysteine proteases (37), or ASIC channels. Prevalently, individual Kunitz toxins act as potassium channel blockers (38), inhibitors of serine proteases (22), or both (39). Mambaquaretin-1 effectively and selectively targets V2R, expanding the repertoire of activities associated with the Kunitz fold. By using the first loop instead of the N-terminal part to interact with V2R, mambaquaretin-1 mimics aprotinin rather than α-DTX in its binding strategy. The Kunitz-fold versatility is revealed by simple residue substitutions in mambaquaretin-1 sequence that significantly improve alternatively $K_v1.1$ and trypsin inhibition. Mambaquaretin-1 is a polypeptide five times bigger than vasopressin. Its great selectivity toward V2R may be related to a binding not confined to the orthosteric pocket. Certain hV2R loops peripheral to the orthosteric pocket are acidic whereas mambaquaretin-1 has a strongly basic character (net charge +9). Charge complementarity may help drive complex formation. The larger interaction surface, compared with vasopressin and vaptans, may also explain why mambaquaretin-1 acts as a full antagonist in three intracellular signaling pathways controlled by V2R activation. It can be speculated that its binding may induce a frizzed and nonfunctional V2R conformation that could have advantages in PKD treatment. The physiological consequences of a complete blockage of V2R will need a more detailed examination. The most promising finding is that mambaquaretin-1 is an efficient renoprotective agent able to slow down the progression of ADPKD in the murine model of the disease without any detectable adverse effects and no apparent toxicity up to 1 μmol/kg BW. The pcy mice treated with mambaquaretin-1 compared with untreated animals had fewer smaller cysts. The renoprotective effect of mambaquaretin-1 could be due to lower cAMP levels in V2R-expressing cells. We demonstrated that mambaquaretin-1 inhibited the dDAVP-induced cAMP production in a dose-dependent manner. All therapeutic effects observed for mambaquaretin-1 were similar to those reported for vaptans. Despite its liver toxicity, tolvaptan is currently the key drug for PKD treatment (40, 41). Mambaquaretin-1, being a natural peptide, is unlikely to share the adverse effects associated with long-term vaptan use. Mambaquaretin-1 being the most selective ligand for V2R is likely to have fewer side effects because of its better selectivity. Animal venoms constitute a natural library of several million molecules, largely unexplored at present as a source of potential drugs. Six peptidic drugs derived from venoms are now on the market. Tens of other candidates are in clinical development and hundreds of patents have been filed, highlighting their therapeutic potential. Mambaquaretin-1 could be added to this list as a promising candidate for PKD treatment.

Materials and Methods

Unless mentioned otherwise, all chemicals were obtained from Sigma-Aldrich.

Identification of Mambaquaretin-1. One gram of *Dendroaspis angusticeps* venom (Latoxan) was separated into 13 peaks by ion exchange as described (42) and the fraction *F* in seven fractions as described in *SI Method 1: Mambaquaretin-1 Identification*.

Peptide Synthesis and Crystal Structure Determination. Mambaquaretin-1 and its variants were synthesized on an Applied Biosystems 433A peptide synthesizer, purified, and folded according to a method previously described (43). Briefly, this involved solid-phase synthesis, using an Fmoc strategy on 50 μmol of Chemmatrix resin loaded with the glycine residue. The linear peptide was folded in the presence of 25% glycerol and oxidized and reduced glutathione (1 mM) in Tris buffer at pH 8 for 16 h at room temperature (13). Crystallization trials were set up for the WT, the truncated mambaquaretin-1 lacking its four first residues, and the KA variant as described in *SI Method 2: Crystallization and Structure Determination*.

Assays. Binding assays were performed using radioactive tracers on cell membrane preparation from CHO cells expressing the receptor of interest as described in *SI Method 3: Radioligand Binding Assay*. Selectivity profiles of mambaquaretin-1 have been subcontracted to a service company as described in *SI Method 4: Selectivity Profile of Mambquaretin-1*. Voltage-gated ion channels pharmacology was performed on *Xenopus* oocytes expressing potassium and sodium channels as described in ref. 44 and in *SI Method 5: Voltage-Gated Ion Channels Pharmacology*. Trypsin inhibition assays were performed by monitoring hydrolysis of the fluorogenic substrate Mca-R-P-K-P-V-E-NVal-W-R-PK(Dnp)-NH_2 as described in *SI Method 6: Trypsin Inhibition Assays*. Cell-based assays were performed on eukaryotic cells expressing

the vasopressin-sensitive receptors as described in *SI Method 7: Cell-Based Assays*. All animal assays were conducted in accordance with the German Animal Protection Law and are described in *SI Method 8: Animal Assays*.

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