Identification of a Forskolin-Like Molecule in Human Renal Cysts

William C. Putnam,* Sarah M. Swenson,* Gail A. Reif,† Darren P. Wallace,† George M. Helmkamp, Jr.,‡ and Jared J. Grantham†‡

**Midwest Research Institute, Kansas City, Missouri; and †Kidney Institute and ‡Department of Biochemistry and Molecular Biology, Kansas University Medical Center, Kansas City, Kansas*

Renal cyst enlargement is increased by adenosine cAMP, which is produced within mural epithelial cells. In a search for modulators of cAMP synthesis cyst fluids from 18 patients with autosomal dominant or recessive polycystic kidney disease (PKD) were analyzed, and in 15 of them, a stable lipophilic molecule that increased cAMP levels, stimulated transepithelial chloride and fluid secretion, and promoted the proliferation of human cyst epithelial cells was characterized. With the use of HPLC-mass spectrometry, a bioactive lipid with the same mass spectral fingerprint, the same chromatographic retention time, and the same biologic properties as forskolin, a widely known, potent adenylyl cyclase agonist, has been isolated and identified within the cyst fluid. Forskolin is synthesized by the plant *Coleus forskohlii*, but its appearance or compounds like it have not been reported in animals. The origin of forskolin in patients with PKD was not revealed by this study. Synthesis by mural cyst epithelial cells or an exogenous source are the most likely possibilities. Forskolin is sold for weight management and as a cardiovascular tonic in health stores and through the Worldwide Web. It is concluded that forskolin may have a role in promoting the enlargement of cysts in autosomal dominant PKD and recommended that patients avoid oral and parenteral preparations that contain this compound.

J Am Soc Nephrol 18: 934–943, 2007. doi: 10.1681/ASN.2006111218

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the progressive enlargement of innumerable cysts (1). The fluid of some cysts contains a potent lipophilic secretogogue/mitogen that (*1*) stimulates cAMP formation in renal epithelial cells, thereby increasing transepithelial chloride and fluid secretion (2), and (*2*) stimulates the proliferation of mural epithelial cells (2–6). Cell proliferation and fluid secretion act in concert to expand individual cysts and thereby cause progressive enlargement of the kidneys (1). In a protracted study, we identified a molecule with chemical features of forskolin, a plant diterpene that commonly is used as a reagent, that is a strong candidate for the elusive cyst fluid agonist.

Materials and Methods

ADPKD Cyst Fluid Collection

Cyst fluids were obtained from discarded ESRD kidneys (one autosomal recessive PKD [ARPKD] and 17 ADPKD, 2 wk to 56 yr old; institutional review board exemption #4) in 14 different states, packed in wet ice, and shipped to the PKD Biomaterials Research Core by expedited delivery (Table 1). Relatively clear fluids from several surface cysts were aspirated, pooled, and centrifuged to remove cellular material, and the supernatant was aliquotted and stored at −10 to −25°C. Sample AD1 (Table 1) was a pooled collection of cyst fluids from kidneys of five different individuals and served as a high-activity reference. Forskolin, purchased from Fisher Scientific (Fair Lawn, NJ) and Sigma (St. Louis, MO), was shown in previous studies from this laboratory to stimulate the formation of cAMP, transepithelial anion and fluid secretion, and cell proliferation in renal epithelia cells, including human ADPKD mural cyst cells and T-84 cells (2,7–12).

Determination of Biologic Activity

Cyst fluid lipids were extracted with ethyl acetate, and the organic phase was dried under nitrogen. Chloroform:methanol (1:1, vol/vol) extraction was equally effective. Polarized, confluent monolayers of T-84 and ADPKD cells on permeable supports were mounted in modified Ussing chambers in isotonic Ringer's solution and maintained at 37°C (2). Cyst fluid extracts were dissolved in 100 μl of DMEM/F12 + 0.1% decomplemented FBS. The anion secretory activity of the original cyst fluid was estimated from the short-circuit current (Isc response (μAmp/cm^2) relative to forskolin (10 μM; *i.e.* percentage of forskolin response [Table 1] (2). As used in these experiments, Isc measures net chloride secretion to a major extent (10,11,13,14)

Mass Spectrometric and Evaporative Light Scattering Detection Experiments

The mass spectrometry (MS) experiments used three chromatographic techniques to introduce the cyst fluid extracts into the MS: Direct-infusion MS (DI-MS), normal-phase liquid chromatography MS (NP-HPLC-MS), and reversed-phase liquid chromatography MS (RP-HPLC-MS).

NP-HPLC chromatography was used to duplicate conditions in a previous study (2). The NP-HPLC analyses were conducted using concurrent MS (NP-HPLC-MS) and evaporative light scattering detection (NP-HPLC-ELSD). The RP-HPLC chromatographic conditions

Received November 8, 2006. Accepted December 7, 2006.

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. Jared J. Grantham, The Kidney Institute, Mailstop 3018, Kansas University Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160. Phone: 913-588-7605; Fax: 913-588-9251; E-mail: jgrantha@kumc.edu

ISSN: 1046-6673/1803-0934

Table 1. Boactivity and relative amounts of a forskolin-like molecule in cyst fluids[a]

Sample	Date Collected	State	Gender	Isc RP (% Forskolin Response)	HPLC-MS (Signal Intensity)
AD8	1993	VA	F	105	3
AD21	1990	OH	F	103	3
AD2	1991	GA	M	96	3
AD20	1991	OR	F	77	3
AD6	1994	KS	M	52	3
AD1[b]	1991	KS, WI, OK, DC	3F, 2M	49	3
AD3	1993	MO	M	40	3
AD24	1994	FL	M	15	1
AD27	1995	NY	M	7	1
AD28	1995	AZ	M	0	1
AD29	1995	NY	Unknown	0	0
AD13	2000	NJ	M	0	0
AD39	1999	MS	M	0	0
AR1	1998	MO	F	25	2

[a]Isc, short-circuit current; MS, mass spectrometry.
[b]Samples from five kidneys pooled.

were developed subsequent to the NP conditions to optimize chromatographic performance and used only MS detection.

DI-MS Experiments

The mass-to-charge ratios (*m*/z or ions) associated with commercial forskolin were obtained by DI-MS on an API-3000 Tandem Triple-Quadrupole Mass Spectrometer equipped with an electrospray ionization source. Solutions of forskolin were introduced into the MS with a Harvard Apparatus syringe pump (Holliston, MA).

Ethyl acetate extracts of cyst fluids were analyzed by high-resolution DI-MS at Washington University Resource for Biomedical and Bioorganic Mass Spectrometry, an National Institutes of Health Research Resource. In these experiments, a quadrupole–time-of-flight MS was used in negative ion mode.

NP-HPLC-MS and NP-HPLC-ELSD

Chromatographic separation was achieved using a Waters μPorasil (10 μm, 125A) normal-phase column (3.9 × 300 mm), isocratic elution with a Shimadzu (SIL-HTc) HPLC module (Columbia, MD), and a mobile phase system of 98:2:0.01 hexane:isopropanol:acetic acid (vol/vol/vol). The flow was set at 1.0 ml/min, and the column effluent was split with approximately 200 μl/min flowing to the MS and approximately 800 μl/min flowing into the ELSD. Before introduction to the mass spectrometer, 25 μl/min of 1:1 (vol/vol) water:isopropanol was added to the split column effluent to enhance ionization. The MS was either an Applied Biosystems/MDS Sciex API-3000 Tandem Triple-Quadrupole Mass Spectrometer (Foster City, CA) or a MicroMass Quattro-LC Tandem Triple-Quadrupole Mass Spectrometer (Waters, Milford, MA). For NP-HPLC-MS, the MS was operated in the full scan, selected ion monitoring, and product ion modes.

RP-HPLC-MS

Chromatographic separation used a Varian Inertsil (5 μm) RP column (2.1 × 50 mm), isocratic elution with a Shimadzu (SIL-HTc) HPLC module and a mobile phase system of 1:1 methanol:water (vol/vol, 200 μl/min flow rate). The MS setup was identical to the NP analyses. MS was conducted in the full scan, selected ion monitoring, product ion, or Multi-Reaction Monitoring modes. In Multi-Reaction Monitoring, the MS monitor responds to a precursor/product pair, a very selective analytic method.

Results

In a series of studies that spanned 14 yr, a lipid fraction that was isolated from ADPKD cyst fluid samples was found to stimulate secretory anion current (Isc) in epithelial monolayers (Table 1) and to promote the proliferation of human ADPKD cyst epithelial cells *in vitro* (2,5,6,12). These biologic activities of cyst fluid samples ranged from nil to levels nearly as great as that caused by a maximally effective concentration of forskolin (10 μM), a reagent that was used coincidentally throughout these studies as a reference agonist. In each bioassay using monolayers that were derived from human ADPKD kidneys, normal human kidney cortex, MDCK, or T-84 cells, forskolin was added to standardize the responsiveness of the respective membranes. Figure 1 illustrates the concentration-dependent Isc response to forskolin of a monolayer of human ADPKD cyst mural epithelial cells. Significant increases in Isc and net fluid secretion were detected at concentrations of forskolin as low as 10^{-8} M.

Nine active cyst fluid samples and four with no detectable biologic activity were used for characterization of the ADPKD agonist by LC-MS. A cyst fluid fraction that contained the biologic activity was purified by preparative thin-layer chromatography (2). This fraction next was subjected to preparative chromatography by NP-HPLC (isocratic), and biologic activity was determined by the Isc method in each peak detected by LC-ELSD (Figure 2). Cyst fluid sample AD2 is a representative example in which the neutral lipid fraction determined by TLC stimulated Isc in T84 cells to a level 96% as great as a maximal concentration of forskolin (Table 1). There were four major peaks and one minor peak in the ELSD chromatogram. It is

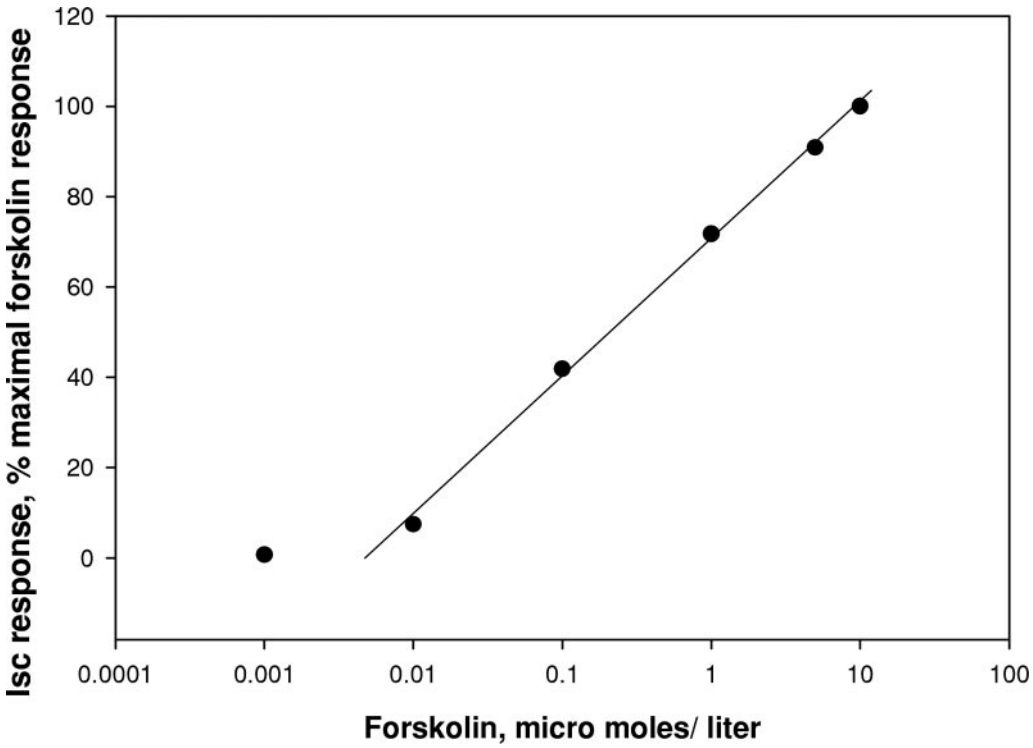

Figure 1. Effect of forskolin on short-circuit current (Isc) of autosomal dominant polycystic kidney disease (ADPKD) epithelial cell monolayers in a typical experiment. Forskolin was added to basolateral medium, and steady-state Isc was recorded after 15 to 20 min (2). Increased Isc was observed at concentrations as low as 10^{-8} M and was maximal at 10^{-5} M. The concentration dependence of cellular cAMP accumulation was similar (2).

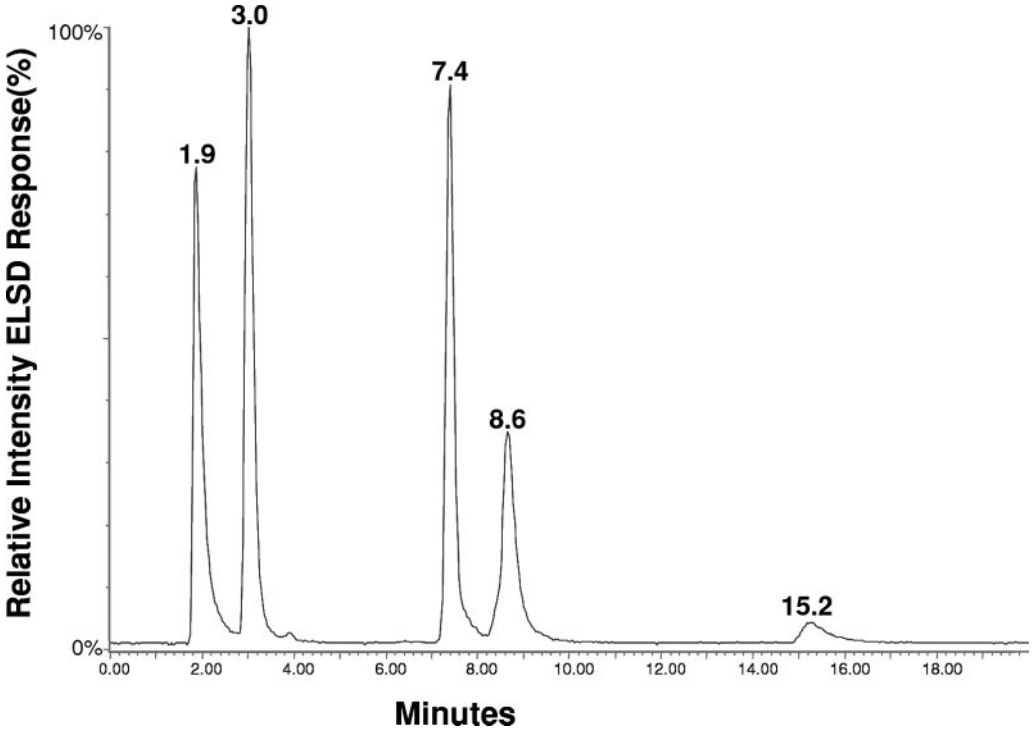

Figure 2. Normal-phase liquid chromatography evaporative light scattering detection (NP-HPLC-ELSD) chromatogram of sample AD2 (Table 1), 50-μl injection volume. Relative intensity is in arbitrary units with 100% set to the value of the greatest peak above baseline in this and subsequent figures. The 8.6- and 15.2-min peaks contained strong biologic activity.

interesting that none of these peaks was observed when an ultraviolet detector was used as opposed to the ELS or the MS detector. The peaks at 8.6 and 15.2 min contained biologic activity.

MS examination (full scan mode, 100 to 1000 *m/z*) of the chromatographic peak at 8.6 min yielded the spectrum depicted in Figure 3A. The LC-MS spectrum of the 8.6-min peak revealed a molecular species with a proposed nominal molecular weight of 410 exhibiting the following mass-to-charge ratios: $[M+H]^+ = 411$, $[M+Na]^+ = 433$, $[M+K]^+ = 449$, and $[2M+H]^+ = 821$. It is interesting that the peak at 15.2 min also produced a mass spectrum similar to the peak at 8.6 min.

Several of the cyst fluids listed in Table 1 had similar chromatograms and mass spectra, and most exhibited these biologically active peaks at approximately 8.6 and 15.2 min. The relative areas of these two peaks were different among the various cyst fluids; however, the mass spectra for each peak (*i.e.*, fragmentation patterns) were identical. Exact comparison of LC-MS responses between samples was complicated by the fact that samples were analyzed on different days during a protracted interval (3 yr), so we used a semiquantitative rating scale (0 = nil detection, 1 = detected, 2 = intermediate, 3 = strong) to judge the relative strengths of the various cyst fluid responses.

To clarify the structure of the putative active lipid, we analyzed cyst fluids by NP-HPLC-MS/MS in product ion mode to determine the collision-induced dissociation products of 411 $[M+H]^+$ (*i.e.*, which fragments are produced when the molecule is broken up). Identical fragmentation patterns were observed in AD8 and AD2 (Figure 3) and in all of the cyst fluids that contained biologically active material. From the repetitive pattern of m/z separated by 18 Da, it was postulated that the molecule contained multiple hydroxyl moieties; however, a complete structure could not be determined readily from the fragmentation pattern alone.

To optimize the chromatography, we developed an RP-HPLC method. This adjustment reduced the analysis time and provided superior chromatography (resolution and stability). The analysis also was simplified by use of selected ion monitoring (SIM) detection for the known ions that were associated with this molecule. Typically, positive electrospray ionization was used (SIM sum of m/z 411($[M+H]^+$), 428($[M+NH_4]^+$), 433($[M+Na]^+$), and 375 (predominate fragment). Figure 4 depicts the RP-HPLC-MS analysis of three ADPKD cyst fluids that contained high biologic activity material and the widely different levels of the two peaks of interest.

To sharpen the molecular weight assignment of the putative lipid, we analyzed ethyl acetate extracts of cyst fluids AD8 and AD20 by DI–quadrupole–time-of-flight MS in the negative-ion mode. Mass-to-charge ratios of 409.2237 and 409.2207 were observed for the deprotonated molecular ions ($[M-H]^-$), respectively. A molecular mass of 409.2226 was postulated for the deprotonated species (from the most probable molecular formula of $C_{22}H_{33}O_7$); therefore, a tentative molecular formula of $C_{22}H_{34}O_7$ was selected for the compound of interest. A database search of this molecular formula turned up forskolin, the same laboratory agent that we had used to standardize the bioassays.

To investigate whether the molecular species in cyst fluid was forskolin, we analyzed reagent-grade forskolin using DI-MS, NP-HPLC-MS, and RP-HPLC-MS. All three MS techniques provided a similar MS m/z pattern for forskolin as was observed in the cyst fluid extracts. It is interesting that the chromatographic analysis of commercial forskolin by both NP-HPLC-MS and RP-HPLC-MS yielded only a single chromatographic peak (Figure 3C).

To explore the nature of the two biologically active chromatographic peaks in cyst fluids, we incubated forskolin in cyst fluid that lacked biologic activity and in water at room temperature (Figure 5). Immediately after addition of commercial forskolin to the inactive cyst fluid blank, a single peak was found by HPLC-MS; however, approximately 4 h later, a second peak (species) that increased in magnitude for at least 144 h developed. The second peak corresponds chromatographically to the second peak that was observed in the active cyst fluids. By contrast, a second peak did not develop after addition of forskolin to nonbuffered HPLC water for the same length of time. The inactive cyst fluid was not equilibrated with CO_2; consequently, the pH exceeded 7.5. To explore the effect of pH on the stability of forskolin, we incubated the commercial compound at room temperature for 18 h in the following buffers: 25 mM potassium hydrogen phthalate (pH 4.0), 25 mM dibasic sodium phosphate/monobasic potassium phosphate (pH 7.0), and 25 mM potassium carbonate/potassium borate (pH 10.0; Figure 6). A single peak at 6.8 min was observed in the pH 4.0 solution. At pH 7.0, a large peak appeared at 6.9 min as well as a small peak at 5.5 min. At pH 10.0, the later peak (7.0 min) was strikingly diminished, whereas the earlier peak (5.5 min) increased to a large extent.

Examination of the mass spectra that were associated with the two HPLC peaks of biologically active cyst fluids revealed a small difference in the mass spectrum of the later eluting peak of the NP chromatograms (this is the earlier peak in the RP chromatograms). The second peak in the NP-HPLC contained much higher levels of species with the mass-to-charge ratios of 821. An *m/z* of 821 Da corresponds to a dimer of forskolin $[2Forskolin+H]^+$. In consideration of the pH-dependent changes in mobility, we think that the second peak of the NP-HPLC probably is a biologically active solution-phase dimer of forskolin.

We determined the sensitivity and the linearity of the RP-HPLC-MS method for quantifying forskolin in cyst fluid and other biologic solutions. Forskolin was added to biologically inactive and spectrally negative cyst fluids. RP-HPLC-MS analysis of these standardized fluids produced a linear response over the concentration range 10 to 1000 ng/ml. The limit of detection (*i.e.*, signal-to-noise >10:1) for forskolin in cyst fluid was approximately 10 ng/ml (0.5 ng for a 50-μl sample injection; 2.4×10^{-8} mol/L).

The relative amounts of forskolin that were determined by LC-MS in 13 cyst fluids were compared with the biologic activity of the respective cyst fluids that were determined by Isc analysis (Table 1). The forskolin candidate was detected by

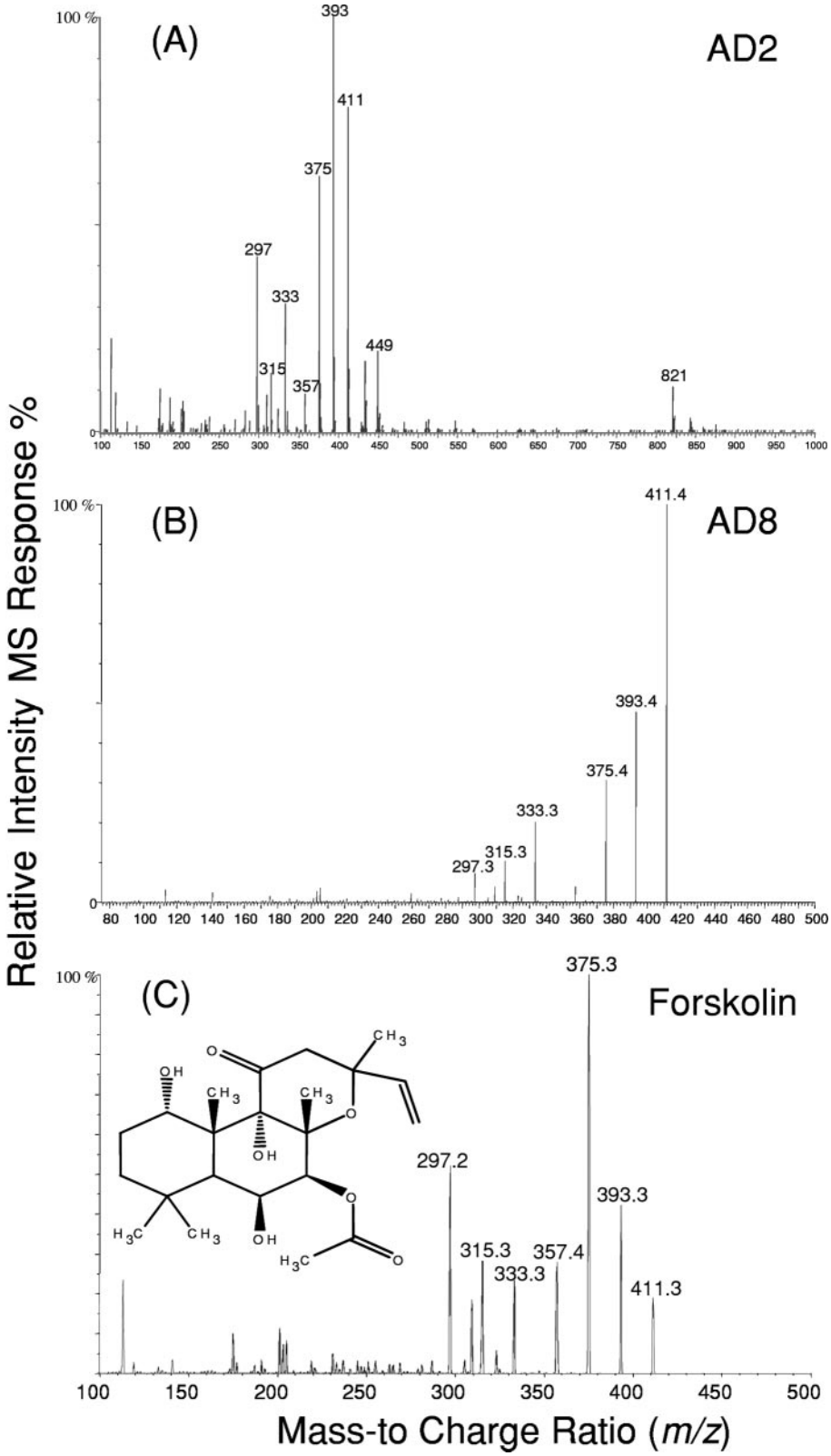

Figure 3. Positive electrospray ionization mass spectrometry (MS) analysis of cyst fluid extracts and forskolin. (A) Mass spectrum associated with the peak observed at a retention time of 8.6 min in NP-HPLC-ELSD chromatogram of HPLC fraction derived from AD2 (Table 1). (B) NP-HPLC-MS/MS product ion mass spectrum of mass-to-charge ratio (*m/z*) 411.4 in 8.6-min peak from HPLC fraction derived from AD8 (Table 1). (C) Direct-infusion MS analysis of commercial forskolin. Daughter-ion fragments match those in AD2 in A and AD8 in B. Inset depicts structure of forskolin with an exact molecular weight of 410.2305.

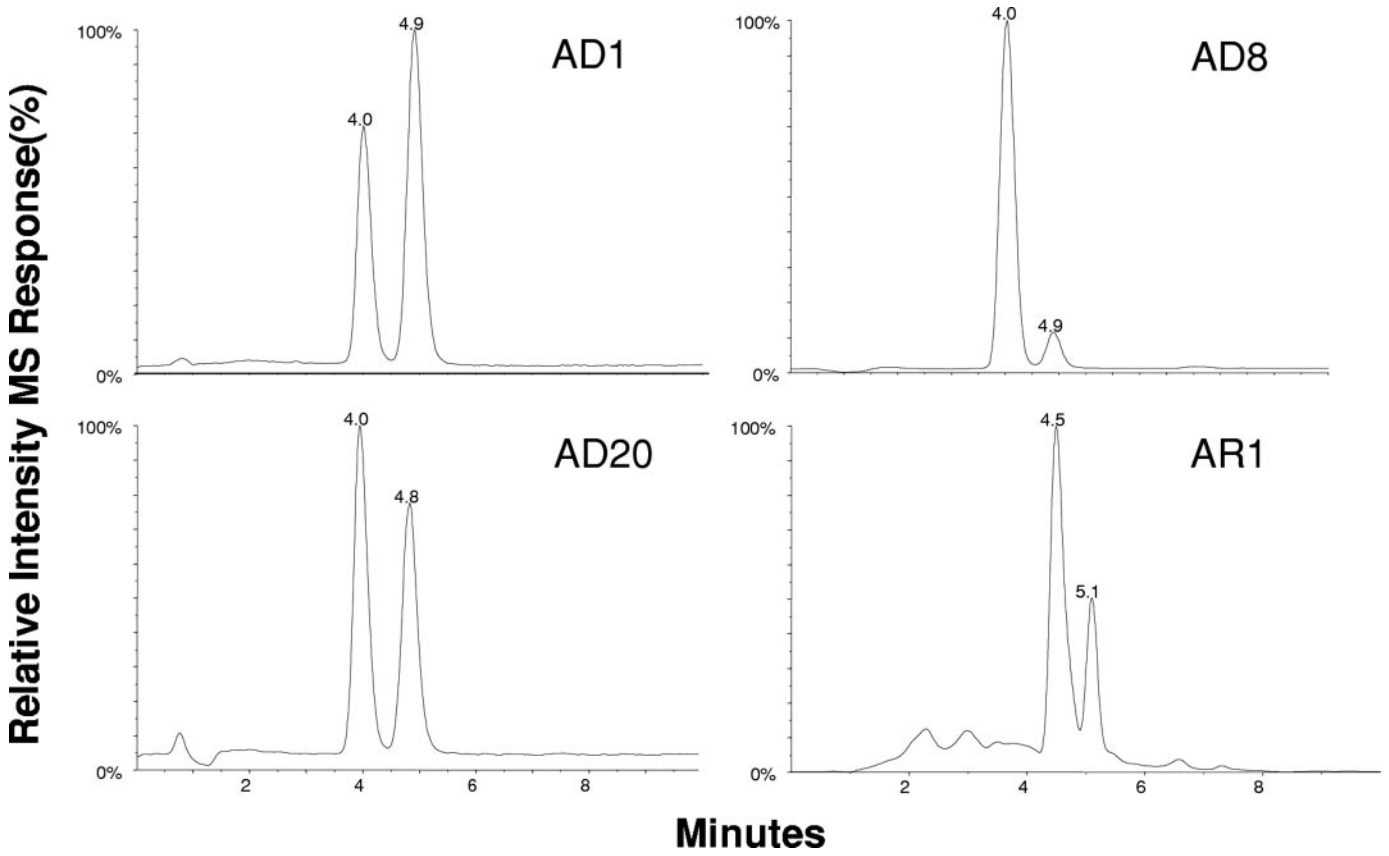

Figure 4. Reversed-phase (RP)-HPLC-MS analysis selected ion monitoring (SIM detection mode) of high-activity ADPKD cyst fluids. Samples AD1, AD8, and AD20 were analyzed with positive electrospray ionization and detection in SIM mode including the sum of *m/z* 411($[M+H]^+$), 428($[M+NH_4]^+$), 433($[M+Na]^+$), and 375 (predominate fragment). Two peaks at approximately 4 and approximately 5 min typically were observed; relative heights of each peak varied from patient to patient. High signal: Baseline noise indicates relatively high levels of candidate molecule. AR1 cyst fluid was analyzed by RP-HPLC-MS in Multi-Reaction Monitoring (MRM) detection mode. In MRM detection mode, the MS monitored precursor/product pairs 411.4 to 375.0, 411.4 to 393.4, and 428.4.5 to 375.0. Two peaks were observed with more baseline noise, consistent with a lower concentration of forskolin-like material than in AD1, AD8, or AD20.

HPLC-MS in 10 of the samples, and in seven of these, the biologic activities, expressed in relation to forskolin, were relatively high. Allowing for dilution factors in sample preparation, we estimate the forskolin concentrations of the original cyst fluids from which these samples were obtained to be in the range of 1 to 20 μM (0.4 to 8 μg/ml). In samples AD24 and AD27, the biologic activities and HPLC-MS intensities were lower; the forskolin candidate was not detected in samples AD13, AD28, or AD29 that lacked detectable biologic activity. In sample AD28, a 411 *m/z* signal was detected by HPLC-MS, but no biologic activity was observed.

To determine whether the forskolin-like molecule is limited to ADPKD cysts, we examined cyst fluid from a 2-wk-old child with ARPKD, a different and more rare genetic condition with a more rapid renal course (15) (Table 1). Isc analysis revealed moderately strong biologic activity, and LC-MS, in multireaction monitoring mode, produced a signal of intermediate intensity (Figure 4).

Discussion

The similarities of the HPLC retention times, MS fingerprints, dimeric behaviors, and biologic activities suggest—but do not prove—that the bioactive lipids in cyst fluid and forskolin are chemically identical. Forskolin, a lipophilic labdane diterpene, increases the enzymatic production of cAMP through direct activation of adenylyl cyclase (16). For decades, forskolin has been an invaluable laboratory reagent for intracellular cAMP activation (17). Considering that mammalian adenylyl cyclases have a forskolin-binding pocket (16), it is reasonable to consider the existence of naturally produced forskolin-like compounds in animals. Nevertheless, evidence for an endogenous source of forskolin in animals has not been reported in more than 16,000 "forskolin" citations in PubMed.

Why has it taken so long to discover an endogenous forskolin-like compound? The answer possibly lies in the small amounts that may be accumulated within normal tissues and body fluids and the extraordinary power of forskolin to activate adenylyl cyclase in vanishingly small amounts. It is fortuitous that ADPKD creates isolated, expanding epithelial sacs filled with relatively stagnant fluid in which chemicals may accumulate. The forskolin-like molecule seems to dimerize in alkaline cyst fluids, and early work suggested that the lipophilic substance may bind reversibly to macromolecules within cysts,

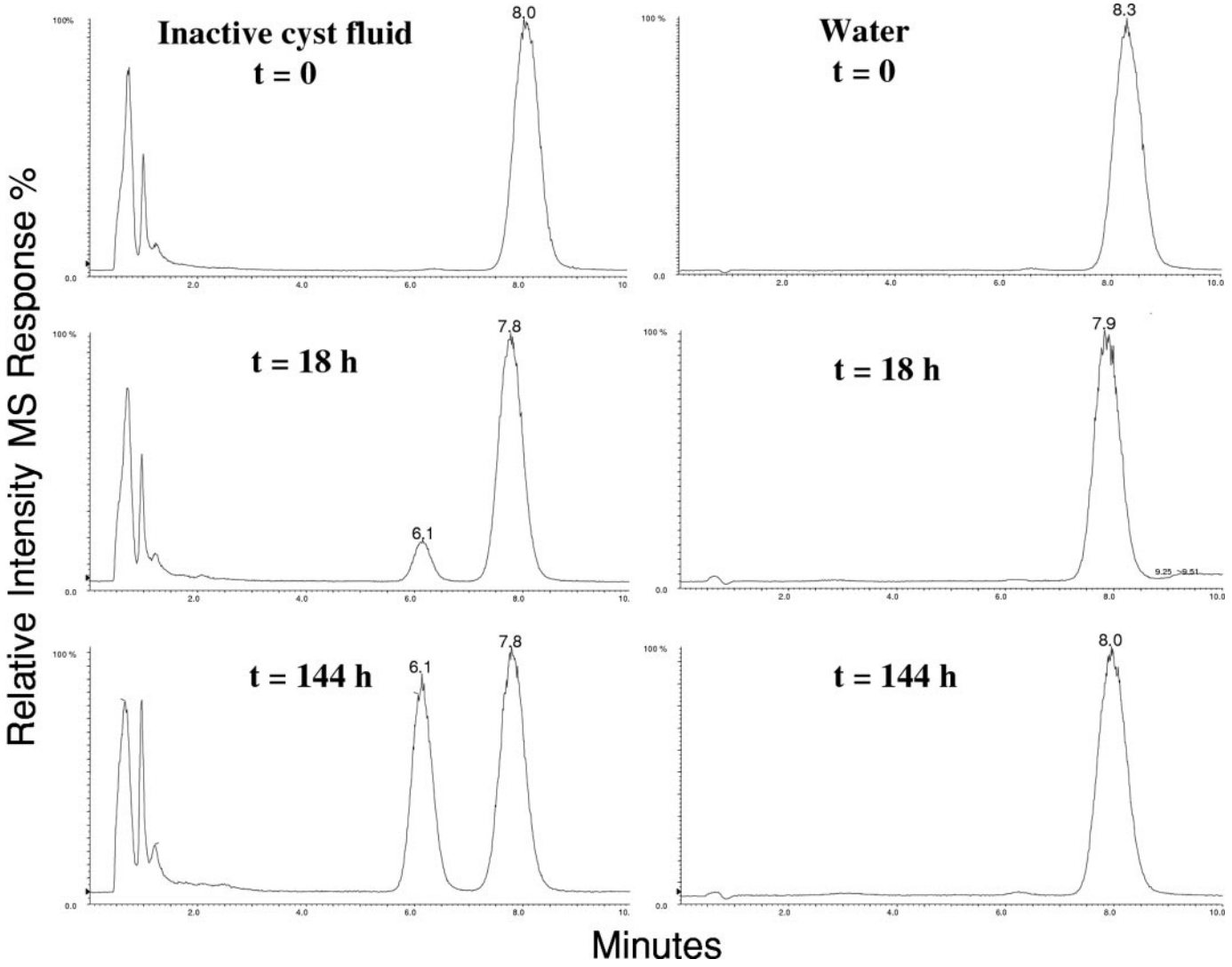

Figure 5. RP-HPLC-MS time course analysis (SIM detection mode) of forskolin after incubation in inactive (blank) cyst fluid or distilled water. Commercial forskolin was spiked into blank cyst fluid or water and incubated for 0, 18, and 144 h at room temperature. Two peaks developed in inactive cyst fluid, but only a single peak developed in water.

factors that would favor trapping. It is in reservoirs such as these that scarce biologically active compounds are likely to be discovered. Because we were searching specifically for a lipid in cyst fluid that activates adenylyl cyclase and had excluded the usual suspects (prostaglandins [2]), we were forced to look for anonymous substances. In light of the fact that we chose forskolin as a reference compound for this series of studies, it is ironic to discover that a forskolin-like substance may be the lipophilic material that had eluded us for more than 14 yr.

Does the forskolin-like compound mimic a xenobiotic, or is it synthesized by renal cells or elsewhere in the body? One of the key branch-point enzymes used in forskolin biosynthesis by the plant *Coleus forskohlii*, all-*trans*-geranylgeranyl diphosphate synthase (18), also is found in rat (19) and human (20) kidneys. Unfortunately, however, none of the synthetic enzymes in the path from all-*trans*-geranylgeranyl diphosphate synthase to forskolin has been reported in plants or animals, precluding a direct examination for *de novo* production. Although unconventional, it is conceivable that forskolin may be synthesized in human kidneys, similar to the appearance of ouabain in adrenal tissue (21) and catecholamines in plants (22). It is interesting to note that the isolation from an animal source of ouabain, another scarce chemical that is synthesized by plants, began with the extraction of 20 kg of fresh bovine adrenal tissue (21). Proof of forskolin synthesis in animal tissues also may be a daunting task if our antecedent experience with this nefarious lipid is any guide.

It is unlikely that forskolin-like activity that was detected in the cyst fluids came from forskolin-containing dietary supplements or from administration of therapeutic products to the patients during hospitalization. First, most of the cyst fluids with the highest biologic activities were collected from 1990 to 1995, before there was widespread, unregulated medicinal use of forskolin in the United States. Second, relatively high levels of forskolin-like activity were discovered in the cyst fluid of a 2-wk-old infant with ARPKD.

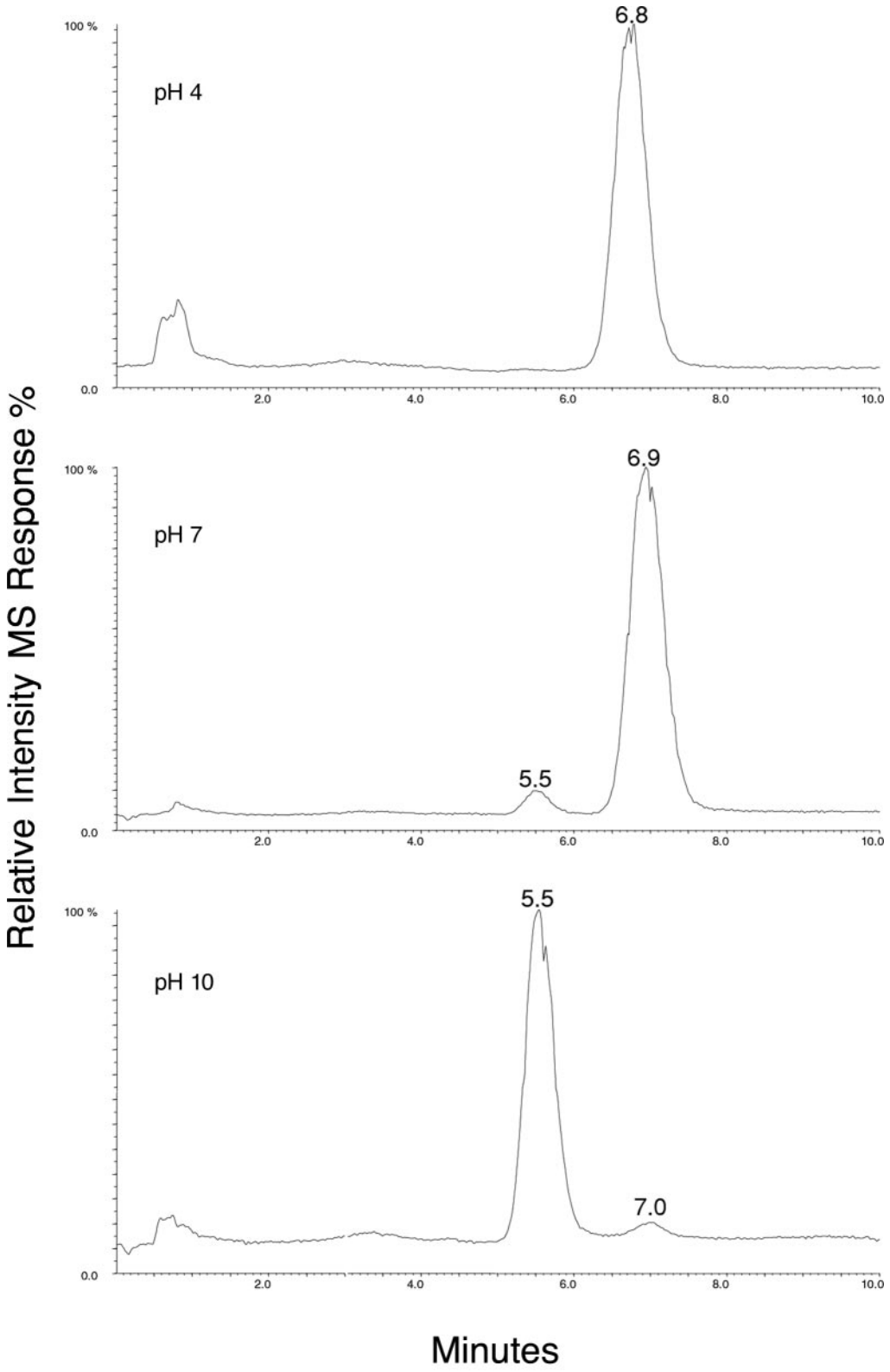

Figure 6. Effect of pH on development of forskolin peaks. RP-HPLC-MS analysis (SIM detection mode) of freshly prepared forskolin when added to buffered water and incubated at room temperature for 18 h. Major peaks were observed at 6.8 (pH 4), 6.9 (pH 7), and 5.5 min (pH 10). Smaller peaks were observed at 5.5 (pH 7) and 7.0 min (pH 10).

cAMP is a major growth factor in PKD progression through its effects to stimulate fluid secretion into the cysts and its mitogenic effect on mural epithelial cells (23). Recent studies of hereditary polycystic disease in rodents showed that suppres-

sion of renal cAMP through the inhibition of vasopressin V_2 receptors (24–26) or suppression of circulating vasopressin levels (27) strikingly reduced renal enlargement that was caused by cyst expansion. In each model, the reduction of cAMP levels decreased the rate of kidney enlargement but did not eliminate growth. The accumulation of forskolin-like material within cyst mural cells may contribute to renal enlargement in the absence of G protein–mediated activation of adenylyl cyclase. More likely, however, is the possibility that relatively low levels of forskolin might potentiate the growth effects of a wide array of receptor-mediated G-protein activators of adenylyl cyclase that are known to have an impact on the kidneys (17).

Conclusion

The disposition of forskolin within body fluids and tissues in mammals or lower species is poorly understood. Nothing in the published literature describes the absorption of forskolin from dietary sources or the renal accumulation or excretion of forskolin in humans or lower animals. Nonetheless, years after the collection of cyst fluid samples that were used in this study, forskolin is marketed in weight loss preparations and as a cardiovascular tonic in health stores and on the Worldwide Web. Although there is much more to learn about the origin, chemistry, pharmacology, and pathophysiologic effects of forskolin in the human body, it is clear that this substance should not be used in any form by patients with PKD.

Acknowledgments

This work was supported by program project grant P50 DK 53763 (J.G.) and PKD Center Grant P01 DK 57301 (J.G., D.W.) from the National Institutes of Health and by grants from the Kansas University Medical Center-Midwest Research Institute Consortium of the Kansas City Life Sciences Institute and the PKD Foundation 112a2r (D.W.).

We thank Hong Li, Jacqueline Tremblay, Richard Grabbe, Lorraine Rome, Leslie Moody, Terry O'Neill, and Anne-Marie Hedge for technical assistance. We thank Texas Tech University Health Sciences Center's School of Pharmacy, where confirmatory mass spectral experiments were conducted. Quadrupole–time-of-flight MS was provided by the Washington University Mass Spectrometry Resource with support from the National Institutes of Health National Center for Research Resources (grant P41RR0954).

Disclosures

None.

References

1. Grantham JJ, Chapman AB, Torres VE: Volume progression in autosomal dominant polycystic kidney disease: The major factor determining clinical outcomes. *Clin J Am Soc Nephrol* 1: 148–157, 2006
2. Belibi FA, Reif G, Wallace DP, Yamaguchi T, Olsen L, Li H, Helmkamp GM Jr, Grantham JJ: Cyclic AMP promotes growth and secretion in human polycystic kidney epithelial cells. *Kidney Int* 66: 964–973, 2004
3. Ye M, Grant M, Sharma M, Elzinga L, Swan S, Torres VE, Grantham JJ: Cyst fluid from human autosomal dominant polycystic kidneys promotes cyst formation and expansion by renal epithelial cells in vitro. *J Am Soc Nephrol* 3: 984–994, 1992
4. Grantham JJ: 1992 Homer Smith Award. Fluid secretion, cellular proliferation, and the pathogenesis of renal epithelial cysts. *J Am Soc Nephrol* 3: 1841–1857, 1993
5. Grantham JJ, Ye M, Davidow C, Holub B, Sharma M: Evidence for a potent lipid secretagogue in the cyst fluids of patients with autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 6: 1242–1249, 1995
6. Grantham JJ, Schreiner GF, Rome L, Grenz L, Joly A: Evidence for inflammatory and secretagogue lipids in cyst fluids from patients with autosomal dominant polycystic kidney disease. *Proc Assoc Am Physicians* 109: 397–408, 1997
7. Ye M, Grantham JJ: The secretion of fluid by renal cysts from patients with autosomal dominant polycystic kidney disease. *N Engl J Med* 329: 310–313, 1993
8. Mangoo-Karim R, Uchic ME, Grant M, Shumate WA, Calvet JP, Park CH, Grantham JJ: Renal epithelial fluid secretion and cyst growth: The role of cyclic AMP. *FASEB J* 3: 2629–2632, 1989
9. Mangoo-Karim R, Uchic M, Lechene C, Grantham JJ: Renal epithelial cyst formation and enlargement in vitro: Dependence on cAMP. *Proc Natl Acad Sci U S A* 86: 6007–6011, 1989
10. Mangoo-Karim R, Ye M, Wallace DP, Grantham JJ, Sullivan LP: Anion secretion drives fluid secretion by monolayers of cultured human polycystic cells. *Am J Physiol* 269: F381–F388, 1995
11. Wallace DP, Grantham JJ, Sullivan LP: Chloride and fluid secretion by cultured human polycystic kidney cells. *Kidney Int* 50: 1327–1336, 1996
12. Grantham JJ, Ye M, Gattone VH 2nd, Sullivan LP: In vitro fluid secretion by epithelium from polycystic kidneys. *J Clin Invest* 95: 195–202, 1995
13. Sullivan LP, Wallace DP, Grantham JJ: Epithelial transport in polycystic kidney disease. *Physiol Rev* 78: 1165–1191, 1998
14. Sullivan LP, Wallace DP, Grantham JJ: Chloride and fluid secretion in polycystic kidney disease. *J Am Soc Nephrol* 9: 903–916, 1998
15. Ward CJ, Hogan MC, Rossetti S, Walker D, Sneddon T, Wang X, Kubly V, Cunningham JM, Bacallao R, Ishibashi M, Milliner DS, Torres VE, Harris PC: The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat Genet* 30: 259–269, 2002
16. Tesmer JJ, Sunahara RK, Gilman AG, Sprang SR: Crystal structure of the catalytic domains of adenylyl cyclase in a complex with Gsalpha. GTPgammaS. *Science* 278: 1907–1916, 1997
17. Insel PA, Ostrom RS: Forskolin as a tool for examining adenylyl cyclase expression, regulation, and G protein signaling. *Cell Mol Neurobiol* 23: 305–314, 2003
18. Engprasert S, Taura F, Kawamukai M, Shoyama Y: Molecular cloning and functional expression of geranylgeranyl pyrophosphate synthase from Coleus forskohlii Briq. *BMC Plant Biol* 4: 18, 2004
19. Ericsson J, Runquist M, Thelin A, Andersson M, Chojnacki T, Dallner G: Distribution of prenyltransferases in rat tissues. Evidence for a cytosolic all-trans-geranylgeranyl diphosphate synthase. *J Biol Chem* 268: 832–838, 1993
20. Kainou T, Kawamura K, Tanaka K, Matsuda H, Kawamukai M: Identification of the GGPS1 genes encoding gera-

nylgeranyl diphosphate synthases from mouse and human. *Biochim Biophys Acta* 1437: 333–340, 1999
21. Schneider R, Wray V, Nimtz M, Lehmann WD, Kirch U, Antolovic R, Schoner W: Bovine adrenals contain, in addition to ouabain, a second inhibitor of the sodium pump. *J Biol Chem* 273: 784–792, 1998
22. Van Alstyne KL, Nelson AV, Vyvyan JR, Cancilla DA: Dopamine functions as an antiherbivore defense in the temperate green alga Ulvaria obscura. *Oecologia* 148: 304–311, 2006
23. Torres VE: Cyclic AMP, at the hub of the cystic cycle. *Kidney Int* 66: 1283–1285, 2004
24. Gattone VH 2nd, Maser RL, Tian C, Rosenberg JM, Branden MG: Developmental expression of urine concentration-associated genes and their altered expression in murine infantile-type polycystic kidney disease. *Dev Genet* 24: 309–318, 1999
25. Gattone VH 2nd, Wang X, Harris PC, Torres VE: Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. *Nat Med* 9: 1323–1326, 2003
26. Torres VE, Wang X, Qian Q, Somlo S, Harris PC, Gattone VH 2nd: Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. *Nat Med* 10: 363–364, 2004
27. Nagao S, Nishii K, Katsuyama M, Kurahashi H, Marunouchi T, Takahashi H, Wallace DP: Increased water intake decreases progression of polycystic kidney disease in the PCK rat. *J Am Soc Nephrol* 17: 2220–2227, 2006