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Details of the Collaborative Activity

2020-21

Name of the Collaborating Institute: Epithelial Systems Biology Laboratory, National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), Bethesda, USA

Name of the Collaborating Department: Yenepoya Research Center

Activities:

Collaborative Research projects were undertaken with Dr. Mark Knepper, Epithelial Systems Biology Laboratory, National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), Bethesda, USA and Dr. Arnab Datta, Yenepoya Research Center and the outcome includes joint research publications.

Joint Publications

1. **Datta A,** Yang CR, Salhadar K, Park E, Chou CL, Raghuram V, Knepper MA. Phosphoproteomic identification of vasopressin-regulated protein kinases in collecting duct cells. *British Journal of Pharmacology*. 2021 Mar;178(6):1426-44.
2. Chen L, Jung HJ, **Datta A**, Park E, Poll BG, Kikuchi H, Leo KT, Mehta Y, Lewis S, Khundmiri SJ, Khan S. Systems Biology of the Vasopressin V2 Receptor: New Tools for Discovery of Molecular Actions of a GPCR. *Annual Review of Pharmacology and Toxicology*. 2021 Sep 27;62
3. **Datta A**, Yang CR, Limbutara K, Chou CL, Rinschen MM, Raghuram V, Knepper MA. PKA-independent vasopressin signaling in renal collecting duct. *Federation of American Societies for Experimental Biology (FASEB)*. 2020

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Annual reviews revisions.

Knepper, Mark A (NIH/NHLBI) [E] <knepperm@nhlbi.nih.gov> Wed, Mar 24, 2021 at 11:49 PM
To: "Chen, Lihe (NIH/NHLBI) [C]" <lihe.chen@nih.gov>, Hyun Jun Jung <hjung24@jhmi.edu>, "arnabdattaju@gmail.com" <arnabdattaju@gmail.com>

Dear Lihe, Hyun Jun and Arnab –

The review of the Annal Review paper asks for a new figure or table summarizing the omic techniques. I am attaching a draft. Please make corrections or additions in red and send back. Arnab will do the edits.

Arnab has already revised the original Table 1 which will become Table 2.

Mark

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Table 1 new.xlsx
11K

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RESEARCH PAPER

BJP BRITISH PHARMACOLOGICAL SOCIETY

Phosphoproteomic identification of vasopressin-regulated protein kinases in collecting duct cells

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Background and Purpose: The peptide hormone vasopressin regulates water transport in the renal collecting duct largely via the V_2 receptor, which triggers a cAMP-mediated activation of a PKA-dependent signalling network. The protein kinases downstream from PKA have not been fully identified or mapped to regulated phosphoproteins.

Experimental Approach: We carried out systems-level analysis of large-scale phosphoproteomic data quantifying vasopressin-induced changes in phosphorylation in aquaporin-2-expressing cultured collecting duct (mpkCCD) cells. Quantification was done using stable isotope labelling (SILAC method).

Key Results: Nine thousand six hundred forty phosphopeptides were quantified. Stringent statistical analysis identified significant changes in response to vasopressin in 429 of these phosphopeptides. The corresponding phosphoproteins were mapped to known vasopressin-regulated cellular processes. The vasopressin-regulated sites were classified according to the sequences surrounding the phosphorylated amino acids giving 11 groups. Among the vasopressin-regulated phosphoproteins were 25 distinct protein kinases. Among these, six plus PKA appeared to account for phosphorylation of about 81% of the 313 vasopressin-regulated phosphorylation sites. The six downstream kinases were salt-inducible kinase 2 (Sik2), cyclin-dependent kinase 18 (Cdk18), calmodulin-dependent kinase kinase 2 (Camkk2), protein kinase D2 (Prkd2), mitogen-activated kinase 3 (Mapk3) and myosin light chain kinase (Mylk).

Conclusion and Implications: In V_2 receptor-mediated signalling, PKA is at the head of a complex network that includes at least six downstream vasopressin-regulated protein kinases that are prime targets for future study. The extensive phosphoproteomic data reported in this study are provided as a web-based data resource for future studies of GPCRs. [Correction added on 4 March 2021, after first online publication: The first sentence in the Key Results was corrected in this current version.]

KEYWORDS
Camkk2, Cdk18, GPCR signalling, aquaporin-2-expressing cultured collecting duct cells, Prkd2, Sik2, V_2 receptor signalling

Abbreviations: AKAP, A-kinase anchoring protein; AMPK, 5′-AMP-activated protein kinase; AQP2, aquaporin-2; CREB, cAMP-responsive element binding protein; CV, co-efficient of variation; dDAVP, 1-deamino-8-D-arginine vasopressin; EThcD, electron-transfer/higher-energy collision dissociation; Fe-NTA, ferric nitrilotriacetate; GO, gene ontology; H-89, N-[2-[[3-(4-bromophenyl)-2-propenyl]amino]ethyl]-5-isoquinolinesulfonamide; HCD, higher-energy collision dissociation; IMCD, inner medullary collecting duct; mpkCCD, murine immortalized cortical collecting duct; PDZ, domain present in PSD-95, Dlg and ZO-1; PH, pleckstrin homology; RRID, research resource identifier; SILAC, stable isotope labelling with amino acids in cell culture.

Systems Biology of the Vasopressin V2 Receptor: New Tools for Discovery of Molecular Actions of a GPCR

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Abstract

Systems biology can be defined as the study of a biological process in which all of the relevant components are investigated together in parallel to discover the mechanism. Although the approach is not new, it has come to the forefront as a result of genome sequencing projects completed in the first few years of the current century. It has elements of large-scale data acquisition (chiefly next-generation sequencing–based methods and protein mass spectrometry) and large-scale data analysis (big data integration and Bayesian modeling). Here we discuss these methodologies and show how they can be applied to understand the downstream effects of GPCR signaling, specifically looking at how the neurohypophyseal peptide hormone vasopressin, working through the V2 receptor and PKA activation, regulates the water channel aquaporin-2. The emerging picture provides a detailed framework for understanding the molecular mechanisms involved in water balance disorders, pointing the way to improved treatment of both polyuric disorders and water-retention disorders causing dilutional hyponatremia.

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RESEARCH ARTICLE

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PKA-independent vasopressin signaling in renal collecting duct

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Abstract

Vasopressin regulates renal water excretion by binding to a $G_{\alpha}s$-coupled receptor (V2R) in collecting duct cells, resulting in increased water permeability through regulation of the aquaporin-2 (AQP2) water channel. This action is widely accepted to be associated with cAMP-mediated activation of protein kinase A (PKA). Here, we use phosphoproteomics in collecting duct cells in which PKA has been deleted (CRISPR-Cas9) to identify PKA-independent responses to vasopressin. The results show that V2R-mediated vasopressin signaling is predominantly, but not entirely, PKA-dependent. Upregulated sites in PKA-null cells include Ser256 of AQP2, which is critical to regulation of AQP2 trafficking. In addition, phosphorylation changes in the protein kinases Stk39 (SPAK) and Prkci (an atypical PKC) are consistent with PKA-independent regulation of these protein kinases. Target motif analysis of the phosphopeptides increased in PKA-null cells indicates that vasopressin activates one or more members of the AMPK/SNF1-subfamily of basophilic protein kinases. In vitro phosphorylation assays using recombinant, purified SNF1-subfamily kinases confirmed postulated target specificities. Of interest, measured IBMX-dependent cAMP levels were an order of magnitude higher in PKA-null than in PKA-intact cells, indicative of a PKA-dependent feedback mechanism. Overall, the findings support the conclusion that V2-receptor mediated signaling in collecting duct cells is in part PKA-independent.

KEYWORDS

AMPK, AQP2, cAMP, mpkCCD, phosphoproteomics, PRM-MS

1 | INTRODUCTION

Body water balance is regulated chiefly by the peptide hormone vasopressin, which acts in the kidney to control the rate of water excretion.[1] Vasopressin regulates water excretion chiefly by controlling the osmotic water permeability of collecting duct cells through changes in the abundance and cellular distribution of the water channel aquaporin-2 (AQP2).[2] The permeability changes, therefore, determine whether luminal water is returned to the general circulation (high vasopressin) or excreted in the urine (low vasopressin). The regulation of osmotic water transport in the renal

Abbreviations: AQP2, aquaporin-2; dDAVP, D-amino D-arginine vasopressin; EThcD, electron-transfer/higher-energy collision dissociation; FA, formic acid; GPCR, G-protein coupled receptor; HCD, higher-energy collisional dissociation; IBMX, 3-isobutyl-1-methylxanthine; nLC-MS/MS, nano liquid chromatography-tandem mass spectrometry; PKA, protein kinase A; PRM, parallel reaction monitoring; SILAC, stable-isotopic labeling with amino acids in cell culture; V2R, vasopressin V2 receptor.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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