CRF 8G (Revised Sep 15)

RGC Reference	HKU6/CRF/11G
please insert ref. above	

The Research Grants Council of Hong Kong
Collaborative Research Fund Group Research Projects
Completion Report

(*for completed projects only*)

Part A: The Project and Investigator(s)

1. Project Title

Strategic research of hormones and their receptors in the water homeostatic axis: from molecular mechanisms to anti-hypertensive drug design

體內水平衡相關的激素及其受體之策略研究：從分子機制到抗高血壓藥物的開發

2. Investigator(s) and Academic Department/Units Involved *(please highlight approved changes in the composition of the project team and quote the date when RGC granted approval of such changes)*

Research Team	Name/Post	Unit/Department/Institution	Average number of hours per week spent on this project in the current reporting period
Project Coordinator	Billy K.C. Chow/ Chair Professor	School of Biological Sciences/ University of Hong Kong	5 h
Co-Principal investigator(s)	YS Chan/Professor	Physiology/HKU	2.5 h
	SK Chung/Professor	Anatomy/HKU	2.5 h
	LTO Lee/ RAP	SBS/HKU	2.5 h
	MCM Lin/Professor	Surgery/CUHK	2.5 h
	WY Lui/Assistant Professor	SBS/HKU	2.5 h
	SSM Ng/RAP	SBS/HKU	2.5 h
	HZ Sun/Professor	Chemistry/HKU	2.5 h
	GSW Tsao/Professor	Anatomy/HKU	2.5 h
	WH Yung/Professor	SBS/CUHK	2.5 h
	KKL Yung/Professor	Biology/BU	2.5 h
	AST Wong/Professor	SBS/HKU	2.5 h
Collaborators/ Others			

3. Project Duration

	Original	Revised	Date of RGC Approval *(must be quoted*)
Project Start Date	30/06/2012	--	--
Project Completion Date	29/06/2015	29/12/2015	09/04/2015
Duration (*in month*)	36	42	
Deadline for Submission of Completion Report	29/06/2016	29/12/2016	

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To unravel mechanisms that regulate fluid and salt homeostasis involving SCT, ANGII and VP by molecular, physiological and electrophysiological approaches.

2. To develop novel pharmacological strategies for resistant hypertension and cardiovascular diseases.

5.2 Revised objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

N.A.

6. Research Outcome

6.1 Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

1. Physiology and electrophysiology

The SCT-/- mice showed systemic and pulmonary hypertension after invasive telemetry pressure monitoring and echocardiographic measurements. The pulmonary vascular remodeling and bronchiolar epithelium changes were observed along with significant apoptosis and fibrosis in the lungs and the heart. The pathologies were related to reduced serum nitric oxide and vascular endothelial growth factor as well as increased plasma aldosterone levels. The 3-month-long secretin treatment was able to ratify the pathologies. We found that secretin could induce aldosterone secretion and release in the rat adrenal cortex. The stimulated release of aldosterone by hyperosmolality and hypovolemia was significantly reduced in SCT-/- mice. Our finding indicates that secretin pathway is tightly related to the aldosterone production in the adrenal cortex. Furthermore, secretin was found to be involved in sodium conservation through the renin angiotensin aldosterone system, and SCTR is important for aldosterone production and release. As the more recent study in rats in vivo demonstrated that the effect of SCT on PVN neurons is heterogeneous, our electrophysiology recordings have confirmed the heterogeneous electrophysiological response to SCT in the PVN.

2. Receptor dimerization and signaling

We have shown the presence of receptor specific hetero-complexes of SCTR and AT1aR since SCTR can form heteromers with AT1aR but not with AT2R. We found that the basal cAMP and Emax value were significantly dropped in SCTR/AT1aR co-transfected cells, while there were no significant differences in EC50 values. The data suggests that the presence of AT1aR in the system stabilizes/favors an inactive conformation of SCTR. The inactive conformation of AT1aR dramatically reduces the efficacy of SCTR within the hetero-complex. We also showed that SCTR selectively heterodimerizes with AVPR2. This interaction of SCTR/AVPR2 was found to modulate the effect of hormones on cellular cAMP responses. A decrease in maximal response and lower potency for Vp were found in SCTR/AVPR2 cells treated.

3. New antihypertensive candidate search

We created a 3D homology model of human SCTR using multiple template approach and validated for structural orientation and disulfide bridges. The 3D structure for secretin and its analogs were also generated from PACAP as primary template and VIP, GIP as supporting templates for human secretin homology modeling and the secretin analogs were generated using secretin homology model. Virtual docking predicts binding at the constrained region of these analogs and loss of binding in the unconstrained region. These results suggest that both the N-terminal and C-Terminal portions are essential for binding and activation. We also demonstrated that the transmembrane peptides were able to inhibit dimerization of SCTR and AT1aR by suppression of hyperosmolality –induced drinking through ICV-injection of ATM-1 peptide. Moreover, we found that SCT/ANGII could induce VP release and this phenomenon was attenuated by ATM-4 and STM-II central injection.

4. Antihypertensive efficacy testing

The potential molecules were prioritized based on binding energies and known functional activity and found that Glycyrrhizic acid (GA) was in the third position in binding affinity. It is the only molecule with secretin-like functions as well and thus screened for binding affinity and functional activity. GA's effects on blood pressure and heart rate were analyzed. The systolic and the diastolic blood pressure was reduced to 48.6 +/- 2.9 % and 38.0 +/- 2.6 % respectively. The heart rate dropped to 50.6 +/- 7.78 % and thus GA was proposed to be a SCT sensitizer to modulate SCT's effects on blood pressure and water/salt homeostasis.

6.2 Potential for further development of the research and the proposed course of action *(maximum half a page)*

The study was able to extend the area of research for the development of pulmonary hypertension and cardiac pathologies. This let us secure HKD 1,100,302 from GRF17127215 to study the details molecular mechanisms underlying the progressive development of pulmonary arterial hypertension in secretin knockout mice. The progress of pulmonary hypertension research is encouraging and it can expend our overall research to pulmonology and cardiology as well.

It was reported that secretin deficiency in heart failure patients and we also found significant apoptosis and fibrosis in the heart of secretin deficient mice. Currently, we are working on these phenotypes and could be flourished into cardiac apoptosis and failure research. Further development will be focused on preclinical experiments in translational aspect such as genetic testing and patient sample collection for pulmonary and systemic hypertension and heart failure.

Since secretin is naturally produced after food intake for counter acidity and can influence on water drinking pattern and body fluid homeostasis, we also would like to expend our research on how changes in food and water intake pattern could influence secretin release and effect on hypertension and heart disease using animal models such as spontaneous hypertensive rats (SHR). This research will be helpful for general public and life style management for the patients with hypertension and cardiovascular diseases.

6.3 Research collaboration achieved *(please give details on the achievement and its relevant impact)*

1. Luncheon meetings

Several luncheon meetings were hosted by PC. Both PC and Co-Is usually discussed the progress of research, possible ways for better collaboration and future potentials.

2. Symposiums

Two-day symposiums with average ten presentations per day were held in every three months. All the postgraduate students and post-doctoral fellows required to present and had a chance to discuss with supervisors from different specialized fields. These symposiums serve as brainstorming sessions and were productive not only for the project itself but also for every participant.

3. Research and training exchange

The studies related to core objectives are carried out in respective laboratories as described in the proposal. Since each lab has its own specialized things such as instruments or skilled persons, the postgraduate students and post-doctoral fellows have to travel other collaborated laboratories for experiments or to learn the necessary techniques.

4. New Collaboration:
   a. Professor David Vaudry, University of Rouen, France.
      Peptide agonist design and synthesis
   b. Professor Lawrence J Miller, Mayo Clinic
      GPCR dimerization and signaling
   c. Prof. Huang Yu, The Chinese University of Hong Kong
      Hypertension studies

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

The cardiovascular diseases (CVDs) are the leading cause of death globally and in Hong Kong. Hypertension is one of the most important risk factors for CVDs, and approximately 1 billion people were affected worldwide. Confident with our knowledge in hormones, body water balance, bioinformatics, and medicine, we performed a well-constructed study to unravel the complicated mechanisms as well as to develop pharmacological strategies for hypertension and heart diseases. We found that secretin deficiency could contribute systemic and pulmonary hypertension as well as heart and lungs pathologies and are related to reduced nitric oxide and vascular endothelial growth factor and increased plasma aldosterone levels. Encouragingly, the secretin treatment could prevent the pathologies. The secretin receptor was important for aldosterone production and deficiency showed impaired aldosterone synthesis. We successfully created a secretin receptor 3D model and showed that both N and C terminals of SCT peptide sequence are important for the receptor binding and activation. Interestingly, Glycyrrhizic acid could stimulate secretin release and subsequently reduce the blood pressure and showed its potential as an antihypertensive. Overall, the study could decipher the enigma of hypertension and heart diseases and bring potentials treatments and preventive ideas as well.

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Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and Journal/Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC	Attached to this report *(Yes or No)*	Acknowledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance	Under Review	Under Preparation (optional)						
			✓	AM Zaw, R Sekar, HKW Law, BKC Chow*	Secretin is an important regulator of nitric oxide-mediated cardiovascular functions.		Yes	Yes	No
	2016			AM Zaw, CM Williams; HKW Law, BKC Chow*	Minimally invasive transverse aortic constriction in mice. **JOVE**		Yes	Yes	No
		✓		Juan Bai, BKC Chow*	Secretin is involved in sodium conservation through the renin-angiotensin-aldosterone system. **FASEB J**		Yes	Yes	No
2016				K Singh, AM Zaw, R Sekar, A Palak, AA Allam, J Ajarem, BKC Chow*	Glycyrrhizic Acid Reduces Heart Rate and Blood Pressure by a Dual Mechanism. **Molecules.** 21(10): 1291.		Yes	Yes	Yes
2016				Hans K. H. Ng, Kaleeckal G. Harikumar, Laurence J. Miller, BKC Chow*	Signaling Modification by GPCR Heteromer and Its Implication on X-Linked Nephrogenic Diabetes Insipidus. **PloS One.** 11(9): e0163086.		Yes	Yes	No
2016				R Sekar, K Singh, AWR Arokiaraj, BKC Chow*	Pharmacological Actions of Glucagon-Like Peptide-1, Gastric Inhibitory Polypeptide, and Glucagon. **Int. Rev. Cell Mol. Biol.** 326: 279-341.		Yes	Yes	Yes

2016				OWH Chua, KKL Wong, BC Ko, SK Chung, BKC Chow, LTO Lee*	Role of nuclear factor of activated T-cells 5 in regulating hypertonic-mediated secretin receptor expression in kidney collecting duct cells. **BBA -Gene Regulatory Mechanism**. 1859(7): 922-32.		Yes	Yes	Yes
2016				JJ Bai, CD Tan, BKC Chow*	Secretin, at the Hub of Water-Salt Homeostasis. **Am J Physiol-Renal. (In Press)**		Yes	Yes	Yes
2016				K Singh, V Senthil, AWR Arokiaraj, J Leprince, B Lefranc, D Vaudry, AA Allam, J Ajarem, BKC Chow*	Structure-Activity Relationship Studies of N- and C-Terminally Modified Secretin Analogs for the Human Secretin Receptor. **PLoS One.** 11(3): e0149359.		Yes	Yes	Yes
2015				JSW On, C Duan, BKC Chow, LTO Lee*	Functional pairing of class B1 ligand-GPCR in cephalochordate provides evidence of the origin of PTH and PACAP/glucagon receptor family. **Mol. Biol. Evol.** 32(8): 2048-59.		Yes	Yes	Yes
2015				JSW On, BKC Chow, LTO Lee*	Evolution of parathyroid hormone receptor family and their ligands in vertebrate. **Front in Endocrinol (Lausanne)** 6(28): 1-6.		Yes	Yes	Yes
2015				HKH Ng, BKC Chow*	Oligomerization of family B GPCRs: exploration in inter-family oligomer formation. **Front in Endocrinol**. 6(10): 1-5.		Yes	Yes	Yes
2014				R Sekar, BKC Chow*	Role of secretin peptide family and their receptors in the hypothalamic control of energy homeostasis. **Horm Metab Res**. 45(13): 945-54.		Yes	Yes	Yes
2014				L Zhang, BKC Chow*	The central mechanisms of secretin in regulating multiple behaviors. **Front Endocrinology** 5:77	Yes (2015)	No	Yes	Yes

2014				JKV Tam, LTO Lee, J Jin, BKC Chow*	Molecular evolution of GPCRs: Secretin/secretin receptors. **J Mol Endocrinology** 52(3):T1-14	Yes (2015)	No	Yes	Yes
2014				R Sekar, BKC Chow*	Secretin receptor-knockout mice are resistant to high-fat diet-induced obesity and exhibit impaired intestinal lipid absorption. **FASEB J** 28(8):3494-505	Yes (2015)	No	Yes	Yes
2014				R Sekar, BKC Chow*	Lipolytic Actions of Secretin in Mouse Adipocytes. **Journal of lipid research** 55(2):190-200	Yes (2013)	No	Yes	Yes
2014				L Zhang, SK Chung, BKC Chow*	The knockout of secretin in cerebellar purkinje cells impairs mouse motor coordination and motor learning. **Neuropsychopharmology** 39(6):1460-8	Yes (2013)	No	Yes	Yes
2014				LTO Lee, SYL Ng, JYS Chu, R Sekar. KG Harikumar, LJ Miller, BKC Chow*	Transmembrane peptides as unique tools to show in vivo action on water intake of a GPCR hetero-complex. **FASEB J** 28(6):2632-44	Yes (2013)	No	Yes	Yes
2013				SYL Ng, LTO Lee, BKC Chow*	Receptor oligomerization: from early evidence to current understanding in class B GPCRs. **Front Endocrinology** 3:175	Yes (2013)	No	Yes	Yes
2013				Y Yuan, BKC Chow, VH Lee, LTO Lee*	Neuron-restrictive silencer factor functions to suppress Sp1-mediated transactivation of human secretin receptor gene. **Biochim Biophys Acta** 1829(2), 231-8	Yes (2013)	No	Yes	Yes
2013				R Sekar, BKC Chow*	Metabolic effects of Secretin. **Gen Comp Endocrinology** 181,18-24	Yes (2013)	No	Yes	No
2013				JKV Tam, BKC Chow, LTO Lee*	Structural and Functional Divergence of Growth Hormone-Releasing Hormone Receptors in Early Sarcopterygians: Lungfish and Xenopus. **PLoS One** 8(1): e53482.	Yes (2013)	No	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each conference abstract)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(year)*	Attached to this report *(Yes or No)*	Acknowledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
August 2016 Leuven, Belgium	Secretin Receptor Alters the Angiotensin II-induced Calcium Influx in Adrenal Zona Glomerulosa via Cross-class GPCR dimerization.	28th Conference of European Comparative Endocrinologists CECE		Yes	Yes	No
August 2016 Leuven, Belgium	Altered postnatal development of the cerebellum in secretin knockout mice	28th Conference of European Comparative Endocrinologists CECE		Yes	Yes	No
2016 Seoul, S Korea Plenary Lecture	Secretin and the development of pulmonary arterial hypertension	8th AOSCE Congress		Yes	Yes	No
12-14 July 2016 Rouen, Normandy, France.	Signaling modification by GPCR heteromer and its implication on X-linked nephrogenic diabetes insipidus	The RegPep2016 International Meeting		Yes	Yes	Yes
21-26 September 2015 Cappadocia, Turkey.	The role of secretin and Its receptor in Angiotensin II-induced Aldosterone Biosynthesis and release	The 12th International Symposium on VIP/PACAP and Related Peptides (Vip-Pacap 2015		No	Yes	Yes
09/2014/ Kyoto, Japan Invited lectures	Molecular interaction of mouse secretin and angiotensin II receptors and their potential implications in water homeostasis	20th Symposium on Regulatory Peptides 2014	Yes (2015)	No	Yes	No
08/2014/ Rennes, France Invited lectures	Structural and Functional Divergence of Growth Hormone-Releasing Hormone Receptors in Early Sarcopterygians	27th Conference of European Comparative Endocrinologists CECE	Yes (2015)	No	Yes	No

03/2014/ Keelung, Taiwan. Invited lecture and Session chair	Transmembrane peptides as unique tools to demonstrate the *in vivo* action of a GPCR hetero-complex of secretin and angiotensin.	7th Intercongress Symposium of Asia and Oceania Society for Comparative Endocrinology (AOSCE)	Yes (2015)	No	Yes	No
08/2013/ Bristol, England. Invited speaker	The potential of secretin as neurohypophysial factor.	10th World Congress on Neurohypophysial Hormones 2013	Yes (2015)	No	Yes	No
08/2013/Pecs	Transmembrane domain peptides as a new class of drug to demonstrate the in vivo function of GPCR hetero-oligomerization in water intake behavior	The 11th International Symposium on VIP, PACAP and Related Peptides	Yes (2015)	No	Yes	No
06/2013/San Francisco	Transmembrane IV of secretin receptor as a molecular determinant in secretin and angiotensin II type 1A receptor dimerization	ENDO 2013	Yes (2015)	No	Yes	No
07/2013/ Barcelona	The role of secretin in modulating GABAergic inhibitory postsynaptic currents of mouse cerebellar Purkinje cells	17th International Congress of Comparative Endocrinology	Yes (2015)	No	Yes	No
07/2013/ Barcelona	The role of secretin in regulating aldosterone synthesis and renal sodium reabsorption.	17th International Congress of Comparative Endocrinology	Yes (2015)	No	Yes	No
06/2013/San Francisco	Interaction studies of different species of secretin and human secretin receptor	ENDO 2013	Yes (2015)	No	Yes	No
06/2013/San Francisco	Secretin receptor knockout mice are resistant to diet-induced obesity and exhibit impaired intestinal lipid absorption	ENDO 2013	Yes (2015)	No	Yes	No
05/2013/ Su Zhou, China Invited speaker	Lipolytic effect of secretin.	Cold Spring Harbor Asia Conferences – Metabolism, Obesity and Obesity-associated Diseases	Yes (2015)	No	Yes	No
10/2012/HK Plenary lecture	The Central Actions of Secretin to Regulate Water Balance.	7th International Huaxia Congress of Endocrinology	Yes (2015)	No	Yes	No
08/2012/ Zürich Plenary lecture	The Function Of Secretin In Regulating Water And Salt Balance In Our Body.	26th Conference of the European Comparative Endocrinologists (CECE)	Yes (2015)	No	Yes	No

06/2012/ Houston	Knockout of Secretin in Purkinje Cells Changes Mouse Motor and Balance Behaviors	ENDO 2013	Yes (2015)	No	Yes	No
05/2012/ Berlin	The endocrine disrupting effect of hypoxia on pituitary cells	6th SETAC World Congress	Yes (2015)	No	Yes	No

10. *Student(s) trained* (please attach a copy of the title page of the thesis)

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Kwok-hin, Ng,	Ph.D.	1/9/2011	24/2/2016
Revathi, Sekar	Ph.D.	1/1/2010	15/4/2014
Senthil, Vijayalakshmi	Ph.D.	1/1/2010	12/11/2014
Stephanie, Ng	Ph.D.	3/1/2011	3/7/2014
Chin Pang, Tam	M.Phil.	9/1/2011	3/7/2014
Li, Zhang	Ph.D.	1/9/2009	30/7/2013

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

Prize: 2010 **Research Output Prize**, By The University of Hong Kong.

2014 Best Oral Presentation Award: HongKong Society of Endocrinology, Metabolism and Reproductio