President's Symposium

President's Symposium 1

Brain and hormones: Their seamless interaction between structure and function from molecular to behavioural level

(March 21, 8:30～10:00, Room A)

PS1–1

Structural studies in neuroendocrine research. Lessons from the past; clues for the future

Morris, John (*Department of Physiology, Anatomy & Genetics, University of Oxford, Oxford, UK)*

Structural studies have played a major role in the understanding of the functions of neuroendocrine systems. As new imaging techniques have been devised it has proved possible to look in greater and greater detail into the structure of neuroendocrine tissues, probing down to the level of individual molecules. While these studies have become increasingly good at providing an answer to "what" and "where" questions, in experiments that use an anatomical end-point it has only been when careful manipulation has been applied to the tissues that real advances in understanding function have been made. Most structural techniques provide only static images - literally single frames in the movie of living tissues. Furthermore, living, behaving organisms comprise many different tissue systems and our scientific exploration is only gradually getting to grips with ways in which the different systems and tissues interact to organise complex behaviours. In generating simple systems to apply Ockham's razor experimentally, we inevitably lose sight of the relative quantitative effect of all the competing signals involved.This presentation will review and analyse the ways in which investigations of the anatomy of neuroendocrine tissues has contributed to some of the milestones of increased understanding of the functional behaviour of neuroendocrine systems and entire organisms. It will also try to peer into the rather opaque crystal ball of the future by drawing lessons from past successes.

(COI: No)

PS1–2

Challenge to visualize/regulate physiological functions of neurohypophysial hormones

Ueta, Yoichi (*Dept. Physiol. Sch. Med. Univ. Occup. and Environ. Health, Kitakyushu, Japan)*

Neurohypophysial hormones, arginine vasopressin (AVP) and oxytocin (OXT) are synthesized in the magnocellular neurosecretory cells (MNCs) localized in the hypothalamic paraventricular (PVN) and the supraoptic nuclei (SON) that project their axon terminals into the posterior pituitary (PP). Recent studies have revealed that AVP and OXT are secreted not only into the systemic circulation from the axon terminals in the PP but also in the central nervous system from the somatodendrites of the MNCs. Nowadays, central actions of AVP and OXT are known such as social behavior, pair-bonding and maternal behavior. We challenged to visualize MNCs that synthesize AVP and OXT with their neuronal activities (the c-fos gene expression) simultaneously in transgenic rats that express the AVP-eGFP (or OXT-mRFP1) fusion gene and the c-fos-eGFP (or mRFP1) fusion gene. Recently, we have also challenged to regulate the neuronal activities of MNCs by a light-activated ion channel in transgenic rats that express the AVP-eGFP and channelrhodopsin 2 fusion gene. These genetic modified animals enable us to visualize/regulate neurohypophysial hormone dynamics in in vitro and in vivo preparation from vesicle, neuronal activity to behavior.

(COI: No)

PS1–3

Vasopressin: from synthesis to secretion

Leng, Gareth; Macgregor, Duncan (*Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK)*

In the forty years since the first electrophysiological recordings from identified vasopressin neurons, our understanding of these cells has come a long way – but how well do we really understand them? One test of our understanding is whether we can express it in a computational model that can reproduce the complex behaviour of vasopressin cells in a manner that is quantitatively precise, but which also can yield novel predictive insights. We have developed a computational model of the vasopressin cell that accounts for its characteristic electrophysiological behaviour (the distinctive phasic patterning of spike activity in individual cells), and, by introducing variability in key parameters we can generate a population of model cells that closely mimics the range of electrophysiological phenotypes observed *in vivo* (MacGregor & Leng, PLoS Comput Biol. 2012; 8(10): e1002740). We extended that spiking model to include a representation of stimulus-secretion coupling derived from experimental data, building a model population that displays the dynamics of secretion from the population as well as from individual cells (Macgregor & Leng, PLoS Comput Biol. 2013; 9(8): e1003187). Now we have extended that model further, to incorporate synthesis of vasopressin and its activity dependent regulation. This enables us to simulate the behaviour of the vasopressin system in response to diverse challenges – including challenges that result in depletion of the pituitary stores of vasopressin. Finally, we are extending the model to simulate dendro-dendritic interactions between vasopressin cells, to better understand how this intercommunication impacts on the physiological behaviour of the vasopressin system.

(COI: No)

PS1-4

Sex steroid feedback and brain programming -a role of kisspeptin neurons-

Tsukamura, Hiroko (*Grad. Sch. Bioagricultural Sci., Nagoya Univ., Nagoya, Japan)*

Mammalian reproductive function is regulated by the hypothalamus-pituitary-gonadal axis. The mechanism is sexually differentiated and the differentiation is considered to be due to the brain programming by perinatal sex steroids. Kisspeptin neurons, which play a critical role in reproduction via controlling gonadotropin-releasing hormone (GnRH)/gonadotropin release, show sexual dimorphism in the rodent brain: many kisspeptin neurons in female anteroventral periventricular nucleus (AVPV) while few in males. The present paper first focuses on the organizational effect of neonatal steroids causing the sexual differentiation of kisspeptin neurons and consequent GnRH/luteinizing hormone (LH) surge generating mechanism. We found that neonatal steroids decrease AVPV kisspeptin expressions, resulting in the failure of GnRH/LH surge in male rats. The neonatal steroids failed to affect kisspeptin neurons in the arcuate nucleus (ARC), which is considered to regulate GnRH/LH pulses. Second, the paper focuses on the functional effect of estrogen in adulthood, which is responsible for estrogen positive and negative feedback effects on GnRH/LH release to induce GnRH/surge and suppress GnRH/LH pulses, respectively. More specifically, the epigenetic mechanism involved in the estrogen feedback on kisspeptin expression in adult rodent brain will be discussed. This work was supported in part by the Research Program on Innovative Technologies for Animal Breeding, Reproduction (REP2002), and Vaccine Development and the Science and technology research promotion program for agriculture, forestry, fisheries and food industry.

(COI: No)

PS1-5

An integrated system of environmental signals on the reproductive neuroendocrine axis

Ozawa, Hitoshi (*Dept. Anat. Neurobiol., Grad.Sch. Med., Nippon Med. Sch., Tokyo, Japan)*

Reproductive neuroendocrine regulations are mainly originated and/or integrated at HPG axis, arising from 1) the hypothalamus, where a group of scattered neurons secretes gonadotropin-releasing hormone (GnRH) for stimulating gonadotropins from the gonadotroph in the anterior pituitary; 2) the anterior pituitary, where gonadotrophs secrete the gonadotropins for promoting the gonadal maturation, and 3) the gonados, which secrete sex steroids. In addition, feedback loops also regulate within this axis for facilitating the homeostatic regulation of reproductive neuroendocrine system in different physiological conditions. Recent advance in our understanding of the controlling GnRH, therefore the functions of gonados came with discover of the kisspeptin and their receptor, GRP54. Kisspeptin neurons are observed in the anteroventral periventral (AVPV) and the arcuate (Arc) nuclei in the rat brain. It is reported that kisspepitn neurons express sex steroid receptors, and the leptin receptor and cortcotropin-releasing hormone receptor. This means that kisspeptin neurons directory receive the negative feedback signal of HPG axis, the energy (nutritional) information, and the stress response signal, then the kisspeptin neurons relay these environmental information to the HPG axis. So, the kisspeptin neurons may be possible to understand integrating neurons of the different physiological information to facilitate the homeostatic regulation of the HPG axis. In the paper, I would like to introduce a new concept of kisspeptin-HPG axis, which is interacted with the energy regulation and the stress response.

(COI: No)

President's Symposium 2

Structure and function of biological membranes: viewed from molecules and their nano-environments

(March 23, 9:00～10:30, Room A)

PS2-1

Crystal Structure of Voltage Sensor Domain Protein

Nakagawa, Atsushi[1,2] ([1]*Inst for Protein Res, Osaka Univ;* [2]*CREST, JST)*

X-ray crystallography is a powerful technique to determine the three-dimensional atomic structures of biological macromolecules, such as proteins. The atomic structure gives valuable information to understand the function of the biological macromolecules.

The voltage-gated ion channels are the members of voltage-sensing protein family, which is regulated by membrane potential changes. The voltage-gated ion channel is consisted from two distinct domains, called a voltage sensor domain (VSD) and a channel domain. When the sensor domain senses membrane potential changes, the S4 helix in the sensor domain changes its orientation and transmits the signal to the channel domain, and open and close the gate that is formed by four domains of the tetramer of the molecules. Recently new protein family, which has VSD but lacks channel domain, has been identified and is named voltage sensor domain protein. Voltage-gated proton channel, named Hv1 or VSOP, is a member of this family. The VSD of Hv1 plays dual roles of voltage sensing and proton permeation. It is required for high-level superoxide production by phagocytes through its tight functional coupling with NADPH oxidase to eliminate pathogens. Hv1 is also expressed in human sperm and has been suggested to regulate motility through activating pH-sensitive calcium channels. The activities of Hv1 also have pathological implications, such as exacerbation of ischemic brain damage and progression of cancer.

The crystal structure of mouse Hv1 (mHv1) in the resting-state was determined at 3.45A resolution, and it provides a novel platform for understanding the general principles of voltage sensing and proton permeation.

(COI: No)

PS2–2

Physiological functions revealed by looking at nanoscale distribution of membrane lipids

Fujimoto, Toyoshi (*Grad. Sch. Med. Nagoya Univ., Nagoya, Japan*)

The biological membrane is a highly dynamic structure, but most physiological reactions occur in restricted areas. Therefore, to study phenomena in the membrane, e.g., signal transduction, it is important to know how membrane molecules, both proteins and lipids, are located in the smallest-possible scale. Electron microscopy is a powerful tool for this purpose, but conventional techniques are not satisfactory because those used for proteins are not applicable to or not appropriate for lipids. One major problem for lipids is that they do not react with aldehydes and remain mobile even after fixation.

To circumvent this "unfixability" problem, we have been working on an EM method utilizing physical fixation. In this method, cells are quick-frozen to stop molecular motion instantaneously; then a membrane is split into two leaflets by freeze-fracturing, after which membrane molecules are immobilized by vacuum evaporation of platinum and carbon; to this physically-stabilized membrane preparation, specific probes are applied to label target molecules.

By using this method, we can observe not only the two dimensional distribution of membrane lipids (and proteins as well) but also the asymmetry that lipids show between the outer and inner leaflets. Because membranes are retained in a stable form in the freeze-fracture replica, they can be subjected to various chemical treatments that may perturb membrane structures when applied to native membranes. I would like to show the results on several different lipids, including gangliosides and phosphoinositides [PI(4, 5)P_2, PI(3)P, PI(3, 5)P_2] and discuss their physiological implications.

(COI: No)

PS2–3

The class III PI3K and phosphatidylinositol 3-phosphate ensure structural and functional integrity of cardiomyocytes

Sasaki, Takehiko[1,2]; Kimura, Hirotaka[1,2]; Eguchi, Satoshi[1]; Sasaki, Junko[1]; Mizushima, Noboru[3] ([1]*Dept Med Biol, Grad Sch Med, Akita Univ, Akita, Japan;* [2]*Res Center for Biosignal, Akita Univ, Akita, Japan;* [3]*Dept Biochem and Mol Biol, Grad Sch Med, Tokyo Univ, Tokyo, Japan*)

Vps34, the catalytic subunit of the class III phosphoinositide 3-kinase complex, phosphorylates phosphatidylinositol to produce phosphatidylinositol-3- phosphate (PtdIns3P). The principal role of PtdIns3P is to recruit cellular proteins to specific membrane domains where they function. To date, more than 100 proteins with a range of functions have been reported to bind to PtdIns3P. Thus, PtdIns3P synthesis by Vps34 is considered important for a variety of cellular activities.Here, we report that Vps34 is involved in the etiology of certain cases of idiopathic cardiomyopathy. Vps34 expression in the heart was markedly reduced in a subset of patients with hypertrophic cardiomyopathy. In accordance with the observation, muscle-specific deletion of *Pik3c3* encoding Vps34 in mice led to thickening of the left ventricular wall, reduced cardiac contractility, bundle branch block and arrhythmia. All these mutants died suddenly between postnatal day 80 (P80) and P110, suggesting a protective role for Vps34 against the occurrence and progression of heart failure. We will present the molecular mechanisms behind these abnormalities and discuss the interplay between autophagy and ESCRT machinery, the two protein degradation systems diverge from PtdIns3P, in the context of myofibril organization.

(COI: No)

PS2–4

Specialized membrane nanodomain organized by lipid modification ~ Synaptic organization regulated by PSD-95 palmitoylation machinery ~

Fukata, Masaki[1,2]; Sekiya, Atsushi[1,2]; Murakami, Tatsuro[1,2]; Yokoi, Norihiko[1,2]; Kobayashi, Kenta[1,3]; Fukata, Yuko[1,2] ([1]*Div Membrane Physiol, NIPS, Japan;* [2]*Dept Physiol, SOKENDAI, Japan;* [3]*Viral Vector, NIPS, Japan*)

Precise regulation of protein assembly at specialized membrane domains is essential for diverse cellular functions including synaptic transmission. However, it incompletely understood how protein clustering at the plasma membrane is initiated, maintained and controlled. Protein palmitoylation, a common reversible lipidation, regulates protein targeting to the plasma membrane. Such modified proteins are enriched in these specialized membrane domains. Recently, we found that endogenous palmitoylated postsynaptic density protein 95 (PSD-95), a representative postsynaptic scaffold, is partitioned into multiple discrete subdomains (nanodomains) in a dendritic spine in cultured hippocampal neurons. PSD-95 in nanodomains undergoes continuous de/repalmitoylation cycles driven by local palmitoylating activity, ensuring the maintenance of compartmentalized PSD-95 clusters within individual spines. Acutely induced plasma membrane insertion of DHHC2 palmitoylating enzyme triggers specific accumulation of PSD-95 at the plasma membrane, and this plasma membrane-inserted DHHC2 is essential for postsynaptic nanodomain formation. Furthermore, we obtained the candidate for the membrane-bound enzyme to depalmitoylate PSD-95 and disperse synaptic PSD-95 clusters. We propose that synaptic palmitoylation machinery defines subsynaptic nanodomains through constituting local palmitoyl cycles on PSD-95 and determines the geometry of postsynaptic densities.

(COI: No)