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General Summary

The research group (Applied molecular medicine, professor Sasaki) continued to work on regenerative medicine for pancreatic islets. The group also collected and accumulated clinical samples to find novel volatile biomarker for inflammatory and metabolic diseases in human, and started to execute multivariate analysis with gas chromatography technique. In 2017, a new research group (Neurosciences, professor Watabe) has joined the institute. This research group focuses on the neuronal mechanisms regulating aversive and affective memory formation and adaptive behaviors, using molecular, cellular, electrophysiological and behavioral techniques.
In addition to performing our own research activities, we continued to engage in an educational laboratory course program with the assignment of students of the third-year grade from the School of Medicine in 2017. We also fulfilled research support duties for registered researchers from the University Hospital at Kashiwa campus so that doctor physician-researchers could work efficiently.

Research Activities

Mechanism of islet injury and beta cell regeneration in diabetes mellitus
Research to elucidate the structure-function relationship of the islet compartment structure and molecules for the cell to cell communication should be helpful to understand the origins of pancreatic islet failure in diabetes mellitus. We have already started a study of "beta cell protection from metabolic stress" through the islet architecture. Experiments in 2016 showed that, in the co-culture conditions of MIN6, a murine established beta cell line, and IMS32, a murine established Schwann cell line, significantly higher in GSIS (glucose-stimulated insulin secretion) or insulin secretory capacity than in MIN6 of a single culture system. When expression of mRNA for the molecule that performs intercellular communication, gapjunction, was knocked-out with RNA interference technology, GSIS was lowered than in the control. This phenomenon was considered as a protective effect from the Schwann cells via intercellular communication.

Search for novel volatile biomarker in breath and skin gas with gas chromatography
Continuing from the previous fiscal year, we established the methodology for analysis of skin-derived gas and breath with gas-chromatography to find novel biomarkers for metabolic or physical stress including systemic inflammation. As many as 200 kinds of volatile organic compounds (VOCs) were detected in human exhaled gas, most of which were turned out to be derived from metabolic substances *in vivo*. We performed analysis of

VOCs in expired gas of patients with inflammation using gas chromatography mass spectrometry (GC-MS) in order to search quantitatively and qualitatively different VOCs between healthy volunteers and patients. Identification of the detected VOC was performed using a mass spectrum database compiled by NIST (National Institute of Standards and Technology). Multivariate analysis was performed to analyze the relevance of the inflammatory disease to the analysis VOCs.

Study of the change of the body components during treatment of diabetes mellitus by sodium-dependent glucose co-transporter SGLT2 inhibitor

In conventional treatment of type 2 diabetes with dietary restrictions and medication, changes of body composition, in particularly possible loss of muscle as well as decrease in fat, have become a problem. Because a novel agent for type diabetes, SGLT2 inhibitor, could cause body fat loss with an advantage of maintaining skeletal muscle mass. We have clarified these issues and report in scientific meeting and papers.

Elucidating the circuitry mechanisms underlying fear memory learning

Avoiding pain and harm is fundamental for survival of human and animals. Aversive stimuli therefore potently induce adaptive behaviors and memory formation. Elucidating neuronal circuitry mechanisms underlying such adaptive behaviors is fundamental to understand brain functions. Furthermore, dysregulation of neuronal circuitry of such aversive behaviors leads to various kinds of anxiety disorders such as post-traumatic stress disorders and other psychiatric diseases.

The amygdala is acknowledged as a critical brain region to attach the aversive valence of nociceptive stimuli as "pain" onto various sensory stimuli, and this association is considered to be mediated *via* synaptic plasticity which is underlying certain forms of learning paradigm such as fear conditioning. While neuronal networks and plasticity mechanisms for fear conditioning have been intensively studied, not much is known about how the emotional value of pain itself is regulated at the circuitry level.

In the previous studies, we have identified one of such nociceptive pathway; neurons in the parabrachial nucleus (PB) in pons form direct monosynaptic projection on the central amygdala (CeA). We found that PB-CeA pathway is necessary and sufficient for fear memory formation, suggesting that the PB-CeA pathway may in involved in some emotional aspect of pain (Sato et al., 2015).

As for research progress in H29, we found that the optogenetic activation of the PB-CeA pathway is perceived as aversive signal for mice using real-time conditioned place avoidance in Y-maze test (in preparation). Also, we have reported that the calcitonin-gene related peptide (CGRP) plays a critical role in the PB-CeA synaptic potentiation observed in the formalin-induced inflammatory pain model using CGRP knock-out mice (Shinohara et al., 2017). As for the collaboration-based progress, we contributed to the memory engram study conducted by Professor Inokuchi at Toyama University (Yokose et al., 2017). Engram theory proposed by Richard Semon and synaptic plasticity theory proposed by Donald Hebb are the two leading theories on the memory study field presented on the 20th century. While synaptic plasticity has been intensely studied, rather conceptual theory of engram has just recently emerged to be experimentally testable due to the tech-

nological advances. Engram is a population of neurons that are activated by learning, and proposed to be reactivated by a part of the original stimuli for memory retrieval. The present study demonstrated that associating old memories with new memories for "memory update" requires specific engram, instead of just activating the all engrams responsible for both old and new memories. A few additional collaborational activities are in progress, including the one with Professor Toya Ohashi using animal model of MGII for contextual fear memory function and therapeutic attempt to rescue the phenotypes. The present research was supported by Grant-in-Aid for Scientific Research (C), Strategic Research Program, CREST to A.M. Watabe and AMED to T. Ohashi.

Publications

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