Molecular imaging opens up a vast new world for neuroscience

September 6 2010, by Yosky Kataoka

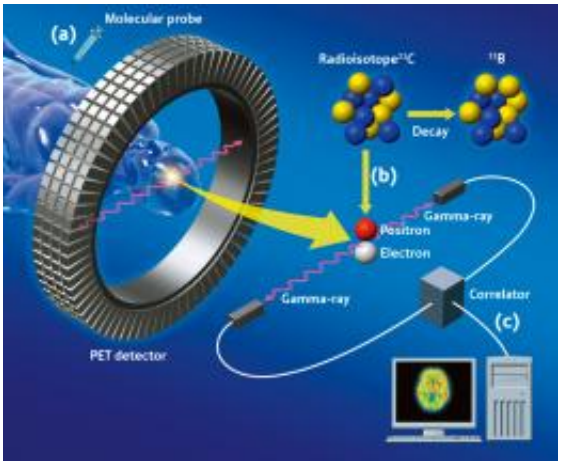

Figure 1. Molecular imaging by PET.

Molecular imaging allows molecules in a living organism to be visualized, and provides a means of observing the distribution and behavior of molecules. One of the most exciting applications of this technology is the ability to examine a patient internally without affecting the subject.

“The biggest advantage of molecular imaging using positron emission tomography lies in its applicability to humans.” says Yosky Kataoka, team leader of the Cellular Function Imaging Laboratory at the RIKEN Center for Molecular Imaging Science. Molecular imaging using positron emission tomography (PET) is expected to contribute to the

diagnosis of disease, as well as to our understanding of pathologic conditions and therapeutic effects, and to the development of new drugs. It is already being used to diagnose Alzheimer's disease and cancer. In October 2009, Kataoka's laboratory announced a groundbreaking achievement that could lead to the development of a diagnostic method for migraine. While many people suffer from migraine, no objective method for diagnosis or treatment has been found so far. Their achievement is attracting attention as a discovery that should dramatically change this situation.

Origins in an experiment conducted ten years ago

Severe pulsing pain on one side of the head. This is the most obvious symptom of a migraine, but it can also be accompanied by vertigo and nausea. If aggravated, this complex of symptoms interferes with daily activities, as any chronic sufferer will attest. "The pain of a migraine is thought to be triggered by repeated contraction and dilation of the blood vessels in the brain. However, the pathology remains unclear, so there is still no objective method for migraine diagnosis," says Yosky Kataoka. "We have made a discovery that will dramatically change the situation."

In October 2009, Kataoka and his colleagues made the headlines with their achievement in 'Unlocking the Mysteries of the Brain with PET'. Their research was published in the November issue of the Journal of Nuclear Medicine in the US. "PET performed on rats enabled us to confirm that a phenomenon known as spreading depression is followed by activation of the microglia, which mediate inflammatory reactions in neural tissue. Our achievement is attracting attention because it is expected to give us an understanding of the pathology of migraines, and their diagnosis and treatment."

Kataoka's link between spreading depression and migraine emerged from an experiment he conducted while at the Osaka Bioscience

Institute. “Everything traces back to an experiment I conducted ten years ago,” he says.

First encounters with spreading depression

In 2000, while investigating how light could be used to control central nervous activities, Kataoka developed a photo-oxidation method to suppress the signaling function of synapses. Neurons in the brain release neurotransmitters from the axon termini, which extend from the neuron body and transmit information to adjacent neurons. In the photo-oxidation method, a photosensitizing dye is administered to the brain, which causes oxidative stress when exposed to light through the generation of reactive oxygen species. This oxidative stress suppresses the signaling function of synapses, but only at the site exposed to light, and the site gradually returns to its original state when the light is removed. This method attracted attention as a groundbreaking approach for examining localized functions of the neural network.

Kataoka later used the photo-oxidation method in a PET study of how the neural function of the monkey cerebral cortex could be suppressed topically. PET is one of the most powerful tools for monitoring the distributions and functions of molecules in living organisms, and involves administering a molecular probe—a site-targeting molecule bearing a radioisotope—to the subject organism. The positrons, or ‘positive electrons’, emitted by the radioisotope collide with ambient electrons to generate gamma rays, which are then counted and visualized (Fig. 1). The molecular probe used by Kataoka was fluorodeoxyglucose (FDG), which is prepared by attaching fluorine-18 (^{18}F) to deoxyglucose. This probe allows the metabolism of glucose to be examined, and is also used in the PET diagnosis of cancer.

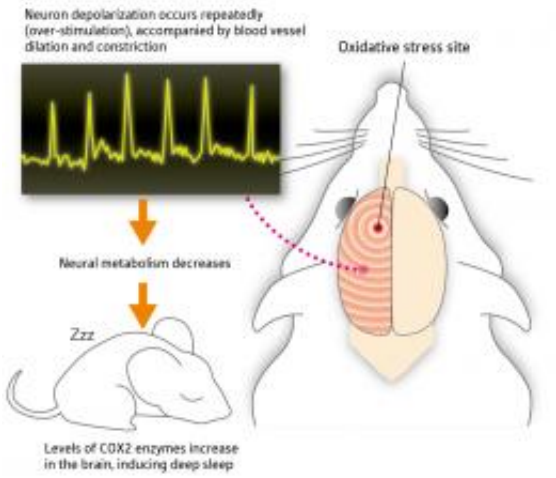

Figure 2. Spreading depression. Starting at the site exposed to oxidative stress (red circle), depolarization propagates in waves at speeds of 2-3 mm per minute. The waves of depolarization spread over the entire hemispherical cortex, but are never transmitted to the opposite hemisphere. Neurons are over-stimulated by the depolarization, and the episode is followed by a reduction in neural metabolism.

“Neurons in action exhibit high levels of glucose metabolism and take up large amounts of FDG. PET imaging revealed a major reduction in FDG uptake at the photo-oxidized sites because the synaptic function had been suppressed. I was planning to announce the findings with the headline: ‘Suppression of central nervous function by photo-oxidation confirmed by PET,’ but I encountered an unexpected and mysterious phenomenon.” PET images taken just after photo-oxidation revealed suppressed neural metabolism only at the photo-oxidized sites. One day later, however, FDG uptake decreased and neural metabolism was suppressed over the entire hemisphere of the cerebral cortex on the photo-oxidized side. At the same time, the opposite hemisphere remained normal. “I could not understand why that happened, and remained puzzled for the next two years.”

It turned out the suppression of neural metabolism over the entire hemisphere cortex was caused by spreading depression. “When neural tissue is exposed to severe oxidative stress, cations flow into the cells, and the potential in the cells rises for a short time; this phenomenon is called depolarization. When a population of cells undergoes depolarization, the phenomenon propagates like waves at a speed of two to three millimeters per minute, causing metabolic suppression in the path of the depolarizing waves. The depolarization waves spread over the entire hemisphere of the cortex, but are never transmitted to the opposite hemisphere. Although spreading depression was discovered back in 1944, it had not attracted the attention of researchers in brain science until only recently.” Kataoka attempted to induce spreading depression artificially using his photo-oxidation method and to examine what occurs in detail.

Deep sleep induced by over-excitation

Rats affected by spreading depression quickly become drowsy and fall asleep. Puzzled by this finding, Kataoka and others examined rats with spreading depression and found that levels of the enzyme cyclooxygenase 2 (COX2) increased after a spreading depression event. COX2 produces large amounts of prostaglandin, a hormone-like substance that induces deep sleep. Kataoka explains, “I suppose that having a deep sleep allows the rat to rest its over-stimulated brain and restore its functions.”

The same effect, drowsiness after tackling something difficult, is also commonly felt by humans, and occurs because prostaglandin is produced as a result of over-stimulation of neurons. However, being busy or being absorbed in something bypasses the sleep impulse. “Going without sleep for a long time is bad for brain functioning,” warns Kataoka. “The brain becomes fatigued, and the nerves are impaired functionally. One human study shows that learning is more effective if you have a sleep after the

learning process. ‘Before an exam, don’t study through the night; instead get a good night’s sleep’ is good advice from the viewpoint of brain science.”

Discovering the dual differentiation of perineuronal progenitor cells

Deep sleep was not the only state found to be caused by spreading depression. “Newborn cells are produced in the brain following spreading depression,” says Kataoka. “We speculate that when the brain takes a rest, it proactively rebuilds neural tissue to increase its resistance to stress.”

In the brain, neurons are responsible for information transmission, and glial cells act to deliver nutrients to the neurons and in their way support information transmission. It had been thought that neurons are not regenerated, except in the hippocampus and olfactory bulb, whereas glial cells are regenerated in all portions of the brain and are renewed in entirety over a year or so.

However, this consensus was upset by a study reported by Kataoka and his colleagues. They examined the brain to determine which cells divide and what kinds of cells they differentiate into, in the context of spreading depression, using a confocal laser microscope (Fig. 3). “We found that dividing cells were present in the vicinity of neurons. But rather than merely occurring in the vicinity of the neurons, the cells actually invaded the neurons. It was commonly believed that the dividing cells are the progenitors of glial cells, and that all progenitor cells differentiate into glial cells. The reality, however, was not like that.” Monitoring dividing cells revealed that most of the progenitor cells underwent self-replication, some became glial cells, and a small number, less than 1%, became juvenile neurons. Surprisingly, the progenitor cells

proved to be capable of differentiating into both neurons and glial cells.

“Cells capable of self-replication and differentiation into both glial cells and neurons seem to be the tissue stem cells of the nervous system. We call the cells ‘perineuronal progenitor cells’, and they are found throughout the entire brain and spinal cord, including the cerebral cortex. Neurons are constantly produced in areas other than the hippocampus and olfactory bulb. This is a major discovery that upsets conventional knowledge.”

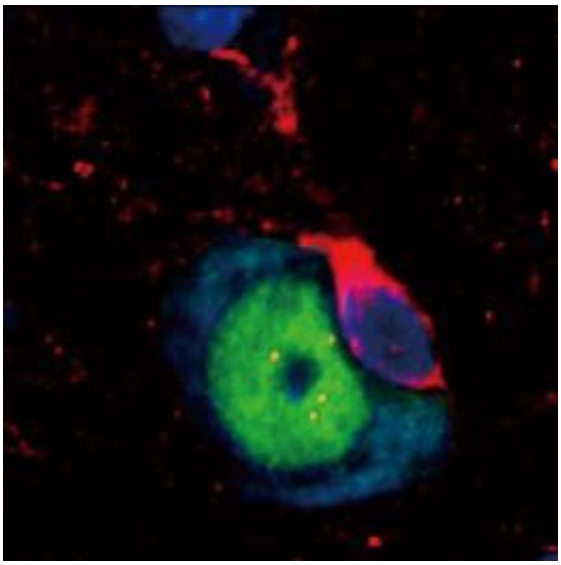

Figure 3. A perineuronal progenitor cell. Photomicrograph of a perineuronal progenitor cell (red) obtained using a confocal laser microscope. The nucleus of a mature neuron is shown in green. The perineuronal progenitor cell divides and propagates by spreading depression. Perineuronal progenitor cells are not only capable of self-replication, but also have pluripotency for differentiation into glial cells and neurons.

Although research into perineuronal progenitor cells has only just begun, some interesting results have already been reported. One example is the

effect of aging. There are two types of cerebral cortex: the limbic cortex involved in the control of instincts and emotions, and the neocortex involved in higher attributes such as information processing and cognition. Examination of rats bred until old age has shown that perineuronal progenitor cells in the neocortex—the part of the brain involved in higher attributes such as information processing and cognition—decreases significantly with age. This is not the case for the limbic cortex—the part of the brain associated with instincts and emotions. The number of neurons, however, was found to remain unchanged, departing from the general opinion that the number of neurons decreases with age. It has also been found that the brain begins to atrophy when the number of perineuronal progenitor cells starts to decline. “Unlike in the neocortex, the perineuronal progenitor cells in the limbic cortex decrease very little in number with age. I can’t help thinking that this may be related to the fact that grandparents continue to feel love towards their grandchildren, while at the same time their capabilities for calculation and memory diminish with age. I suspect that perineuronal progenitor cells are one of the keys to brain senescence. By stopping the reduction in their number, functional impairments that occur with aging may be prevented. With this thought, I am continuing my research.”

Toward PET diagnosis for migraine

Spreading depression was not well-known ten years ago, when Kataoka encountered the phenomenon. However, the situation has changed recently. “The involvement of spreading depression in the onset of migraine has been confirmed in humans, and the phenomenon is attracting attention.”

Scintillating scotoma is one of the symptoms that can affect migraine sufferers at the onset of a migraine attack. This symptom appears to the sufferer as a field of shimmering lights that expands to create what is

commonly known as the migraine aura—localized temporary blindness. When converted to the speed of transmission of nerve excitation in the visual field of the brain, the speed at which the light field expands turns out to be the same as the transmission speed of spreading depression. The feeling of pain only on one side of the head is also consistent with the fact that spreading depression is not transmitted from one cerebral hemisphere to the other. “When spreading depression is transmitted, the blood vessels in the brain contract and dilate repeatedly. During this time, plasma proteins leak from the vessels, causing immune responses in the neural tissue similar to those during inflammation, and these responses are transmitted to the sensory centers to produce pain. This is the pathologic mechanism of migraine suggested by current research.”

Kataoka and his colleagues decided to use PET to confirm that immune responses are actually caused by spreading depression in neural tissue in rats. “Spreading depression is a good target for molecular imaging.” In such experiments, it is important to compare treated animal subjects with their non-treated counterparts. It is difficult, however, to make an individual-based comparison of constantly changing brain activity. Fortunately, in the case of spreading depression, responses are not transmitted to the opposite hemisphere, so that a simultaneous comparison can be made between the left and right hemispheres of the brain in the same individual.

Immune responses in neural tissue are mainly associated with microglia, a type of glial cell. With this in mind, Kataoka and his colleagues constructed a molecular probe by attaching the radioisotope carbon-11 (^{11}C) to PK11195, a molecule that binds only to activated microglia. In the experiments, they induced spreading depression in the left hemisphere of the cerebral cortex, and visualized the distribution of the ^{11}C-PK11195 molecular probe. They found that the molecular probe accumulated in part of the left cerebral cortex. “This demonstrates that microglia activation, or brain immune responses, were caused by

spreading depression."

These are Kataoka's latest findings, and he is already moving on to the next stage. "In collaboration with universities, we are planning to use PET as a diagnostic tool for patients where excess immune responses in the brain are suspected of being involved in nervous diseases. Because 11C-PK11195 is a molecular probe for which safety in humans has already been established, our plan can be carried out after testing has been approved. Provided that microglia activation is confirmed in humans as well, a drug that suppresses immune responses can be used therapeutically. If testing is possible on patients in the future, they will be able to receive the appropriate treatment that distinguishes between migraines and other types of headache."

The idea is emerging that immune responses in the brain are involved in many diseases, including depression, schizophrenia and chronic fatigue syndrome. Additionally, Kataoka's most recent study has yielded results suggesting the possible involvement of perineuronal progenitor cells in immune responses in neural tissue. This finding is attracting attention that will encourage further research into spreading depression and perineuronal progenitor cells, and related medical applications.

Linking perineuronal progenitor cells to regenerative medicine

Kataoka says with a laugh, "I am now going in a totally different direction from what I wanted to do initially. I encountered spreading depression, a phenomenon that was initially difficult to explain, and it has led to investigations into sleep induction, perineuronal progenitor cells and migraine. How can I correlate these different pieces of information? I would like to emphasize this. Realizing that things that have happened unexpectedly are linked and working under the same

single principle makes me very happy. I want to use PET to examine perineuronal progenitor cells and understand their behavior and function. In the future, molecular imaging may help us to understand the mechanisms behind aging, and lead to the discovery of a method to prevent aging-related functional deterioration. Any method that increases the number of perineuronal progenitor cells or boosts their differentiation into neurons will lead to regenerative medicine." However, there is as yet no molecular probe that can be used to monitor perineuronal progenitor cells. "Although it is quite difficult to prepare a new molecular probe and examine molecules using it, there is a good chance that we can do it. Here at the RIKEN Center for Molecular Imaging Science, researchers working in a broad range of fields, including biologists who search for molecules that bind to specific targets, chemists who prepare molecular probes by attaching a radioisotope to molecules, and physicists and technologists who develop equipment and instruments, are readily at hand. This means that we have the chance to do unique work on new and challenging issues."

Provided by RIKEN