Selective detection of α-synuclein and its amyloid by using novel fluorescent dye

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α-Synuclein is a pathological component of Parkinson’s disease by participating in Lewy body formation. Amyloidosis generating the insoluble fibrillar protein deposition has been considered to be responsible for the cell death observed in the neurodegenerative disorder. To investigate the protein aggregation of α-synuclein in vivo as well as in vitro, novel method has been developed by employing a fluorescent probe of JC-1 and fluorescence resonance energy transfer (FRET) analysis with thioflavin-T. Specific interaction of JC-1 to α-synuclein and its amyloids has been elucidated by observing the enhanced JC-1 binding fluorescence. Upon the excitation at 490 nm, the intrinsic fluorescence of JC-1 at 590 nm was augmented specifically with α-synuclein monomer whereas another peak at 527 nm was red-shifted to 538 nm and dramatically enhanced upon the amyloid interaction. By employing various synuclein-related proteins, JC-1 turned out to be selective to the acidic C-terminal region of α-synuclein with an approximate dissociation constant of 2.6 μM. The α-synuclein amyloids were demonstrated to be selectively monitored by employing FRET between thioflavin-T and JC-1 bound to the amyloids because the light emitting at 482 nm by thioflavin-T could be absorbed by JC-1 and emitted at 538 nm upon the excitation at 450 nm. The localization of α-synuclein inside the yeast overexpressing α-synuclein and its modified proteins has been analyzed with JC-1 and the FRET analysis. Based on the data, we have suggested that JC-1 could serve a useful probe to examine α-synuclein and its amyloid formation both in vivo and in vitro.

Role of Na+/H+ exchanger-induced mitochondrial Ca2+ overload in glutamate-mediated excitotoxicity of cortical neurons

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Glutamate excitotoxicity is associated with a variety of neuronal injuries such as stroke. Neuronal cell death caused by excitotoxic levels of glutamate is dependent on Ca2+ influx through glutamate receptors and mitochondrial Ca2+ accumulation, simultaneously. Na+/H+ exchangers (NHE) have been recently reported to be implicated in ischemia/reperfusion injury, based upon that the inhibition of NHE demonstrated protective effects on cardiac and brain ischemia. However, the precise role of NHE and its mechanism of protection against brain ischemic injuries have not been clearly elucidated. Here, we investigated to confirm the protective effects of NHE inhibition against excitotoxicity in neurons and to elucidate the underlying mechanism of the protection. We have used cariporide, a well-known NHE inhibitor, to investigate the role of NHE inhibitors inhibited mitochondrial dysfunction, showing mitochondrial membrane potential loss, cytochrome c release, caspase-3 activation and accumulation of mitochondrial ROS. These results suggest that neuronal death caused by excitotoxicity occur by mitochondrial Ca2+ overload. Thus, we suggest that NHE inhibition protects neurons against excitotoxicity and that the protective effects of NHE inhibition occur by inhibiting mitochondrial death pathway to preserve the functional integrity of mitochondria in neurons.

Resveratrol: more reliable fluorescent probe to quantitatively detect amyloids

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Amyloidosis producing insoluble fibrillar protein aggregates is the common pathological feature of various neurodegenerative disorders such as Parkinson’s and Alzheimer’s disease in which α-synuclein and Aβ participate to form Lewy bodies and senile plaques, respectively. To develop novel analytical tool for the amyloidosis, resveratrol, the major phenolic constituent of red wine and isolatable from grapevines, has been employed to respectively. To develop novel analytical tool for the amyloidosis, resveratrol, the major phenolic constituent of red wine and isolatable from grapevines, has been employed to

Regulation of the differentiation of excitatory synapses by the SALM family of PSD-95-interacting cell adhesion-like molecules

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Synaptic cell adhesion molecules (CAMs) are known to play key roles in various aspects of synaptic structures and functions, including early differentiation, maintenance, and plasticity. We herein report the identification of a family of cell adhesion-like molecules termed SALM that interacts with the abundant postsynaptic density (PSD) protein PSD-95. SALM2, a SALM isoform, distributes to excitatory, but not inhibitory, synaptic sites. Knockdown of SALM2 expression in mouse primary hippocampal neurons disrupted excitatory synapses and dendritic spines. Methylated expression of SALM2 disrupts excitatory synapses and dendritic spines. Bead-induced direct aggregation of SALM2 results in colocalizing of PSD-95 and other postsynaptic proteins, including GSK and AMPA receptors. Knockdown of SALM2 by RNA interference reduces the number of excitatory synapses and dendritic spines and the excitatory postsynaptic density, but not amplitude, of miniature excitatory postsynaptic currents. These results suggest that SALM2 is an important regulator of the differentiation of excitatory synapses.

Protein kinase C (PKC) increase the microtubule dynamics on axonal transport in hippocampal neurons

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Microtubules in developing neurons are major structural components in both neuronal morphology and axonal transport. Microtubules and microtubule-associated proteins regulate the neuronal survival and functions including signaling, and intracellular trafficking between synapses and cell bodies to support neurotransmission. Tau is a member of neuronal microtubule-associated proteins that plays a key role in regulating microtubule dynamics, axonal transport and all these functions of tau are modulated by site-specific phosphorylation. Thus, it is reported that many neurodegenerative diseases lead to typical abnormalities by the changes of microtubules or microtubule-associated proteins consisting of neuronal cytoskeleton and axonal transport. Recently, it is known that activation of PKC promotes microtubule assembly in growth cones. However, it is not clearly established yet how PKC is correlated with microtubule dynamics on axonal transport in developing neurons or neurodegenerative disease. We have studied the effect of protein kinase C on the microtubule dynamics in developing neurons. The activation of PKC by PMA or Bysatanol I increased elongation of microtubules and microtubule assembly. The specific inhibitor of PKC, Go6976 or Rottlerin, blocked the neurotubule outgrowth. In these results, we suggest that activation of protein kinase C (PKC) may be involved in regulation of microtubule dynamics and tau on axonal transport in hippocampal neurons.

Protective effects of benzoazolone on lipopolysaccharide-induced neurotoxicity by inhibiting microglia activation

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An inflammation response in the central nervous system mediated by activation of microglia is a key event in the early stages of the development neurodegenerative diseases. We have synthesized and examined various benzoazolone derivatives against the neuroinflammation. Benzoazolone derivatives are highly interesting molecules for drug development, because they already have been shown to be useful for treating various diseases including neurodegenerative disorders. In this study, we have investigated the protective effects of KHG21834, a benzoazolone derivative, on lipopolysaccharide (LPS)-stimulated murine BV-2 microglia. KHG21834 was a selective inhibitor of proinflammatory cytokine production by activated glia. KHG21834 significantly suppressed the LPS-induced upregulation of tumor necrosis factor-α and interleukin-1β in a dose-dependent manner. The production of inducible nitric oxide synthase (iNOS) was also studied in LPS-stimulated BV-2 cells as a model of microglia activation. KHG21834 dose dependently attenuated nitrite production and iNOS protein expression in LPS-stimulated murine BV-2 microglia. In addition, the neuroprotective effect of KHG21834 was similarly observed in vivo. These results demonstrate a potent suppressive effect of KHG21834 on proinflammatory responses of microglia, suggesting a therapeutic potential for this compound in neurodegenerative diseases accompanied by microglial activation.