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Confocal microscopy for dynamic morphology of living tissue/ cells: with special reference to Ca^{2+} dynamics in peripheral nervous system

Y Satoh*, T Saino, H Akutsu-Yamauchi

Anatomy (Functional Morphology), Iwate Medical University, Morioka, Japan

Adenosine-5'-triphosphate (ATP) extracellularly which is released from neuronal and non-neuronal tissues interacts with cell surface receptors to produce a broad range of physiological responses. The present study addressed the issue of whether the cells of peripheral nerves (e.g. autonomic nerves: the superior cervical ganglia (SCG) and myenteric plexus, somatic nerves: dorsal root ganglion (DRG) and ischiatic nerve), respond to ATP. To this end, we observed the dynamics of the intracellular calcium ion concentration ($[Ca^{2+}]_i$) of neurons, satellite cells, Schwann cells and perineurium of rat and golden hamster. Real-time confocal laser scanning microscopy (Nikon RCM/Ab) was used for the imaging of $[Ca^{2+}]_i$ dynamics.

SCG: ATP produced an increase of $[Ca^{2+}]_i$ in both neurons and satellite cells; initially, ATP elicited $[Ca^{2+}]_i$ increase in satellite cells, subsequently, a $[Ca^{2+}]_i$ change in neurons was observed. P1 purinoceptor agonists had no effect on this process, but P2 purinoceptor agonists induced $[Ca^{2+}]_i$ increase and suramin totally inhibited ATP-induced $[Ca^{2+}]_i$ dynamics in both neurons and satellite cells. Ca^{2+} response of neurons occurred at first in cytosol, and then Ca^{2+} wave propagated into nuclei. In satellite cells, Ca^{2+} channel blockers and the removal of extracellular Ca^{2+}, but not thapsigargin-pretreatment, abolished ATP-induced $[Ca^{2+}]_i$ dynamics. In contrast, thapsigargin-pretreatment abolished ATP-induced $[Ca^{2+}]_i$ dynamics in neurons. Reactive blue-2 (P2Y antagonist) inhibited the ATP-induced reaction on neurons alone. Uridine-5'-triphosphate (UTP; P2Y agonist) caused a $[Ca^{2+}]_i$ increase in neurons and α,β-methylene ATP (P2X agonist) caused a $[Ca^{2+}]_i$ increase in satellite cells. We concluded that neurons of SCG respond to extracellular ATP mainly via P2Y purinoceptors and that satellite cells respond via P2X purinoceptors.

Myenteric plexus: We determined that ATP induced $[Ca^{2+}]_i$ increase in enteroglia, and only a part of enteric neurons responded to ATP. However, the subtype of purinoceptors could not be examined, because ATP-induced muscle contractions moved the specimens, therefore it was hard to apply different stimulants.

Dorsal root ganglion: In contrast to SCG, DRG neurons showed morphological heterogeneity. In accordance with it, Ca^{2+} response of DRG was not uniform. Generally, satellite cells covering small neurons often respond to ATP, ATP could stimulate the satellite cells of large neurons in a few cases. UTP elicited responses of some satellite cells. Only a part of neurons showed $[Ca^{2+}]_i$ increase via purinoceptors. The heterogeneity on ATP-induced Ca^{2+} signaling of DRG was incredible.

Ischiatic nerve bundle: Injuries of peripheral tissue stimulate nerves. Peripheral nerves are surrounded by perineurium, therefore the response of perineurium may be a first event of nerve stimulation at tissue injuries. ATP induced a $[Ca^{2+}]_i$ increase of perineurial cells and naked Schwann cells. If perineurium was intact and the Schwann cells were under the perineurial sheet, extra-nerve ATP could not elicit Ca^{2+} response of Schwann cells. Ca^{2+} channel blockers and removing of extracellular Ca^{2+}, but not thapsigargin-pretreatment, abolished ATP-induced $[Ca^{2+}]_i$ dynamics. This indicated that the $[Ca^{2+}]_i$ increase was due to an influx of extracellular Ca^{2+}. ATP also elicited an increase of $[Ca^{2+}]_i$, but P1 receptor agonists had few effects on $[Ca^{2+}]_i$ dynamics. Suramin (P2 antagonist) totally inhibited ATP-induced $[Ca^{2+}]_i$ dynamics, but reactive blue 2 did not. UTP induced no significant change in $[Ca^{2+}]_i$, but α,β-methylene ATP caused a $[Ca^{2+}]_i$ increase. In conclusion, perineurial cells respond to extracellular ATP mainly via P2X receptors.

Conclusion: ATP can modulate the functions of non-excitatory components of peripheral nerves (e.g. Satellite cells, Schwann cells, enteroglia and perineurium) via P2 receptors. The subtype of purinoceptors may considerably differ in each tissue.

*Corresponding author
E-mail: yisatoh@iwate-med.ac.jp