

Astrocyte plasma membrane extensions are dynamically modulated by purines

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Astrocytes have emerged as important players in keeping physiological brain homeostasis because of their activity in forming tripartite synapse and regulating brain vessels function. Such connections are mediated by plasma membrane (PM) extensions, expressing aquaporin-4 (AQP-4) and K⁺ inward rectifying (Kir) channels, regulating both water and K⁺ homeostasis. Among several transmitters released by astrocytes, adenosine and purines lately have been involved as key regulators in several neurodegenerative diseases, as epilepsy and ischemia. Therefore, in the present study, we explored the effect of purines in modulating PM extensions growth and formation in isolated hippocampal astrocytes. In our model, cells were stimulated with the selective P2Y₁ agonist 2-methylthioadenosine diphosphate (2MeSADP, 100 μM). To validate functional receptors we monitored calcium flux, mobilized by Gq-mediated pathway coupled to P2Y₁ receptor, in living cells loaded with Fluo-4 calcium indicator and stimulated with 2MeSADP. In a different set of live cell imaging experiments, cells were stained with the PM dye CellMask Orange to evaluate PM extension growth and length. Stimulation with 2MeSADP determined in 20 min both growth and elongation of PM extensions, prevented by 5 min treatment with the selective P2Y₁ antagonist MRS 2179 (1 μM). Then, we explored the effect of calcium on astrocytes PM extension growth by chelating calcium in buffer solution with EGTA 10 μM. Cells were exposed 10 min to chelating agent followed by activation with 2MeSADP and they did not show either intracellular calcium increase or PM extension growth. To block intracellular calcium increase, we performed experiments with phospholipase C inhibitor U73122 (3 μM). Cells were treated with U73122 up to 20 min and monitoring cells over time showed a dramatic decrease of PM extensions in length and number. These data suggested that a reduction in PM extensions might influence both AQP-4 expression and Kir function. To test this hypothesis, we performed western blot analysis to evaluate levels of AQP-4 and no changes were observed in protein expression in all conditions tested in imaging experiments. About Kir function, we performed whole-cell configuration recordings in isolated astrocytes in control conditions and after U73122 treatment. In a voltage steps protocol eliciting Kir current, we observed a dramatic increase in current through K⁺ channels at negative potentials (-180 mV to -100 mV) within 3 min treatment with U73122. At positive potentials (20 mV to 60 mV), K⁺ current was reduced over time up to 10 min. Taken together, these data show a dynamic modulation in astrocytes of PM extension growth by purinergic receptor P2Y₁ and its Gq-mediated pathway, suggesting a mechanism for keeping functional and effective astrocytes buffering activity.