MODULATION OF ASTROCYTE PROCESSES BY GPCR: CHANGES IN CELLULAR ACTIVITY

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Astrocyte processes are ultrathin plasma membrane structures that interact with synapses. Neuronal elements of the tripartite synapse, enwrapped by astrocyte peripheral tips, are finely regulated. At the same time, neurotransmitters released in the synaptic cleft may modulate astrocyte activity. It has been shown that process reduction or deficiency in astrocytes might be involved in aging, chronic stress or neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Several G-protein coupled receptors are expressed in astrocytes and the aim of this study was to elucidate whether metabotropic activation/deactivation might influence astrocyte processes function. Among different receptors, activation of purinergic P2Y1 receptor and its Gq-mediated pathway promoted process growth and formation in isolated cells. Living hippocampal astrocytes were stained with plasma membrane dye CellMask Orange and process elongation was observed following stimulation with P2Y1 agonist 2-methylthioadenosine diphosphate (2MeSADP, 100 microM). Such effect was blocked by pre-treatment with the selective P2Y1 antagonist MRS 2179 (1 microM) and by inhibition of Gq effector, phospholipase C, with U73122 (3 microM). Since P2Y1 receptor activation induces calcium mobilization in astrocytes, cells were incubated with different calcium chelators such as BAPTA-AM, 20 microM, and EGTA, 10 microM. Calcium rises in astrocytes differ among specific intracellular areas. Both chelators were effective in blocking somatic calcium mobilization, as shown by no significant changes in fluorescence intensity of Fluo-4 calcium indicator upon Gq-mediated pathway stimulation. However, only in presence of EGTA, P2Y1 receptor activation did not elicit process growth, suggesting a different role of calcium in soma and peripheral tips of astrocytes. Changes in number and length of processes might influence cell activity. It is known that astrocyte processes express aquaporin-4 (AQP-4) and K+ inward rectifying (Kir) channels, regulating both water and K+ homeostasis. AQP-4 was identified in astrocyte processes by immunofluorescence experiments and, since western blot analysis did not show any changes in AQP-4 protein level, a redistribution of this protein was hypothesized in conditions were number and length of processes were reduced. Moreover, Kir function was monitored by whole-cell configuration recordings in isolated astrocytes by applying a 20 mV voltage-step protocol (from -180 mV to +60 mV). In conditions where a reduction in process number and length was observed by imaging experiments, a dramatic increase in current through K+ channels at negative potentials (-180 mV to -100 mV) was recorded. Evidence of such effect was observed within 3-5 min treatment with P2Y1 antagonist MRS 2179 and phospholipase C inhibitor U73122. Taken together, these data suggest that astrocyte processes are modulated by purinergic signaling mainly by P2Y1 receptor and its Gq-mediated pathway, and effects of such influence will affect astrocytes buffering activity.