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Tripartite synapses: bidirectional communication between astrocytes and neurons

Gertrudis Perea and Alfonso Araque

Instituto Cajal, CSIC, Madrid, Spain

Astrocytes posses a form of excitability based on intracellular Ca^{2+} variations. Using Ca^{2+} imaging and electrophysiological techniques in cultured rat hippocampal cells we determined the electrophysiological consequences of elevating astrocytic Ca^{2+} levels on neurons. Astrocytic Ca^{2+} elevations caused a slow inward current (SIC) in adjacent neurons that was mediated by ionotropic glutamate receptor activation. Glutamate (Glu) was released by astrocytes because the insensitivity of the SIC to tetanus toxin, which prevents neuronal exocytosis. Furthermore, thapsigargin, injection of BAPTA, and photolysis of Ca^{2+} cages demonstrated that a Ca^{2+} elevation in astrocytes is both necessary and sufficient to stimulate Glu release. To further investigate the mechanisms underlying the astrocytic Glu release we analysed the effects of disrupting astrocytic vesicle proteins. We found that Glu release requires an electrochemical gradient necessary for Glu uptake in vesicles, because bafilomycin A_1 reduced neuronal responses. Furthermore, neuronal responses were inhibited by injection of astrocytes with the light chain of Botulinum B that cleaves the SNARE protein synaptobrevin. These results demonstrate that the Ca^{2+}-dependent Glu release from astrocytes is a SNARE protein-dependent process that requires the presence of functional vesicle-associated proteins, suggesting that astrocytes store Glu in vesicles and that it is released by exocytosis.

We also analysed the effects of astrocytic Ca^{2+} elevations on synaptic transmission. Ca^{2+} elevation in astrocytes increased the frequency of excitatory as well as inhibitory miniature postsynaptic currents (mPSCs), without modifying their amplitudes. This AP5-sensitive astrocytic-induced increase of mPSC frequency was due to activation of NMDA receptors, which are located extrasynaptically because it persisted after blockage of synaptic receptors by MK-801. Therefore, astrocytes modulate spontaneous synaptic transmission by increasing the probability of transmitter release through the activation of NMDA receptors. In addition, stimulation of astrocytes transiently reduced the magnitude of action potential-evoked excitatory and inhibitory postsynaptic currents through the activation of metabotropic Glu receptors.

These results demonstrate that astrocytes regulate neuronal excitability and synaptic transmission (for a review, see Araque *et al.* 2001).

Considering the relevance of Ca^{2+} elevations in astrocytes, our work has recently focused on the mechanisms involved in the astrocytic Ca^{2+} responses to synaptic activity. Hippocampal astrocytes respond with Ca^{2+} elevations to synaptically released Glu (Porter & McCarthy, 1996). We investigated in rat hippocampal slices whether astrocytes respond to a different synaptically released neurotransmitter by an extrinsic pathway. We stimulated the alveus, which contains glutamatergic axons as well as cholinergic afferents from the septum and diagonal band of Broca, and recorded currents and Ca^{2+} levels of astrocytes located in the stratum oriens. The stimulation evoked an inward current due to Glu transporter activity, and increased the Ca^{2+} levels in astrocytes. The responses were enhanced by 4-AP, and abolished by TTX or Cd^{2+}, indicating that they were due to synaptically released neurotransmitter. Ca^{2+} variations were unaffected by Glu receptor antagonists, but were abolished by atropine, indicating that astrocytes respond to acetylcholine released by synaptic terminals. These results show the existence of cholinergic neuron-to-astrocyte signalling, and suggest that astrocytes are a target of axonal inputs from different brain areas.

Although stratum oriens astrocytes express Glu receptors, the alveus stimulation did not evoke Glu-mediated Ca^{2+} elevations. We investigated whether a different glutamatergic pathway could activate those receptors. We recorded astrocytic responses to the stimulation of glutamatergic Schaffer collaterals (SC). This stimulation induced an inward current mediated by Glu transporters, and evoked astrocytic Ca^{2+} elevations that were abolished by Glu receptor antagonists.

These results indicate that while stratum oriens astrocytes showed acetylcholine-mediated Ca^{2+} elevations after alveus stimulation, astrocytes responded with Ca^{2+} elevations to Glu released by SC. Therefore, astrocytes show functional subcellular domains and may discriminate between the activity of different synaptic terminals belonging to different axon pathways.

In conclusion, these results support the existence of a complex bidirectional communication between astrocytes and neurons, and indicate an important active role of astrocytes in the physiology of the nervous system.

Araque *et al.* (2001). *Annu Rev Physiol*

Porter & McCarthy (1996). *J Neurosci*

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Kainate receptors and synaptic plasticity in the hippocampus

G.L. Collingridge

University of Bristol, Bristol, UK

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Group III metabotropic glutamate receptor signalling in glutamate release modulation

J. Sánchez-Prieto, R. Shigemoto and C. Millán

Departamento de Bioquímica, Facultad de Veterinaria, Universidad Complutense, Madrid-28040, Spain and National Institute for Physiological Science, Myodaiji, Okazaki 444-8585, Japan

Glutamate activates two types of receptors, ligand gated ion channels and G protein-coupled metabotropic glutamate receptors (mGluRs). At least eight mGluR subtypes have been cloned to date, and these receptors can be placed into three major groups on the basis of their pharmacology, second messenger coupling and sequence homology. Group III mGluRs consist of four different subtypes (mGluR4, 6, 7 and 8) and are activated by the selective agonist L(+)-2-amino-4-phosphonobutyrate (L-AP4). The localization of these receptors within presynaptic active zone is consistent with their role as autoreceptors mediating the feedback inhibition of glutamate release. By using biochemical and immunocytochemical techniques as well as Ca^{2+} imaging from single nerve terminals we have determined the contribution of the different signalling pathways (inhibition of Ca^{2+} channels activity, decrease in cAMP levels) to glutamate release inhibition in a preparation of cerebrocortical nerve terminals. In addition, we have studied the distribution of the

distinct group III mGluRs in subpopulations of nerve terminals depending upon the type or combination Ca^{2+} channels present.

In nerve terminals from adult rats (2 months) we find that L-AP4 (1 mM) inhibited the Ca^{2+}-dependent evoked release of glutamate by 25 %. This inhibition of release was mediated primarily by activation of mGluR7, which exhibited low affinity for the agonist L-AP4. This inhibitory effect was largely prevented by pertussis toxin but was insensitive to the inhibitor of protein kinase C (PKC), bisindolylmaleimide, and protein kinase A (PKA), H-89. Furthermore, this inhibition was associated with reduction in N-type Ca^{2+} channel activity in the absence of any detectable change in cAMP levels. In the presence of forskolin, however, L-AP4 decreased the levels of cAMP. The activation of this additional signalling pathway was very efficient in counteracting the facilitation of glutamate release induced either by forskolin or by the β-adrenergic receptor agonist isoprenaline. The specific inhibition by mGluR7 of the release component associated with the activity of N-type channels was unexpected because these Ca^{2+} channels support glutamate release to a lesser extent (29.9 %) than P/Q-type Ca^{2+} channels (72.7 %). However, imaging experiments to measure Ca^{2+} dynamics in single nerve terminals have revealed a specific co-localization of mGlu7 receptors and N-type Ca^{2+} channels. Ca^{2+} channels were distributed in a heterogeneous manner in individual nerve terminals as they contained N-type (31.1 %; conotoxin GVIA sensitive) P/Q-type (64.3 %; agatoxin-IVA sensitive) terminals or terminals insensitive to these two toxins (4.6 %). Interestingly, the great majority of the responses to L-AP4 (95.4 %) were located in N-type channels containing nerve terminals. This specific co-localization of mGlu receptor 7 and N-type Ca^{2+} channels could explain the failure of the receptor to inhibit the release component associated with P/Q-channels and also reveals the existence of a specific mechanism of targeting to place the two proteins in the same subset of nerve terminals.

In cerebrocortical nerve terminals from young rats (3 weeks), two mGluRs with high and low affinity for L-AP4 were identified by immunocytochemistry as mGluR4 and mGluR7, respectively. Ca^{2+} imaging experiments showed that voltage-dependent Ca^{2+} channels are distributed in a more heterogeneous manner than in adult animals. Thus, presynaptic terminals contained only N-type (47.5 %; conotoxin GVIA-sensitive), P/Q-type (3.9 %; agatoxin-IVA-sensitive), or both N- and P/Q-types of Ca^{2+} (42.6 %) channels, although the remainder of the terminals (6.1 %) were insensitive to these two toxins. Interestingly, mGluR4 was largely (73.7 %) located in nerve terminals expressing both N- and P/Q-type Ca^{2+} channels (N-P/Q terminals), whereas mGluR7 was predominantly located (69.9 %) in N-terminals. This specific co-expression of different group III mGluRs and Ca^{2+} channels may endow synaptic terminals with distinct release properties and reveals the existence of a high degree of presynaptic heterogeneity.

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Genetic and proteomic studies of learning

S.N. Grant

University of Edinburgh, Edinburgh, UK

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Non-canonical signalling by ionotropic kainate receptors

J. Lerma, J.L. Rozas and A.V. Paternain

Instituto Cajal. CSIC. Av. Doctor Arce 37, 28002-Madrid

Alterations of glutamatergic neurotransmission have been related to the neuronal damage observed after episodes of ischaemia and hypoglycaemia, as well as to the etiology of a series of neurological conditions including epilepsy, Alzheimer's disease, Huntington's chorea and amyotrophic lateral sclerosis. The cloning of a large number of glutamate receptor proteins and the discovery of their structural relationships have paved the way to most of our current understanding of the biophysical properties and the physiological role of each subtype in the mammalian brain. Of the glutamate receptor subtypes, NMDA, AMPA and kainate receptors, the latter is by far the less well understood. A decade ago, our understanding of the molecular properties of kainate receptors and their involvement in synaptic physiology was essentially nill, despite the observation that kainate administration in experimental animals induces seizures and patterns of neuronal damage closely resembling those observed in epileptics. For this and other reasons, it has become important to understand the physiology of these receptors in brain function. The discovery of a specific AMPA receptor antagonist, GYKI53655, has made such studies feasible. A plethora of recent studies have shown that kainate receptors are key players in the modulation of transmitter release, important mediators of the postsynaptic actions of glutamate, and possible targets for the development of antiepileptic and analgesic drugs.

Consistent with a role in epilepsy, we found that kainate depresses GABA inhibitory transmission in the rat hippocampus. GABA is the major inhibitory neurotransmitter in the brain, and its activity is crucial in maintaining neuronal excitability at normal levels. It has been also found that the effect of kainate on GABA release is sensitive to the presence of pertussis toxin, pointing to the involvement of G proteins in this regulatory process. Moreover, protein kinase C (PKC) inhibitors can also block the action of kainate, further delineating the signalling pathway involved in the modulation of inhibitory neurotransmission. The recruitment of the PKC pathway is not secondary to the depolarisation of the terminal since reducing the influx of sodium ions does not affect the action of kainate. Therefore, it has been proposed that there is a physical link between kainate receptors and the G protein involved in the process, a link that could be either direct or through an intermediary molecule. Lastly, the binding of SYM 2081 decreases after incubation of hippocampal membranes with pertussis toxin and a similar effect of the toxin has been observed on the kainate-mediated inhibition of GABA release from synaptosomes, further suggesting a direct coupling between kainate receptors and G proteins in the mammalian brain.

We have tried to find a suitable model to further delineate this non-canonical signalling by ionotropic kainate receptors and found that in cultured dorsal root ganglion (DRG) cells, kainate could increase intracellular Ca^{2+} in the absence of this

extracellular cation. We also found that kainate receptor activation was able to depress K^+-induced intracellular Ca^{2+} accumulation in a G-protein- and PKC-dependent manner. As it was the case for inhibition of GABA release in hippocampal slices, the inhibition of Ca^{2+} currents was largely independent of kainate receptor ion channel activity. Therefore, DRG neurons are a suitable model to study G protein-coupled kainate receptors and determine subunits and proteins involved.

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