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Burst Activity and Synaptic Mechanisms in a Hypothalamic Network Grown in Culture

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Summary

In a cultured network of rat embryonic hypothalamic cells, synaptic interaction is through $GABA_A$-receptors, that mediate inhibition by an increase in Cl^- conductance, and AMPA-receptors, that mediate excitation by an increase in monovalent cationic conductance. Changes in the balance of inhibition and excitation towards a predominance of excitation lead to phasic synchronous activity of the cells. Synaptic interaction through these receptors is thus capable of modulating neurosecretion rapidly.

Key words

Cultured hypothalamic neurones – Burst activity – Synaptic mechanisms

Hormone secretion is coupled to electrical activity of neurosecretory cells, and bursts of action potentials in hypothalamic neurones are a key factor for efficient hormone release (Cazalis *et al.* 1985). The demands of hormone secretion require instantaneous regulation of the properties of bursting cells in the network. This regulation may be accomplished through fast synaptic mechanisms that control hypothalamic neurones. It has been suggested that GABA and glutamate are the dominant transmitters in neuroendocrine regulation in the hypothalamus (van den Pol *et al.* 1990). To examine the mechanisms which underlie the fast synaptic coupling by these transmitters, we studied the properties and development of GABAergic and glutamatergic synaptic transmission in a rat hypothalamic network grown in culture with respect to the occurrence of synchronized burst activity (Misgeld and Swandulla, 1989, Swandulla and Misgeld 1990).

Whereas the majority of cells recorded after 7 to 14 days in culture (DIC) generated action potentials only upon current injection, some 20 % of cells exhibited spontaneous repetitive burst discharges. In contrast, cells recorded from 21 to 84 DIC displayed pronounced but irregular spontaneous activity. This irregular activity shifted to cycles of rhythmic burst activity with a mean frequency of 0.18 Hz when $GABA_A$ antagonists were present in the dish. The bursts of action potentials were triggered by summating depolarizing postsynaptic potentials which increased in amplitude when hyperpolarizing the membrane. Injection of brief current pulses (10 ms) elicited bursts that had the same appearance as bursts occurring spontaneously in the same cell indicating that short synaptic activation is sufficient for these cells to generate a full-blown burst.

Synaptic currents generated by the network were composed of inhibitory and excitatory postsynaptic currents. IPSCs were generated by an increase in Cl-conductance

and blocked by picrotoxin (20 μM) or bicuculline (20 μM). From 7 DIC on all cells were sensitive to GABA (5 – 20 μM) or muscimol (0.5 – 5 μM) and responded in a dose-dependent manner. Reversal potential of the currents induced by GABA or muscimol were identical and depended on the transmembrane Cl-gradient. As in other preparations (Bormann *et al.* 1987), the Cl-channel involved exhibited partial permeability for organic anions. Because burst activity was observed in cells > 14 DIC only in the presence of $GABA_A$ antagonists, it is concluded that GABAergic inhibitory synapses are well suited to decouple hypothalamic neurones.

EPSPs were generated by an increase in nonselective conductance for monovalent cations. They were blocked by the non-NMDA receptor antagonist kynurenic acid (0.5 μM) or CNQX (1 μM), but not by the NMDA antagonist APV (up to 20 μM) or the nicotinic antagonist mecamylamine (up to 500 μM). Although EPSCs could be seen in cells as early as 7 DIC, there was a development in the sensitivity to the non-NMDA-receptor agonists AMPA and quisqualate within the initial 3 weeks in culture. Both, AMPA and quisqualate activated a nonselective cationic conductance. The inward currents generated by quisqualate in high (> 1 μM) concentrations desensitized rapidly. Desensit-ization by quisqualate also blocked EPSCs, but kainate (20 μM) which also induced an inward current failed to do so. This indicated that the AMPA-type quisqualate receptor involved in the generation of EPSCs does not exhibit a significant kainate sensitivity. Focal applications of GABA and quisqualate revealed that the receptors for quisqualate, but not those for GABA, are located on cell dendrites rather than the cell body. Thus, the presumed function of the two receptors, i.e. coupling into synchronized burst activity by AMPA-type glutamate receptors and decoupling by $GABA_A$ receptors according to the demands of hormone secretion is facilitated by their properties and cellular location.

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