| The Cerebellum 2004; 3: pp 39–64 | 39

Taylor & Francis
healthsciences

Creating Coordination in the Cerebellum

Catania, 2-4 October 2003

Pharmacology of the metabotropic GluR-mediated current at the climbing fiber to Purkinje cell synapse

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Different forms of synaptic plasticity in the cerebellum are mediated by metabotropic glutamate receptors (mGluRs). At parallel fiber (PF) to Purkinje cell (PC) synapses activation of mGluR gives rise to a well known slow synaptic current inhibited by antagonists of mGluR1. The distribution of mGluR types in the climbing fiber (CF) to PC synapses is not well known. Only recently an mGluR1-mediated all-or-none postsynaptic current was also demonstrated at the CF–PC synapse (Dzubay & Otis, Neuron 2002; 36: 1159).

Using whole cell patch-clamp recordings from PCs in rat cerebellar slices with AMPA receptors blocked (NBQX, 10 or CNQX, 20 μM) and impaired glutamate uptake (TBOA, 100 μM) we demonstrate a more complex pharmacology of a current obtained by single or train (10 or 100 Hz) CF stimulation. The mGluR1 specific antagonist CPCCOEt, 100 μM in a group of cells suppressed this response while in a similar number of other cells it induced a potentiating effect. The antagonists of mGluR groups II and III (LY341495 and MSOP, respectively) predominantly suppressed the current. In addition, 100 μM MSOP did not occlude the inhibition by 0.2 μM LY341495.

The ambiguous effect of CPCCOEt was checked by measuring the paired-pulse depression of the CF EPSC, which was not changed with the antagonist. The paired-pulse plasticity was also not changed by CPCCOEt in low (0.5 mM) external Ca^{2+} (used to prevent saturation of AMPARs), thus excluding a presynaptic effect. However, CPCCOEt induced a rise in the amplitude (by ~25%) as well as a prolongation of the decay time of CF EPSCs at normal 2 mM Ca^{2} , i.e. under conditions of AMPAR saturation (11.7 ⊥ 0.7 ms *vs.* 15.8 ⊥ 1.5 ms), thus indicating an effect of postsynaptic origin. In 0.5 mM Ca^{2+} the decay of CF EPSCs was faster (7.5 ⊥ 1.2 ms) but it was also prolonged by CPCCOEt (8.8 ⊥ 1.2 ms). However, the CF EPSC amplitude was not significantly affected indicating an underlying Ca^{2+}-dependent mechanism.

Thus, the pharmacology of the PC mGluR-mediated response points to a dual postsynaptic role of mGluR1 giving rise to a slow postsynaptic current but also regulating other presumably mGluR-dependent currents via second messenger molecules and Ca^{2+}_i. The additional electrophysiological role of mGluR II & III types was also indicated. Such a complex regulatory mechanism may have an important role in the mGluR-dependent forms of homosynaptic plasticity and motor learning at the CF-PC synapse.

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DOI 10.1080/14734220310020164

Electrophysiological and optical recording studies of responses induced by stimulation of the inferior olive and parallel fibers in the cerebellum-pons-medulla preparation isolated from neonatal rats

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The cerebellum-pons-medulla preparation isolated from neonatal rat is a unique *in vitro* preparation that preserves intact neuronal networks including the inferior olive nucleus, pontine nuclei and cerebellar nuclei. This preparation is not only useful for pharmacological approaches, but also advantageous for analyzing the activity of cerebellar cells, keeping neuronal connections to the outside of the cerebellum. Moreover, using optical imaging method, we can identify the responded area projecting from/to stimulation site. In this study, we examined the synaptic responses of Purkinje cells induced by stimulation of inferior olive nucleus/parallel fibers using whole cell patch clamp method and optical imaging.

Cerebellum, pons and medulla were removed from 0- to 8-days-old rats, the preparations were cut rostrally as including the cerebellum and pons and cut caudally at the level of the rostral roots of hypoglossal nerves. The isolated preparation was put caudal surface up in the chamber and superfused with modified Krebs solution equilibrated with 95% O_2 and 5% CO_2, 26–27 °C. Stimulation electrodes were put into the inferior olive nucleus and on the surface of cerebellar cortex softly. The neurons responding to both inferior olive nucleus (IO) and parallel fibers (PF) stimulation were considered Purkinje cell, and were recorded intracellularly using whole cell patch clamp method. The recorded neurons were stained with Lucifer Yellow and identified their locations and morphology. For optical imaging, we used MiCAM01 system which was developed by Dr Ichikawa and colleague (Laboratory for Brain-Operative Device, Brainway Group, RIKEN). Isolated cerebellum-pons-medulla preparation from neonatal rat was stained with a voltage sensitive dye (Di-4 ANEPPS or Di-2 ANEPEQ, 0.2 mg/ml) for 30 min. Then the preparation was put into the recording chamber and fluorescence changes corre-