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# DEVELOPMENTAL LOCALIZATION OF POTASSIUM CHLORIDE CO-TRANSPORTER 2 IN GRANULE CELLS OF THE EARLY POSTNATAL MOUSE CEREBELLUM WITH SPECIAL REFERENCE TO THE SYNAPSE FORMATION

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**Abstract—In the adult CNS, GABA is the predominant inhibitory neurotransmitter, mediating the hyperpolarization of membrane potential and regulating the glutamatergic activity. In the immature CNS, on the other hand, GABA mediates depolarization and is involved in controlling morphogenesis. This developmental shift in GABA actions from depolarization to hyperpolarization occurs as a result of decreasing the intracellular chloride ion ($Cl^-$) concentration ($[Cl^-]_i$) which is regulated by the potassium ($K^+$)-$Cl^-$ co-transporter 2 (KCC2). To clarify the time-course of changes in the GABA actions during development, we examined the developmental localization of the KCC2 in the granule cells of the postnatal mouse cerebellum using specific antibodies against KCC2. The granule cell precursors and migrating granule cells were devoid of immunoreactivity against KCC2 antibodies. At postnatal day 3 (P3), the KCC2-immunolabeling was negative in the internal granular layer, although synaptophysin-positive mossy fiber terminals were detected. At P5, we first detected the KCC2-immunolabeling at the somata of granule cells and their dendrites before granule cells received inhibitory input from Golgi cells. Almost all KCC2-positive dendrites (more than 98%) attached to and formed synapses with mossy fiber terminals. As development proceeded, the number of KCC2-positive granule cells increased, and all granule cells became positive by P21. These results suggested that GABAergic transmission on granule cells might shift from excitation to inhibition after the synapse formation, and the excitatory synapse-formation and related factors might be the triggers for the expression and localization of the KCC2 in the granule cells. Furthermore, it was also suggested that formation of the GABAergic synapses and GABAergic transmission were not necessary for the KCC2-expression in the mouse cerebellar granule cells *in vivo*.** 

**Key words: NKCC1, GABA, synaptophysin, mossy fiber, synaptic glomerulus.**

In the adult CNS, GABA is the predominant neurotransmitter, mediating fast inhibitory synaptic transmission and regulating the excitatory activity of neurons (Olsen and Tobin, 1990; Macdonald and Olsen, 1994). During brain development, on the other hand, GABA is an excitatory transmitter, serves as a trophic factor and is involved in controlling morphogenesis, such as regulating cell proliferation, cell migration, axonal growth, synapse formation, steroid-mediated sexual differentiation and cell death (Ben-Ari, 2002; McCarthy et al., 2002; Owens and Kriegstein, 2002; Represa and Ben-Ari, 2005).

These developmental changes in the actions of GABA occur as a result of a negative shift in the chloride ion ($Cl^-$) reversal potential, which is mainly regulated by two different chloride co-transporter families, sodium ion ($Na^+$)-potassium ($K^+$)-$2Cl^-$ co-transporters (NKCCs) and $K^+$-$Cl^-$ co-transporters (KCCs) (Ben-Ari, 2002; Owens and Kriegstein, 2002; Payne et al., 2003). In two NKCCs, only NKCC1 is detected in the mammalian CNS. The NKCC1 is dominantly expressed in the immature brain and acts to maintain a high intracellular $Cl^-$ concentration ($[Cl^-]_i$). Under the high $[Cl^-]_i$, the activation of ionotropic GABA receptors mediates the depolarization of the membrane potential, and GABA acts as an excitatory transmitter. As development proceeds, $[Cl^-]_i$ is gradually decreased by the KCCs-expression, and GABA becomes an inhibitory transmitter. Among four isoforms of KCCs, KCC1 and KCC2 are expressed in the CNS (Kanaka et al., 2001). The KCC1 is ubiquitously localized in the mammalian tissue (Gillen et al., 1996), and its expression-patterns do not developmentally change in both cerebral cortex (Wang et al., 2002) and cerebellum (Mikawa et al., 2002). Taken together, it is considered that KCC1 is the "housekeeping" isoform involved in cell volume regulation (Gillen et al., 1996; Williams et al., 1999). In contrast, the KCC2 is specifically expressed in neurons, and abundant in the mature CNS (Williams et al., 1999; Kanaka et al., 2001). Changes in the revels of KCC2 correlate with the modification of GABA actions. The transfection of KCC2 into hippocampal neurons converts the actions of GABA from excitatory to inhibitory. GABA is excitatory in the KCC2-knockout mice (Hubner et al., 2001; Ben-Ari, 2002; Payne et al., 2003). These results indicate that not KCC1 but KCC2 plays a key role in decreasing $[Cl^-]_i$, the molecular switch from NKCC1 to KCC2 drives the $Cl^-$ influx in response to ionotropic GABA receptor activation and expression of the KCC2 might be the beginning of the GABAergic inhibition.

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*Abbreviations:* ABC, avidin–biotin–peroxidase complex; $Cl^-$, chloride ion; $[Cl^-]_i$, intracellular chloride ion concentration; IgG, immunoglobulin G; KCC, potassium chloride co-transporter; $K^+$, potassium ion; lp, leading process; NKCC, sodium potassium chloride co-transporter; P, postnatal day; PB, phosphate buffer; PF, parallel fiber; tp, trailing process.

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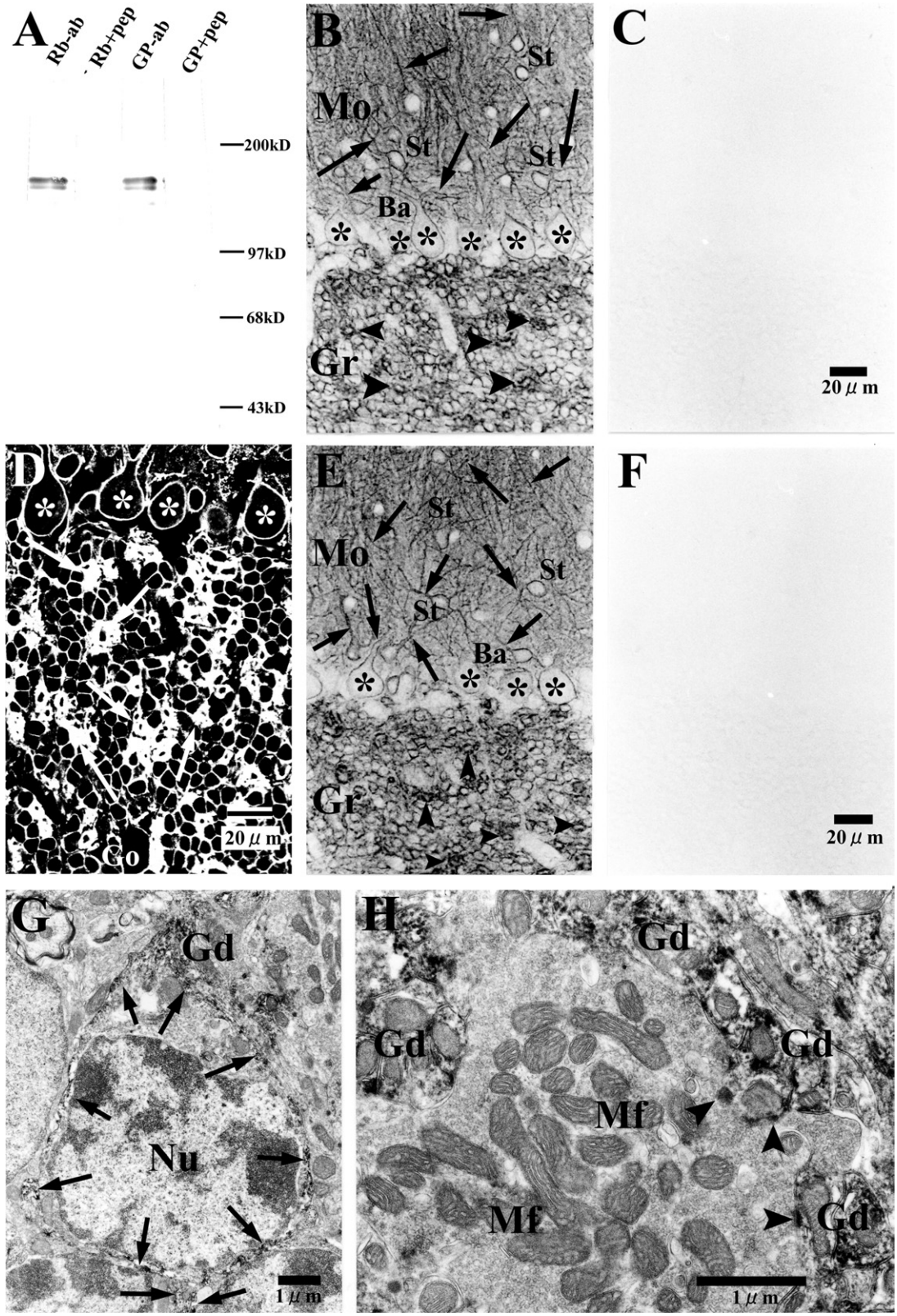


**Fig. 1.** Western blot analysis of the KCC-antibodies (A) and immunohistochemical localization of the KCC2 in the adult cerebellar cortex (B–H). (A) Western blot analysis with rabbit (Rb-ab) and guinea-pig (GP-ab) KCC2 antibodies and the antibodies preincubated with the KCC2-peptide, which was used for immunization (Rb+pep and GP+pep). The position and molecular weights of standards (kD) are shown on the right. (B, C) Immunohistochemical localization of the KCC2 in the adult mouse cerebellar with rabbit antibody. The immunolabeling was detected at the dendrites (black arrows)