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ISONIAZID-INDUCED REDUCTION IN GABAERGIC NEUROTRANSMISSION ALTERS THE FUNCTION OF THE CEREBELLAR CORTICAL CIRCUIT

M. CARTA,[a,b]* L. MURRU,[a,b] E. BARABINO,[a,b] G. TALANI,[a,b] E. SANNA[a,b] AND G. BIGGIO[a,b,c]

[a]*Department of Experimental Biology "Bernardo Loddo," Section of Neuroscience, University of Cagliari, Cittadella Universitaria, SS 554, Km 4,500, 09042 Monserrato (CA), Italy*

[b]*Center of Excellence for the Neurobiology of Dependence, University of Cagliari, Cittadella Universitaria, SS 554, Km 4,500, 09042 Monserrato (CA), Italy*

[c]*CNR (Centro Nazionale Ricerche), Institute of Neuroscience, Section of Neuropsycopharmacology, Cittadella Universitaria, SS 554, Km 4,500, 09042 Monserrato (CA), Italy*

Abstract—The cerebellar cortex contributes to the control of movement, coordination, and certain cognitive functions. The cerebellar network is composed of five different types of neurons that are wired together in a repetitive module. Given that four of these five neurons synthesize and release GABA, this inhibitory neurotransmitter plays a central role in regulation of the excitability and correct functioning of the cerebellar cortex. We have now used isoniazid, an inhibitor of glutamic acid decarboxylase, the enzyme responsible for the synthesis of GABA, to evaluate the contribution of GABAergic transmission in different types of cerebellar cortical neurons to the functioning of the cerebellar circuit. Parasagittal cerebellar slices were prepared from 28- to 40-day-old male rats and were subjected to patch-clamp recording in the voltage- or current-clamp mode. Exposure of the tissue slices to isoniazid (10 mM) resulted in a decrease in the level of GABAergic transmission in Purkinje cells and a consequent increase in the firing rate of spontaneous action potentials that was apparent after 40 min. In granule neurons, isoniazid reduced both tonic and phasic GABAergic currents and thereby altered the flow of information across the cerebellar cortex. Our data support the notion that the amount of GABA at the synaptic level is a major determinant of the excitability of the cerebellar cortex, and they suggest that isoniazid may be a useful tool with which to study the function of the cerebellar network.

Key words: GABA, glutamic acid decarboxylase, cerebellum, excitability, isoniazid, synapse.

*Correspondence to: M. Carta, Department of Experimental Biology "Bernardo Loddo," Section of Neuroscience, Cittadella Universitaria, ss 554, Km 4.5, Monserrato, Cagliari 09123, Italy. Tel: +39-070-675-4179; fax: +39-070-675-4166.
E-mail address: mcarta@unica.it (M. Carta).

Abbreviations: ACSF, artificial cerebrospinal fluid; CFS, cerebrospinal fluid; EGTA, ethyleneglycol-bis-(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid; eIPSC, evoked inhibitory postsynaptic current; EPSP, excitatory postsynaptic potential; $GABA_A$, GABA type A; GAD, glutamic acid decarboxylase; Hepes, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; INH, isonicotinic acid hydrazide; mIPSC, miniature inhibitory postsynaptic current; MPA, 3-mercaptopropionic acid.

The cerebellar cortex plays an important role not only in the regulation of motor systems and coordination, but also in the control of higher functions such as attention and emotion (Schmahmann and Sherman, 1998). This region of the brain contains only five different types of neuron, the inhibitory (GABAergic) Purkinje, stellate, basket, and Golgi cells and the excitatory (glutamatergic) granule cells (Fig. 1A). Purkinje neurons, the only output of the cerebellar cortex, receive excitatory inputs from two different types of afferent fibers, mossy and climbing fibers. Mossy fiber input is relayed via granule cells, which receive an effective inhibitory input from Golgi neurons. Climbing fibers directly stimulate the proximal dendrites of Purkinje cells, which also receive inhibitory input from basket and stellate cells, both of which are interneurons of the molecular layer (Ito, 1984; Llinas, 1990). Given that four of the five neuronal types in the cerebellar cortex synthesize and release GABA, the availability of this neurotransmitter at the synaptic level is likely important for the flow of information in the cerebellar neuronal circuit and likely influences movement and motor learning (Biggio et al., 1977a; Hausser and Clark, 1997; Watanabe et al., 1998; Hamann et al., 2002).

GABA is synthesized from glutamate by two isoforms of glutamic acid decarboxylase, GAD65 and GAD67, and it is released from presynaptic terminals in an action potential-dependent manner (McIntire et al., 1997; Reimer et al., 1998; Cherubini and Conti, 2001). In the cerebellar cortex, GABA released into the synaptic cleft elicits phasic postsynaptic currents by activating specific subtypes of synaptic GABA type A ($GABA_A$) receptors localized on Purkinje cells ($\alpha_1\beta_n\gamma_2$ receptors) and granule cells ($\alpha_{1,6}\beta_n\gamma_2$ receptors) (Wisden et al., 1996; Wall and Usowicz, 1997). In addition, GABA also induces tonic currents by activating high-affinity extrasynaptic $GABA_A$ receptors of the $\alpha_6\beta_n\delta$ subtype localized on granule cells (Brickley et al., 1996, 2001; Tia et al., 1996; Wall and Usowicz, 1997; Hamann et al., 2002; Rossi et al., 2003).

Autoantibodies to GAD have been detected in the cerebrospinal fluid (CSF) of individuals with progressive cerebellar ataxia (Meinck et al., 2001; Takenoshita et al., 2001; Lauria et al., 2003; Vianello et al., 2003). Moreover, exposure of rat cerebellar slices to diluted (1:100) human CSF containing antibodies to GAD from such individuals induced a reduction both in the synthesis of GABA and in its release from the terminals of interneurons onto Purkinje cells (Takenoshita et al., 2001; Mitoma et al., 2003).

We have now investigated the effects of a reduction in inhibitory control by GABA on the function of the cerebellar cortex by the application of electrophysiolog-

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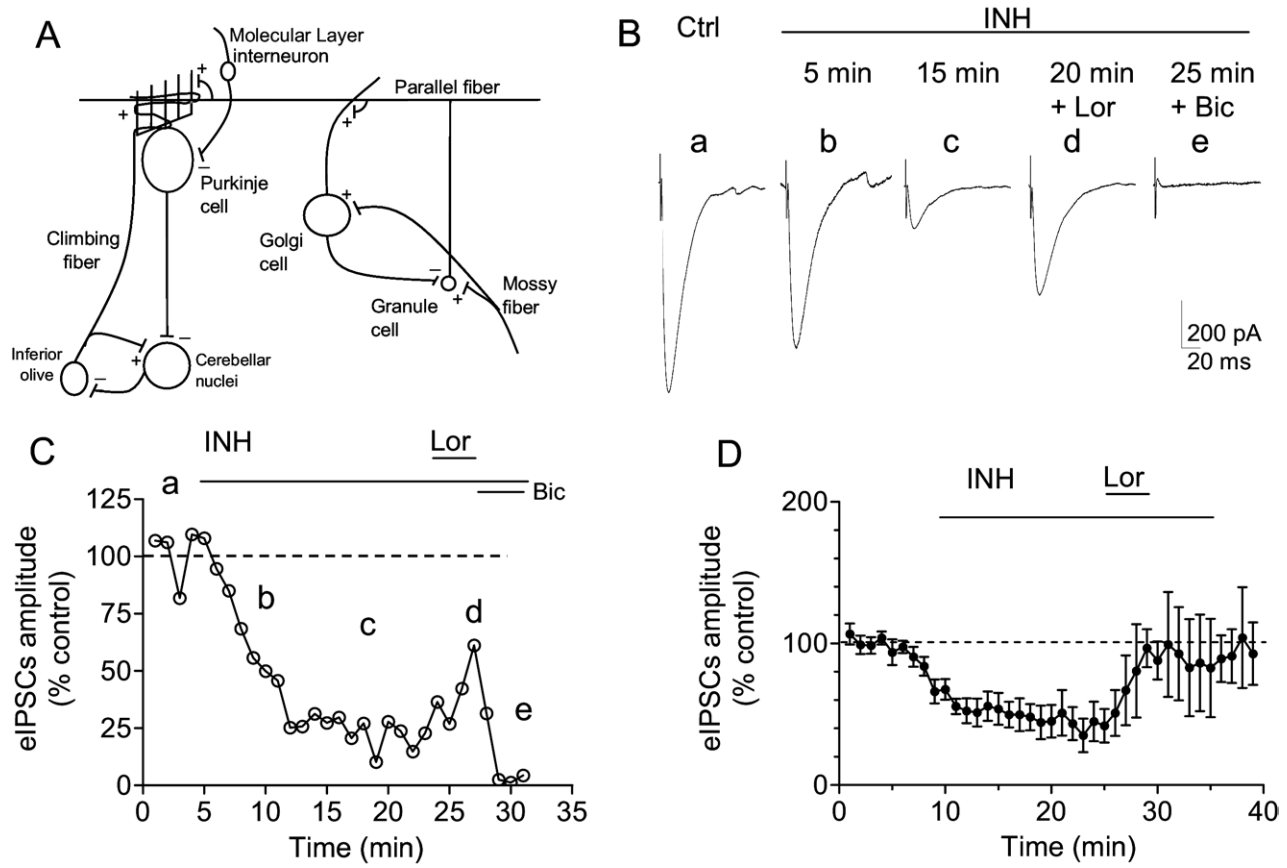

Fig. 1. Effect of isoniazid acid hydrazide (INH) on the amplitude of eIPSCs recorded from Purkinje neurons. (A) Simplified representation of the cerebellar circuit relevant to the present study. Mossy fibers, which originate in the spinal cord and brain stem, provide excitatory inputs to granule and Golgi cells. Granule cells also receive inhibitory input from Golgi cells and provide excitatory inputs to Purkinje cells and molecular-layer interneurons via parallel fibers. Molecular-layer interneurons provide inhibitory input to Purkinje cells. (B) Averaged traces of three to five $GABA_A$ receptor-mediated currents recorded from a single cerebellar Purkinje neuron. eIPSCs were elicited by stimulation of molecular-layer interneurons near the recorded Purkinje cell in the presence of kynurenic acid (3 mM). After a control (Ctrl) period of 5 min (a), application of INH (10 mM) resulted in a gradual decrease in the amplitude of eIPSCs (b, c). Application of lorazepam (Lor, 3 μM) partially reversed the inhibitory effect of INH (d). Finally, application of bicuculline (Bic, 20 μM) abolished all eIPSCs. (C) Time course of the effects of INH, Lor, and bicuculline on eIPSC amplitude in the cell studied in (B). The times at which the traces a through e in (B) were recorded are indicated. Data are expressed as a percentage of the control response. (D) Summary of the effects of INH and Lor on eIPSC amplitude. Data are means±S.E.M. (n=6).

ical techniques to cerebellar slices and with the use of isonicotinic acid hydrazide (isoniazid), an inhibitor of the GAD cofactor pyridoxal phosphate that markedly reduces the brain concentration of GABA and induces tonic–clonic seizures in rats (Horton et al., 1979). Indeed, we previously showed that a reduction in synaptic GABA concentrations induced by systemic administration of isoniazid in rats was associated with augmentation of neuronal activity, measured by the increase in the amount of cGMP, in the cerebellar cortex, and that these latter effects were selectively and potently inhibited by systemic administration or local injection of diazepam (Biggio et al., 1977a,b).

Our present electrophysiological results support the notion that GABA is an important determinant of the excitability of the cerebellar cortex and ensures the correct functioning of this brain region. The inhibition of GAD activity by isoniazid may represent a valuable experimental model with which to study the molecular and functional changes associated with a reduction in the synthesis and release of GABA at the synaptic level.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley CD rats were obtained from Charles River (Como, Italy). After their arrival at the animal facility, the rats were allowed to acclimatize to the new housing conditions for at least 1 week. They were housed six per cage under an artificial 12-h light/dark cycle (lights on from 08:00 to 20:00 h) and at a constant temperature of 22°±2 °C and relative humidity of 65%. They had free access to water and standard laboratory food at all times. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experimental protocols were also approved by the Animal Ethics Committee of the University of Cagliari. These procedures were conformed to international guidelines on the ethical use of animals, and every attempt was made to minimize the number of animals used and their suffering.