Progress in Brain Research, Vol. 148
ISSN 0079-6123

CHAPTER 25

Role of calcium binding proteins in the control of cerebellar granule cell neuronal excitability: experimental and modeling studies

D. Gall[1], C. Roussel[1], T. Nieus[2], G. Cheron[3], L. Servais[1,3], E. D'Angelo[2] and S.N. Schiffmann[1,*]

[1]*Laboratoire de Neurophysiologie (CP601), Faculté de Médecine, Université Libre de Bruxelles, Route de Lennik 808, B-1070 Brussels, Belgium*
[2]*Department of Cellular and Molecular Physiology and Pharmacology, University of Pavia and INFM, Via Forlanini 6, I-27100 Pavia, Italy*
[3]*Laboratoire d'Electrophysiologie, Université de Mons-Hainaut, B-7000 Mons, Belgium*

Keywords: calretinin; calcium; binding protein; cerebellar granule cell; excitability; calcium; mathematical model

Abstract: Calcium binding proteins, such as calretinin, are abundantly expressed in distinctive patterns in the central nervous system but their physiological function remains poorly understood. Calretinin is expressed in cerebellar granule cells which provide the major excitatory input to Purkinje cells through parallel fibers. Calretinin deficient mice exhibit dramatic alterations in motor coordination and in Purkinje cell firing recorded in vivo throught unknown mechanisms. In the present paper, we review the results obtained with the patch clamp recording techniques in acute slice preparation. This data allow us to investigate the effect of a null mutation of the calretinin gene on the intrinsic electroresponsiveness of cerebellar granule cells at a mature developmental stage. Calretinin deficient granule cells exhibit faster action potentials and generate repetitive spike discharge showing an enhanced frequency increase with injected currents. These alterations disappear when 0.15 mM of the exogenous fast calcium buffer BAPTA is infused in the cytosol to restore the calcium buffering capacity. Furthermore, we propose a mathematical model demonstrating that the observed alterations of granule cell excitability can be explained by a decreased cytosolic calcium buffering capacity dur to the absence of calretinin. We suggest that calcium binding proteins modulate intrinsic neuronal excitability and may therefore play a role in the information processing in the central nervous system.

Introduction

Calcium regulates a large variety of neuronal functions, including neurotransmitter release, ionic channel permeability, enzyme activity, and gene transcription. Hence, the cytosolic calcium concentration must be tightly regulated. Cytoplasmic calcium binding proteins play a key role in this regulation leading to specific adjustments of neuronal signaling. Among these, calretinin is the only calcium binding protein known to be expressed in cerebellar granule cells (Résibois, and Rogers, 1992; Marini, et al., 1997) whereas the structurally related calbindin

*Corresponding author. Tel.: +32 2 555 6407; Fax: +32 2 555 4121; E-mail: sschiffm@ulb.ac.be

DOI: 10.1016/S0079-6123(04)48025-X

is exclusively expressed in Purkinje cells. Granule cells form the largest neuronal population in the mammalian brain. They process information entering into the cerebellar cortex through the mossy fibers (Ito, 1984) and convey major excitatory afference to Purkinje cells through the parallel fibers. The involvement of cerebellum in motor coordination has long been recognized and, interestingly, it has recently been shown that calretinin deficient mice (Cr−/−) were impaired in motor coordination tests and displayed alterations in Purkinje cell activity recorded in vivo (Schiffmann et al., 1999). As calretinin is not expressed in Purkinje cells the latter study lacked direct evidence for intrinsic cerebellar electrophysiological alterations at the cellular level due to the Cr−/− mutation. This is particularly relevant since granule cells show a calcium-dependent regulation on their intrinsic excitability (Gabbiani et al., 1994; D'Angelo, et al., 1997, 1998, 2001), so that alteration in calcium-buffering is expected to affect action potential generation. Thus, in addition to addressing a question specific to cerebellar physiology, Cr−/− mice may serve as a model for understanding the regulation of calcium dependent control of neuronal excitability. In the present chapter, we review the experimental evidence showing that the electroresponsiveness of granule cells from Cr−/− mice is altered. Furthermore, we present a mathematical model providing a link between the observed alterations in granule cell electroresponsiveness and the decreased cytosolic Ca^{2+} buffering capacity due to the absence of calretinin. Our results suggest a critical role for calretinin in regulating granule cell excitability and signal coding at the input stage of the cerebellum.

Results

Alteration of granule cell intrinsic membrane excitability

Recordings were made using the perforated patch technique in order to minimize unwanted alteration of the endogenous Ca^{2+} buffering capacity. Intrinsic granule cell electroresponsiveness was investigated in current clamp recordings. The resting potential of WT and Cr−/− granule cell was not significantly different (-64.8 ± 5.5 mV, $n = 5$ vs. -65.9 ± 5.3 mV, $n = 8$, NS). Neither WT nor Cr−/− granule cells generated spontaneous action potentials. Active cell membrane properties were evaluated by measuring the voltage response while injecting steps of depolarizing current of increasing intensities in the granule cell soma. Above a critical value of the injected current (4.4 ± 1.6 pA for WT, $n = 5$ vs. 4.9 ± 1.0 pA for Cr−/−, $n = 8$, NS) fast repetitive spiking was obtained with a threshold of spike prepotential of -58.0 ± 2.3 mV for WT granule cells ($n = 5$) and of -58.7 ± 2.0 mV for Cr−/− granule cells ($n = 8$, NS). Action potentials occurred in regular trains showing little or no adaptation and frequency increased with the intensity of the injected current. The average frequency was measured over the whole duration of current injection (1 s) and was used to construct current–frequency plots (Fig. 1A). At low current intensities, the current–frequency plots were interpolated with a straight line. As the threshold current and the maximal frequency varies substantially from cell to cell, the evaluation of the slope factor of the linear part of the current frequency plots was used as a normalized measure of excitability. Using such an analysis, we observed a significant increase in the slope of the current frequency plots (4.8 ± 0.2 Hz pA^{-1} for WT, $n = 5$, and 6.6 ± 0.7 Hz pA^{-1} for Cr−/−, $n = 8$, $p < 0.05$) indicating that the excitability of Cr−/− granule cells was increased. In addition, Cr−/− granule cells showed a 23% decrease in the action potential half-width evaluated at the threshold potential where fast repetitive spiking is obtained (1.02 ± 0.06 ms for WT, $n = 5$, and 0.78 ± 0.06 ms for Cr−/−, $n = 8$, $p < 0.05$). Besides changes observed in Cr−/− mice, it should be noted that the spike shape and the frequency of spike discharge in WT mice were similar to those reported previously (D'Angelo, et al., 1998). The action potential undershoot amplitude was deeper for Cr−/− than WT granule cells, and this change did not reach statistical significance (-4.0 ± 0.7 mV for WT, $n = 5$, and -6.4 ± 1.7 mV for Cr−/−, $n = 7$, $p = 0.2406$, NS). The fact that the latter change fails to be statistically significant probably reflects the higher sensitivity of this parameter towards cell to cell variations compared to more robust temporal parameters like the slope factor of current–frequency relationship or the action potential half-width.

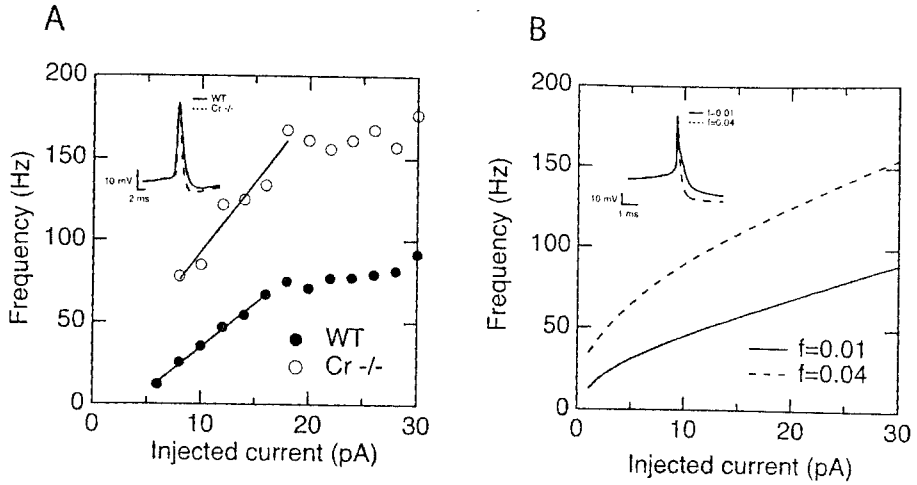

Fig. 1. Evaluation of the intrinsic excitability of a cerebellar granule cell in wild type and Cr−/− mice. Using the perforated patch configuration, the average action potential frequency was measured over the total time of current injection (1 *s*) at increasing intensities of the injected current. From these data, current-frequency plots could be constructed (A). At low current intensities, granule cells showed a linear encoding of stimulus intensity. The slope of the linear part of current frequency plots is used as a measure of the intrinsic granule cell excitability. The Cr−/− granule cell showed an increased excitability and faster action potentials compared to the WT (A, inset). Panel (B) shows the effect of a reduction of the Ca^{2+} buffering capacity on the intrinsic excitability of the granule cell model. The increase in the parameter *f* mimicks the decrease in cytosolic Ca^{2+} buffering capacity due to the absence of calretinin (see text for details). The corresponding current–frequency plots are shown. As observed experimentally, the slope of the current–frequency plot is markedly increased and action potentials are faster (B, inset).

Relationship between calretinin deficiency and altered excitability

To investigate the role of cytosolic Ca^{2+} buffering on intrinsic granule cell excitability, we have used a mathematical model. Calcium dynamics have a profound influence on Ca^{2+}-activated K^+ current (I_{K-Ca}) activation thereby regulating spike discharge. A typical approach to investigate Ca^{2+} dynamics (Traub and Llinas, 1979) is to model Ca^{2+} and I_{K-Ca} and to adapt Ca^{2+} dilution and removal in order to match the firing pattern. This approach has previously been applied to cerebellar granule cell model (Gabbiani et al., 1994; Maex and Schutter, 1998; D'Angelo et al., 2001). Here, in order to focus our attention on Ca^{2+} dynamics, we have reduced the number of gating variables and currents involved in action potential generation. We have also modified the equation governing the Ca^{2+} dynamics to take into account variations in the concentration of endogenous Ca^{2+} buffers. As granule cells have a compact electrotonic structure (Silver et al., 1992; D'Angelo et al., 1993, 1995, 2001) a single compartment model was used. Following the classical Hodgkin and Huxley (1952) approach, the membrane can be considered as a leaky capacitor and the membrane potential dynamics are governed by the current balance equation:

$$C_m \frac{dV}{dt} = -I_{Na} - I_{K-V} - I_{Ca} - I_{K-Ca} \qquad (1)$$

where C_m is the cell capacitance, I_{Na} is a voltage-dependent Na^+ current, I_{K-V} a delayed rectifier K^+ current, I_{Ca} a high-threshold voltage dependent Ca^{2+} current, and I_{K-Ca} a Ca^{2+} activated K^+ current. These ionic currents have been shown to be the core of action potential generation in cerebellar granule cells (D'Angelo et al., 1998) when the excitable response has assumed its mature pattern (D'Angelo et al., 1997). The interplay between I_{Na} and I_{K-V} is the basic mechanism giving rise to the action potentials and the presence of I_{Ca} and I_{K-Ca} allows coupling between intracellular calcium dynamics and membrane potential dynamics. The complete expression for the different ionic currents and all parameter values are the same as in Gall et al. (2003). To complete the model, the following balance equation

gives the evolution of the free calcium concentration (in μM) in a submembrane shell of thickness d in a cell of surface area A.

$$\frac{d\mathrm{Ca}}{dt} = f - \left[\frac{I_{\mathrm{Ca}}}{2FAd} - \beta_{\mathrm{Ca}}\mathrm{Ca}\right] \qquad (2)$$

where the dimensionless parameter f represents the calcium buffering capacity of the cytosol inside the submembrane shell due to the presence of fast calcium binding proteins (binding is assumed to be instantaneous). In particular, calretinin is a fast calcium buffer, the mean free lifetime for a Ca^{2+} ion in presence of a physiological concentration of the protein being of the order of microseconds (Edmonds et al., 2000), three orders of magnitude faster than the time scale of the evolution of the variables of the system. Therefore any alteration in the calretinin level corresponds to a modification of f. Moreover, as this parameter can be seen as the ratio of *free* Ca^{2+} concentration to *bound* Ca^{2+} concentration (Chay and Keizer, 1983; Gall et al., 1999; Gall and Susa, 1999), a decrease in cytosolic Ca^{2+} buffering capacity due to the absence of calretinin can be mimicked by an increase in the value of f. In the absence of data for cerebellar granule cells, the numerical values used here for the cytosolic Ca^{2+} buffering capacity (in the order of 0.01) have been set in agreement with values reported for other neuronal types (Tatsumi and Katayama, 1993; Stuenkel, 1994; Helmchen et al., 1996), with the notable exception of cerebellar Purkinje cells which show a calcium binding ratio an order of magnitude higher (Fierro and Llano, 1996). The parameter β_{Ca} describes Ca^{2+} removal corresponding to diffusion, action of ionic pumps, and slow buffers. Calcium dynamics were adapted to yield Ca^{2+} transients in the μM range, similar to those reported from Gabbiani et al. (1994). This model should be seen as a minimal model allowing us to qualitatively understand the impact of variations in cytosolic Ca^{2+} buffering capacity on the electroresponsiveness of an excitable cell. Nevertheless, parameter values have been chosen in order to reproduce the relevant aspects of the cerebellar granule cell electroresponsiveness. In our model, calcium buffering capacity, due to the action of fast calcium buffers, is represented by the dimensionless parameter f giving the ratio of *free* Ca^{2+} concentration to *bound* Ca^{2+} concentration (Chay and Keizer, 1983; Gall et al., 1999; Gall and Susa, 1999). A decrease in cytosolic Ca^{2+} buffering capacity due to the absence of calretinin can be simulated by an increase in the value of f. The observed alterations in Cr−/− mice can be mimicked by a four-fold decrease in the cytosolic Ca^{2+} buffering capacity, raising the action potential frequency from 55.1 Hz ($f = 0.01$) to 105.7 Hz ($f = 0.04$) in good agreement with the experimental data (Fig. 1B). The linear slope increase in current frequency plots from 3.0 Hz pA^{-1} for $f = 0.01$ to 5.2 Hz pA^{-1} for $f = 0.04$ is also close to the experimental values. The action potential half-width undergoes a 41% decrease when f is increased from 0.01 to 0.04. This action potential shortening reflects a more pronounced activation of $I_{\mathrm{K-Ca}}$ due to faster Ca^{2+} dynamics taking place when the cytosolic Ca^{2+} buffering capacity is decreased. This greater $I_{\mathrm{K-Ca}}$ activation also leads to a 28% increase in the action potential undershoot. Therefore the model suggests that faster Ca^{2+} dynamics can fully explain the increased excitability observed in Cr−/− mice through increased activation of Ca^{2+}-activated K^+ current. Similar results (Fig. 2) were obtained with more complex models by Maex and Schutter (1998) and by D'Angelo et al. (2001) modified to include Ca^{2+} dynamics as described by Eq. (2). This demonstrates that the modulation of intrinsic excitability by calcium buffering properties is a robust effect and does not depend strongly on detailed assumptions underlying the different theoretical models. It is important to note that a change in the dynamics leading to the activation of $I_{\mathrm{K-Ca}}$ is needed to obtain hyperexcitability in the model. A simple increase in the maximal conductance due to Ca^{2+}-activated K^+ channels ($\bar{g}_{\mathrm{K-Ca}}$) leads to the opposite effect, lowering the spike frequency (not shown).

GABA-A antagonist picrotoxin can unmask increased excitability in Cr−/− granule cells

Granule cells are excited by glutamatergic mossy fiber synapses and inhibited by GABAergic Golgi cell synapses. Without GABA-A receptor inhibitors, tonic granule cell inhibition is observed (Armano et al., 2000). Therefore, we have characterized the electroresponsiveness of cerebellar granule cells in the

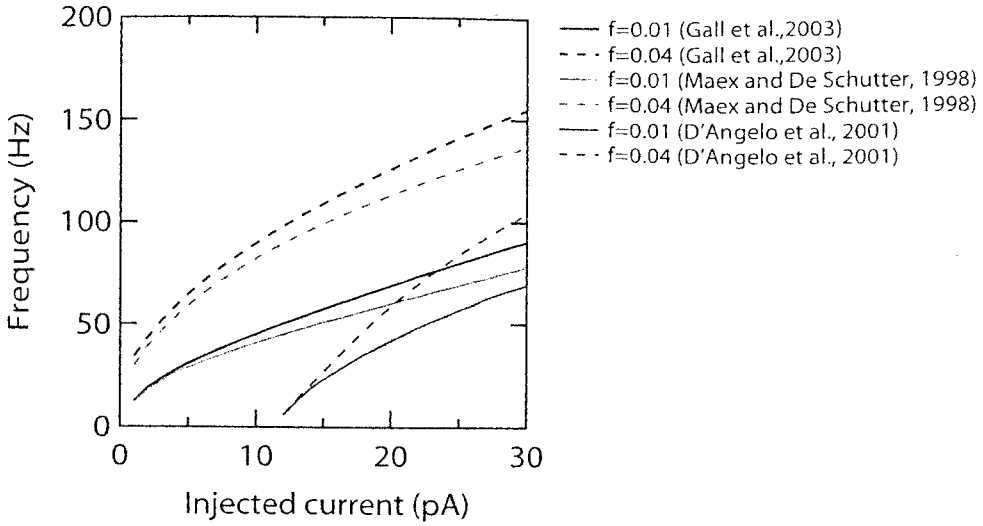

Fig. 2. Effect of a reduction of the Ca^{2+} buffering capacity on the intrinsic excitability in different granule cell models. Current-frequency plots for three granule cell models with a cytosolic Ca^{2+} buffering capacity parameter f of 0.01 (solid lines) and 0.04 (dashed line). All models shows a similar increase in excitability when the calcium beffering capacity is decreased.

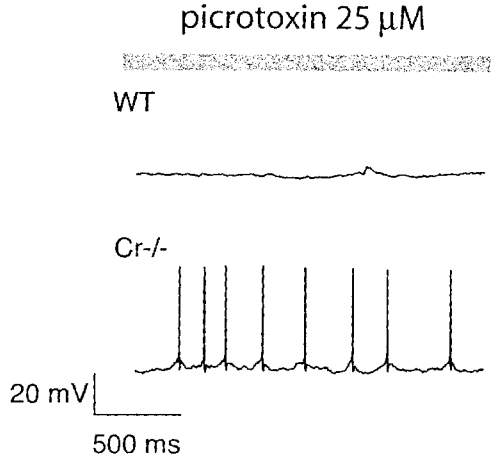

Fig. 3. GABA-A blockade by picrotoxin induces spontaneous firing in Cr−/− granule cells. In the presence of 25 µM picrotoxin, spontaneous electrical activity is seen in Cr−/− granule cells but not in wild type granule cells (WT).

presence of the GABA-A receptor blocker picrotoxin (25 µM). In these conditions, we found that six out of seven Cr−/− granule cells showed spontaneous electrical activity (Fig. 3), but no such activity was present in WT granule cells ($n=5$, $p<0.05$, Fischer's test). In addition, an increase in the intrinsic excitability was also observed since the slope of the current–frequency plots was significantly high in mutant mice (3.4 ± 0.6 Hz pA^{-1} for WT, $n=6$, and 7.3 ± 1.0 Hz pA^{-1} for Cr−/−, $n=5$, $p<0.05$). Interestingly, the effects were not detected when the measure was repeated in the presence of 10 µM bicuculline methobromide which is also a GABA-A blocker (Fig. 4). This can be explained by the fact that bicuculline methobromide also acts as a blocker of Ca^{2+}-dependent K^+ channels (Seutin and Johnson, 1999). These results, therefore, reinforce the idea that this current is indeed mediating the effect of altered Ca^{2+} buffering capacity on granule cell excitability.

Discussion

In this review, we have shown that the absence of calretinin increases cerebellar granule cell excitability without altering passive electrical membrane properties. In addition, we have used a mathematical model in order to examine whether the observed alterations of Cr−/− cerebellar granule cell electroresponsiveness could be linked to the decreased Ca^{2+} buffering capacity determined by the absence of calretinin. We challenged with a decreased cytosolic Ca^{2+} buffering capacity, the model correctly predicts all the changes in Cr−/− granule cell electroresponsiveness that are

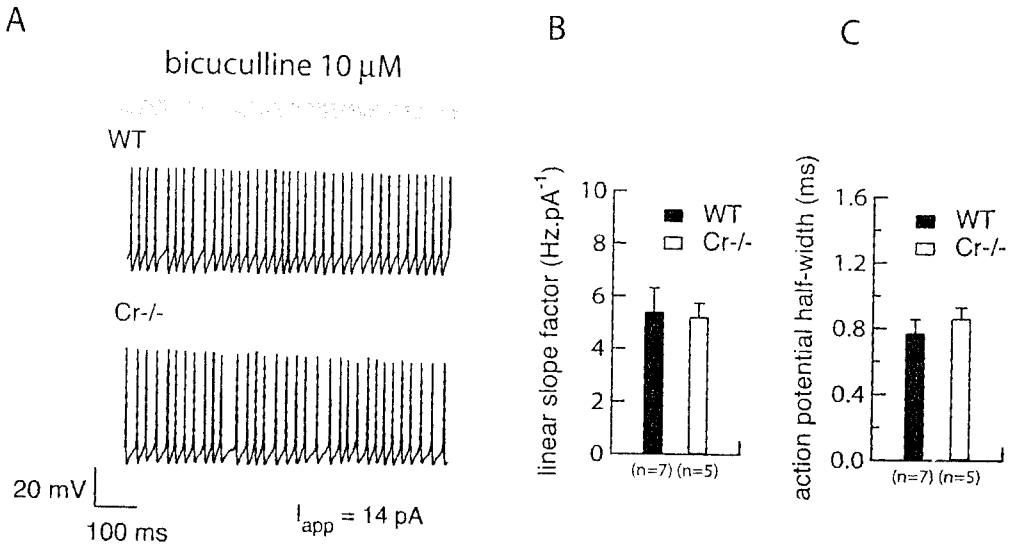

Fig. 4. No change in the intrinsic excitability of the granule cell in presence of bicuculline. In presence of 10 µM bicuculline methobromide, the Cr−/− granule cell show similar action potential frequency (A, bottom) compared to the WT (A, top) cell for the same injected current from a holding potential of −80 mV. Histograms report slope factor of the current–frequency plots for WT vs. Cr−/− (B) and corresponding durations of action potentials at half-amplitude (C) evaluated at the threshold potential where fast repetitive spiking is obtained. In these conditions, Cr−/− granule cells ($n = 7$) do not show a significantly increased excitability of faster action potentials compared to WT ($n = 5$).

observed experimentally. Our model should not be seen as a complex model of the granule cell like the one proposed by D'Angelo et al. (2001) but rather as an abstract model focusing on a specific cell property, cytosolic Ca^{2+} buffering. The purpose of this model is to demonstrate the basic mechanism linking alteration of the intrinsic excitability in Cr−/− mice to alterations in the cytosolic Ca^{2+} buffering capacity. Thus, on a broader perspective, the conclusions drawn from our simulations can be applied to other neuronal types provided that the mechanisms of excitability are essentially the same as in cerebellar granule cells and that the conductance of the Ca^{2+}-activated K^+ channels is sufficient to obtain a strong coupling between excitability and Ca^{2+} dynamics during the spike generation. Whereas it is general knowledge that increasing Ca^{2+} activated K^+ current slows down the firing rate, faster Ca^{2+} dynamics through reduced Ca^{2+} buffering has the opposite effect. The model suggests that this is due to direct control of I_{K-Ca} by the Ca^{2+} transient, speeding-up spike repolarization when the calcium buffering capacity is decreased. In this view, in addition to their obvious role in Ca^{2+} homeostasis, Ca^{2+}-binding proteins could play an active role in modulating neuronal intrinsic excitability and therefore neuronal plasticity. Although information storage is usually believed to be mediated by long-term modifications in the strength of synaptic transmission activity-dependent changes in the neuronal intrinsic excitability also take place, causing forms of nonsynaptic plasticity. Such activity-dependent changes in intrinsic excitability have been shown to occur in cerebellar granule cells (Armano et al., 2000) and neurons of the deep cerebellar nuclei (Aizenman and Linden, 2000). It would be interesting to know whether activity-dependent modifications in the localization or in the level of expression of Ca^{2+} binding proteins are involved. Whereas expression changes are still controversial (see review by Baimbridge et al. (1992)), changes in the localization of calretinin have been shown to occur in neurons (Hack et al., 2000).

In conclusion, calretinin deficiency increases the intrinsic excitability of cerebellar granule cells. The increased granule cell electroresponsiveness may explain the electrophysiological and behavioral alterations observed in vivo on alert Cr−/− mice.

indicating the critical role of granule cells in the information processing in the cerebellar cortex. On a broader perspective, we suggest that modulation of neuronal excitability by Ca^{2+} binding proteins could play a functional role in the control of information coding and storage in the central nervous system.

Acknowledgments

David Gall is postdoctoral Researcher at the Belgian Fonds National de la Recherche Scientifique (FNRS). Celine Roussel is supported by the Belgian Fonds pour la Recherche dans l'Industrie et l'Agriculture (FRIA). This work was also supported by the Queen Elisabeth Medical Foundation (FMRE-Neurobiology 02–04), Fund for Medical Scientific Research (FRSM-Belgium 3.4507.02), Fondation David et Alice Van Buren, Action de Recherche Concertee (2002–2007) and by the European Commission's 5th Framework Program for research (CEREBELLUM BIO4CT98-0182 and SPIKEFORCE IST35271).

References

Aizenman, C.D. and Linden, D. (2000) Rapid, synaptically driven increase in the intrinsic excitability of cerebellar deep nuclear neurons. Nat. Neurosci., 3: 109–111.

Armano, S., Rossi, P., Taglietti, V. and D'Angelo, E. (2000) Long term potentiation of intrinsic excitability at the excitability at the mossy fiber-granule cell synapse of rat cerebellum. J. Neurosci., 20: 5208–5216.

Baimbridge, K.G., Celio, M. and Rogers, J. (1992) Calcium-binding proteins in the nervous system. Trends Neurosci., 15: 303–308.

Chay, T.R. and Keizer, J. (1983) Minimal model for membrane oscillations in the pancreatic β-cell. Biophys. J., 42: 181–190.

D'Angelo, E., Filippi, G.D., Rossi, P. and Taglietti, V. (1995) Synaptic excitation of individual rat cerebellar granule cells in situ: evidence for the role of NMDA receptors. J. Physiol. (Lond.), 484: 397–413.

D'Angelo, E., Filippi, G.D., Rossi, P. and Taglietti, V. (1997) Synaptic excitation of Ca^{2+} action potentials in immature rat cerebellar granule cells in situ. J. Neurophysiol., 78: 1631–1642.

D'Angelo, E., Filippi, G.D., Rossi, P. and Taglietti, V. (1998) Ionic mechanism of electroresponsiveness in cerebellar granule cells implicates the action of a persistent sodium current. J. Neurophysiol., 80: 493–503.

D'Angelo, E., Nieus, T., Maffei, A., Armano, S., Rossi, P., Taglietti, V., Fontana, A. and Naldi, G. (2001) Theta-frequency bursting and resonance in cerebellar granule cells: experimental evidence and modeling of a slow K^+-dependent mechanism. J. Neurosci., 21: 759–770.

D'Angelo, E., Rossi, P. and Tagiletti, V. (1993) Different proportions of N-methyl-D-aspartate and non-N-methy-D-aspartate receptor currents at the mossy fibre-granule cell synapse of developing rat cerebellum. Neuroscience, 53: 121–130.

Edmonds, B., Reyes, R., Schwaller, B. and Roberts, W. (2000) Calretinin modifies presynaptic calcium signaling in frog saccular hair cells. Nat. Neurosci., 80: 2521–2537.

Fierro, L. and Llano, I (1996) High endogenous calcium buffering in Purkinje cells from rat cerebellar slices. J. Physiol., 496: 617–625.

Gabbiani, F., Mitgaard, J. and Knoepfel, T. (1994) Synaptic integration in a model of cerebellar granule cells. J. Neurophysiol., 72: 999–1009.

Gall, D., Gromada, J., Susa, I., Herchuelz, A., Rorsman, P. and Bokvist, K. (1999) Significance of Na/Ca exchange for Ca^{2+}-buffering and electrical activity in mouse pancreatic β-cells. Biophys. J., 76: 2018–2028.

Gall, D., Roussel, C., Susa, I., D'Angelo, E., Rossi, P., Bearzatto, B., Galas, M.-C., Blum, D., Schurmans, S. and Schiffmann, S. (2003) Altered neuronal excitability in cerebellar granule cells of mice lacking calertinin. J. Neurosci., 23: 9320–9327.

Gall, D. and Susa, I. (1999) Effect of Na/Ca exchange on plateau fraction and $[Ca]_i$ in models for bursting in pancreatic β-cells. Biophys. J., 77: 45–53.

Hack, N.J., Wride, M., Charters, K., Kater, S. and Parks, T. (2000) Developmental changes in the subcellular localization of calretinin. J. Neurosci., 20: RC67.

Helmchen, F., Imoto, K. and Sakmann, B. (1996) Ca^{2+} buffering and action potential-evoked Ca^{2+} signaling in dendrites of pyramidal neurons. Biophys. J., 70: 1069–1081.

Hodgkin, A.L. and Huxley, A. (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol., 117: 500–544.

Ito, M. (1984) Granule cells. In: The Cerebellum and Neuronal Control. Raven, New York, pp. 74–93.

Maex, R. and Schutter, E.D. (1998) Synchronization of Golgi and granule cell firing in a detailled network of the cerebellar granule cell layer. J. Neurophysiol., 80: 2521–2537.

Marini, A.M., Strauss, K. and Jacobowitz, D. (1997) Calretinin containing neurons in rat cerebellar granule cell cultures. Brain Res. Bull., 42: 279–288.

Résibois, A. and Rogers, J. (1992) Calretinin in rat brain: an immunohistological study. Neurosci., 46: 101–134.

Schiffmann, S., Cheron, G., Lohof, A., D'Alcantara, P., Meyer, M., Parmentier, M. and Schurmans, S. (1999) Impaired motor coordination and Purkinje cell excitability in mice lacking calretinin. Proc. Natl. Sci. USA, 96: 5257–5262.

Seutin, V. and Johnson, S. (1999) Recent advances in the pharmacology of quaternary salts of bicuculline. Trends Pharmacol. Sci., 20: 268–270.

Silver, R.A., Traynelis, S. and Cull-Candy, S. (1992) Rapid time course of miniature and evoked excitatory currents at cerebellar synapses in situ. Nature, 355: 163–166.

Stuenkel, E.L. (1994) Regulation of intracellular calcium and calcium buffering properties of rat isolated neurohypophysial nerve endings. J. Physiol., 481: 251–271.

Tatsumi, H. and Katayama, Y. (1993) Regulation of the intracellular free calcium concentration in acutely dissociated neurons from rat nucleus basalis. J. Physiol., 464: 165–181.

Traub, R.D. and Llinas, R. (1979) Hippocampal pyramidal cells: significance of dendritic ionic conductances for neuronal functioning and epileptogenesis. J. Neurophysiol., 42: 476–496.