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ARTICLE

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Modulation by Zn^{2+} and Cd^{2+} of $GABA_A$ receptors of rat cerebellum granule cells in culture

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Abstract This study aims to characterize more closely the different populations of $GABA_A$ receptors present on the cerebellar granule cells of the rat. The effects of two divalent cations, Zn^{2+} and Cd^{2+}, on GABA-activated chloride currents were studied using the whole-cell patch-clamp technique. Zinc cations inhibit differently the peak and the steady-state current elicited by 10 μM GABA. In fact, Zn^{2+} appears to be more potent in inhibiting the steady-state component, with a lower IC_{50}. The inhibition of the peak component is of the competitive type, whereas the inhibition of the steady-state one is mixed, being partly competitive and partly allosteric. In addition, Cd^{2+} has an inhibitory effect on GABA-activated chloride currents. In terms of the peak component, its effect is limited in extent with a maximal inhibition of only 26%, but with a high affinity (IC_{50} as low as 0.03 μM). The steady-state component is inhibited by 20% independently from the Cd^{2+} concentration, in the 10^{-2}–10^{2} μM range. In this case, the inhibitory mechanism appears to be of the competitive type for the peak component and of the allosteric type for the steady-state one. We suggest these data are a further confirmation that the rapidly and slowly desensitizing components of the GABA-activated chloride currents, corresponding respectively to the peak and the steady-state components, are made up of two different receptor populations.

Keywords $GABA_A$ receptors · Current inhibition · Zinc cations · Cadmium cations · Cerebellar granule cells

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Introduction

Considerable attention has been devoted to the effects of heavy metal cations on $GABA_A$ receptor associated chloride currents. The effects of Zn^{2+} on both native and recombinant $GABA_A$ receptors have been focused on in particular (Smart and Constanti 1990; Celentano et al. 1991; Smart et al. 1991; Kilic et al. 1993; White and Gurley 1995; Frye et al. 1996; Martina et al. 1996; Saxena and Macdonald 1996; Trombley and Shepherd 1996; Wooltorton et al. 1997; Fisher and Macdonald 1998; Gingrich and Burkat 1998; Horenstein and Akabas 1998; Krishek et al. 1998; Strecker et al. 1999; Barberis et al. 2000; Sharonova et al. 2000). Some interest has also been devoted to the effects of Cu^{2+} (Trombley and Shepherd 1996; Sharonova et al. 2000) and to those of some divalent cations (Arakawa et al. 1991; Narahashi et al. 1994; Schwartz et al. 1994). Interest in the study of the effects of Zn^{2+} stems from its presence in the brain and particularly in the cortex and limbic system (Fredrickson 1989). An additional interesting feature is its presence in nerve endings (Holm et al. 1988; Danscher et al. 2001) and the possibility of being released after stimulation (Assaf and Chung 1984; Howell et al. 1984). The same applies to Cu^{2+} (Kardos et al. 1989). In the case of Zn^{2+}, it is worthwhile recalling that a putative Zn^{2+} transporter is found on the surface of glutamate-containing vesicles (Wenzel et al. 1997). Indeed, the effects of Zn^{2+} on glutamate NMDA receptors have also been thoroughly studied (Peters et al. 1987; Westbrook and Mayer 1987; Legendre and Westbrook 1990; Chen et al. 1997). Another good reason to study the effects of Zn^{2+} on $GABA_A$ receptors is the attempt to understand the biophysical mechanism of its inhibitory action on different types of such receptors (Gingrich and Burkat 1998; Barberis et al. 2000).

In this report we used zinc and cadmium cations to test their effects on the GABA-activated chloride currents in cultured rat cerebellar granules in order to gain different, although related, information. This enabled us

to characterize in more detail the different populations of $GABA_A$ receptors present in cerebellar granule cells (Robello et al. 1999; Cupello and Robello 2000). In fact, previous experiments have shown that different types of $GABA_A$ receptors which can be present in cerebellar granules are differently sensitive to Zn^{2+} (Saxena and Macdonald 1996; Krishek et al. 1998). The results of this study show somewhat different effects of Zn^{2+} and Cd^{2+} on two granule cell $GABA_A$ receptor populations endowed with different desensitization kinetics.

Materials and methods

Primary cultures of rat cerebellum granules

Granule cells were prepared from cerebella of 8-day-old Wistar rats following the procedure of Levi et al. (1984), as previously described (Robello et al. 1993). Cells were plated at a density of 1×10^6 per dish on poly-L-lysine-coated glass coverslips placed in 20 mm plastic dishes and kept at 37 °C in a humidified 95% air/5% CO_2 atmosphere. Experiments were performed at room temperature between days 5 and 12 after plating.

Patch clamp recordings

In all the experiments, membrane currents were measured with the standard whole-cell patch-clamp technique by an EPC-7 (List medical) as previously described (Robello et al. 1993). Patch pipettes were prepared from borosilicate glass capillaries (type 1406129, Hilgenberg, Malsfeld, Germany) using a programmable Sachs and Flaming puller model PC-84.

The holding potential was set to –80 mV in all the experiments reported, which resulted as the most suitable condition to record the total chloride current elicited by different GABA concentrations. Run-down phenomena of chloride currents were prevented by the presence of ATP in the internal solution.

Ionic currents were registered with a Labmaster D/A, A/D converter driven by pClamp software (Axon Instrument, Burlingame, Calif., USA). Analysis was performed with p-Clamp and SIGMA PLOT (Jandel Scientific, Erkrath, Germany) software. Data are given as mean ± SEM. Statistical comparisons were made by Student's *t*-test.

Solutions

All chemicals were purchased from Sigma (St Louis, Mo., USA). The standard external solution consisted of (in mM): 135 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, 10 glucose. The pH was adjusted to 7.4 using NaOH. The pipette filling (internal) solution contained (in mM): 142 KCl, 10 HEPES, 2 EGTA, 4 $MgCl_2$, 3 ATP. The pH was adjusted to 7.3 with Trizma base.

GABA was dissolved in distilled water (10 mM) and then diluted with the external solutions to the desired concentration just before the experiments were conducted. Once diluted, GABA was applied together with the external solution to the cell bath by steady perfusion (~3 mL/min gravity flow). Stock solutions of zinc and cadmium chlorides were diluted to the desired final concentration in the internal or external solution.

Results

Effects of Zn^{2+} on the peak and steady-state components of the GABA-activated chloride current

The results in Fig. 1 show that 50 μM Zn^{2+} reduces the chloride current activated by 10 μM GABA in granule cells registered in the whole-cell configuration. Washing out zinc for 2 min is enough to restore the control chloride current. The effects of different concentrations of zinc cations on respectively the peak and the steady-state components of the GABA-activated chloride current are shown in Fig. 2A.

The extent of the inhibition by Zn^{2+}, $I\% = 100\times[I_{Cl}(GABA) - I_{Cl}(GABA + Zn^{2+})]/I_{Cl}(GABA)$, of the peak and the steady-state components of the chloride current (I_{Cl}) activated by 10 μM GABA is a function of the concentration (C). The data were fitted by the equation:

$$I\% = I\%_{max}\left[C^n/\left(C^n + IC_{50}^n\right)\right] \quad (1)$$

where $I\%_{max}$ is the maximal inhibition, IC_{50} represents the $[Zn^{2+}]$ at which the inhibition is $0.5\times I\%_{max}$, and n is the Hill coefficient. The data show that for the peak component, $I\%_{max} = 51 \pm 5\%$, $IC_{50} = 13 \pm 3$ μM and $n = 0.6 \pm 0.1$; for the steady-state component one obtains $I\%_{max} = 73 \pm 13\%$, $IC_{50} = 5 \pm 1$ μM and $n = 0.6 \pm 0.3$. The difference in the $I\%_{max}$ values was not statistically significant ($P = 0.11$). However, the difference of the IC_{50} values was significant, $P < 0.02$. Pre-treatment of the cells with 10 μM Zn^{2+} for 30 s changed the inhibition of the peak chloride current from $29 \pm 3\%$ to $52 \pm 2\%$ ($P = 0.0121$). Pre-treatment for up to 2 min did not significantly change the inhibition (from $52 \pm 2\%$ to $60 \pm 4\%$).

Recovery from the zinc effect; absence of an effect by intracellular Zn^{2+}

The recovery percentage of the chloride peak current after a 2 min wash is complete for $[Zn^{2+}]$ up to 100 μM

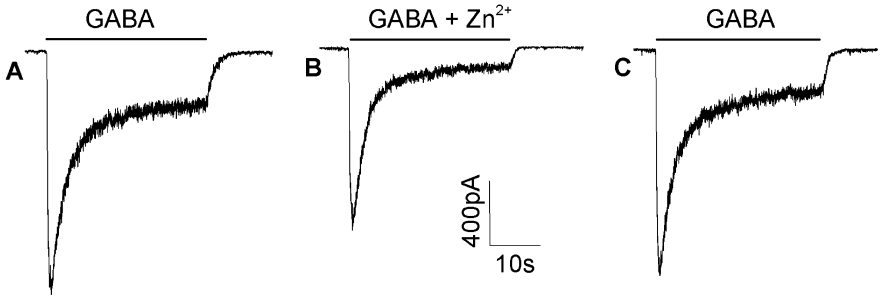

Fig. 1A–C Effect of zinc on the granule cells chloride current activated by 10 μM GABA. The current was activated in whole-cell recording in a granule cells voltage clamped at –80 mV. **A** 10 μM GABA, control, in standard solution. **B** Co-application of 10 μM GABA and 50 μM zinc. **C** 10 μM GABA after 2 min washout with standard solution

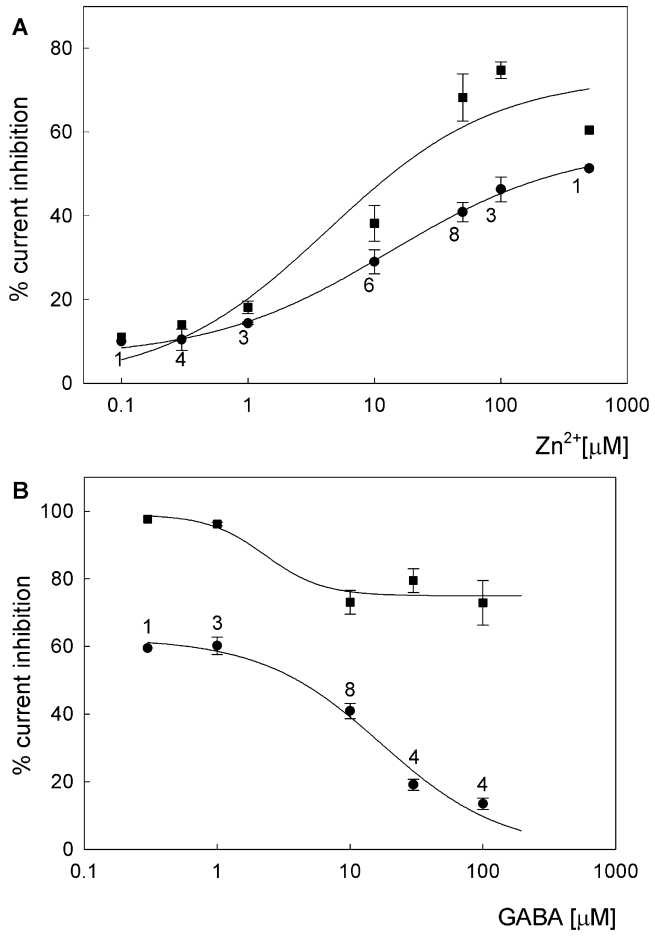

Fig. 2 **A** Dose-response curve. Semi-logarithmic plot of the peak (*circles*) and steady-state (*squares*) current inhibition as a function of zinc concentration. Each cell was treated with the same dose of GABA (10 μM) and different concentrations of zinc. The points represent mean ± SEM and the number of cells tested is indicated. Experimental points can be fitted using Eq. (1). The best fit gave $I\%_{max} = 51 \pm 5\%$, $IC_{50} = 13 \pm 3$ μM, $n = 0.6 \pm 0.1$ for the peak inhibition, and $I\%_{max} = 73 \pm 13\%$, $IC_{50} = 5 \pm 1$ μM, $n = 0.6 \pm 0.3$ for the steady-state. **B** Dose-response curve. Semi-logarithmic plot of the inhibition of the peak (*circles*) and steady-state (*squares*) current in the presence of 50 μM zinc as a function of GABA concentration. Each point represents mean ± SEM and the number of cells tested is indicated. Theoretical fitting is obtained using Eq. (2). The best fit gave $I\%_{max} = I\%_{con} = 62 \pm 5\%$, $IC_{50} = 17 \pm 5$ μM, $n = 1 \pm 0.3$ and $I\%_{res} = 0$ for peak current inhibition. The parameters for inhibition of the steady-state current were: $I\%_{max} = 99 \pm 11\%$, $IC_{50} = 2.9 \pm 0.2$ μM, $n = 2.2 \pm 0.4$, $I\%_{res} = 75 \pm 2\%$, $I\%_{con} = 24 \pm 2\%$

(100 ± 3% of the control peak current). When 10 μM Zn^{2+} was added to the intracellular medium, no inhibition of GABA-activated peak chloride current was found, even with a pre-treatment with intracellular 10 μM zinc for up to 12 min.

GABA dose-response curve for the current activated in the presence of zinc

The inhibitory effect by 50 μM Zn^{2+} on the peak and steady-state chloride currents activated by different GABA concentrations is a function of the GABA concentration (C), as shown in Fig. 2B. The data were fitted by the equation:

$$I\% = \mathrm{I}\%_{con}\left[1 - \mathrm{C}^{n}/\mathrm{left}(\mathrm{C}^{n} + \mathrm{IC}_{50}^{n})\right] + \mathrm{I}\%_{res} \qquad (2)$$

where the maximal inhibition at low [GABA] is $I\%_{max} = I\%_{con} + I\%_{res}$; IC_{50} represents the [GABA] at which the inhibition is $0.5I\%_{con} + I\%_{res}$, n is the Hill coefficient, $I\%_{res}$ is the residual inhibition when [GABA] tends to high values in the respect of IC_{50} and $I\%_{con}$ is the inhibition component which depends on the GABA concentration.

From the data we could evaluate for the peak component an IC_{50} of 17 ± 5 μM, an $I\%_{max}$ of 62 ± 5%, an n coefficient of 1 ± 0.3 and $I\%_{res}$ is zero. Obviously, in this case all inhibition depends on [GABA] and $I\%_{max} = I\%_{con}$. For the steady-state component, IC_{50} is 2.9 ± 0.2 μM, $I\%_{max}$ is 99 ± 11%, n is 2.2 ± 0.4 and $I\%_{res}$ is 75 ± 2%. In this case, $I\%_{con} = 24 \pm 2\%$. The statistical comparisons give: $P < 0.01$ for IC_{50}, $P < 0.005$ for $I\%_{max}$, $P < 0.02$ for n, $P < 0.000001$ for $I\%_{res}$ and $P < 0.00001$ for $I\%_{con}$.

The effect of 10 μM Cd^{2+} on the chloride current activated by 10 μM GABA

Cd^{2+} (10 μM) applied together with an equimolar concentration of GABA resulted in a reduction of both peak and steady-state components of the chloride current (Fig. 3). Pre-treatment with Cd^{2+} give a greater inhibition. For instance, treatment with 1 μM Cd^{2+} for 30 s caused a greater inhibition of the peak component (35 ± 1.4% versus 22 ± 1.5%, $P = 0.012$). In the presence of 100 μM Cd^{2+} in the intracellular medium, no inhibition of GABA-activated peak chloride current was found, the current activated by GABA in this case being 96 ± 2% in relation to the control.

The recovery of the control level for the peak component after a 2 min washout of Cd^{2+} was complete (101 ± 2% in relation to the control).

Effects of various Cd^{2+} concentrations on the peak and steady-state currents elicited by 10 μM GABA

The effects of various concentrations of Cd^{2+} on the peak chloride current elicited by GABA are shown in Fig. 4A as the percent inhibition. The data were fitted to the same equation used above for Zn^{2+} inhibition (Eq. 1). The results were: $I\%_{max} = 26 \pm 1\%$; $IC_{50} = 0.03 \pm 0.01$ μM; $n = 0.4 \pm 0.1$. Thus, the data showed that the inhibitory effect on the peak component is very potent. The effect on the steady-state component seems to be constant and independent of the Cd^{2+} concentration (~20% inhibition in the whole 10^{-2}–10^{2} μM Cd^{2+} concentration range).

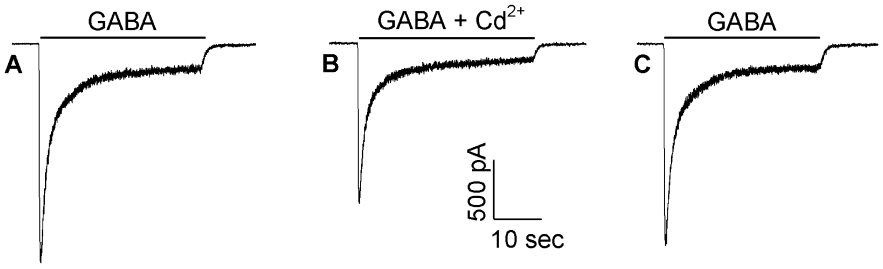

Fig. 3A–C Effect of cadmium on the granule cells chloride current. Traces were clamped at –80 mV. **A** Current activated by 10 μM GABA, control, in standard solution. **B** Current activated by co-application of 10 μM GABA and 10 μM cadmium after 2 min from control registration. **C** Current activated by 10 μM GABA after 2 min washout with standard solution

Effect of 1 μM Cd^{2+} at various [GABA]

The inhibitory effect on the peak current by 1 μM Cd^{2+} was constant at GABA concentrations between 1 and 10 μM. However, at [GABA] > 10 μM the inhibition began to decline (Fig. 4B). Data are fitted by Eq. (2) and the parameter values are: $IC_{50} = 340 \pm 51$ μM, $I\%_{con} = 22 \pm 1\%$, $n = 1.0 \pm 0.2$ and $I\%_{res} = 0$. Also, in this case, $I\%_{max} = I\%_{con}$. For the steady-state component: IC_{50} is 8.9 ± 1.5 μM, $I\%_{max}$ is $24 \pm 5\%$, n is 1 ± 0.2 and $I\%_{res}$ is $8 \pm 1\%$. In this case, $I\%_{con} = 16 \pm 2\%$. The statistical comparisons give: $P < 0.000005$ for IC_{50}, $P < 0.01$ for $I\%_{con}$ and $P < 0.000001$ for $I\%_{res}$. The differences were not significant for $I\%_{max}$ and n.

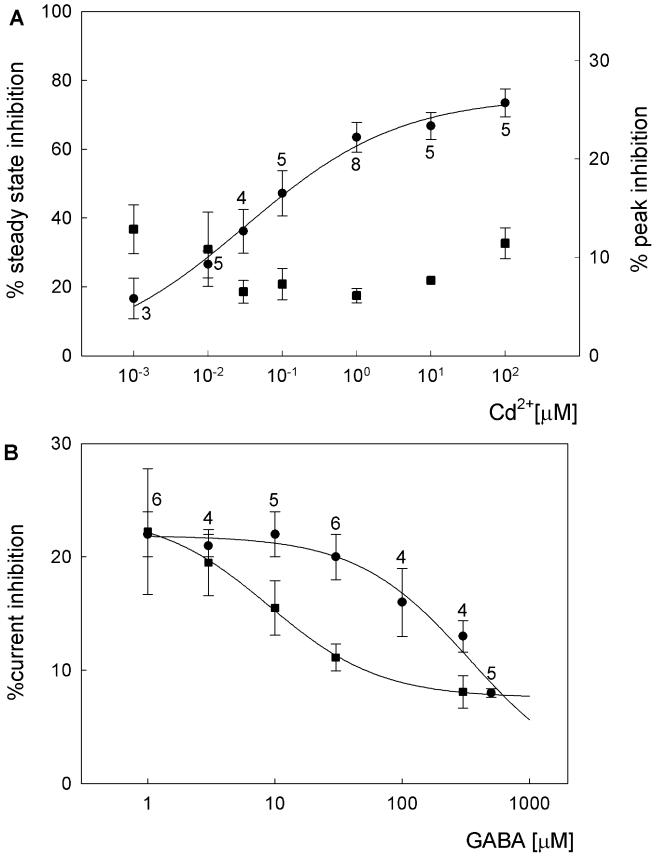

Fig. 4 A Dose-response curve. Semi-logarithmic plot of the inhibition of the peak (*circles*) and steady-state (*squares*) current activated by 10 μM GABA, as a function of cadmium concentration. Each cell was voltage clamped at –80 mV. Each point represents mean ± SEM and the number of cells tested is indicated. Mathematical fitting is obtained using Eq. (1). The best fit gave $I\%_{max} = 26 \pm 1\%$, $IC_{50} = 0.03 \pm 0.01$ μM, $n = 0.4 \pm 0.1$ for peak current inhibition. **B** Dose-response curve. Semi-logarithmic plot of the inhibition of the peak (*circles*) and steady-state (*squares*) current in the presence of 1 μM cadmium as a function of GABA concentration. Each cell was treated with the same dose of cadmium and different concentrations of GABA. Each point represents mean ± SEM and the number of cells tested is indicated. Mathematical fitting is obtained using Eq. (2). The best fit gave $I\%_{max} = I\%_{con} = 22 \pm 0.7\%$, $IC_{50} = 340 \pm 51$ μM, $n = 1.0 \pm 0.2$, $I\%_{res} = 0$ for peak current inhibition. The parameters for inhibition of the steady-state current were: $I\%_{max} = 24 \pm 5\%$, $IC_{50} = 8.9 \pm 1.5$ μM, $n = 1.0 \pm 0.2$, $I\%_{res} = 8 \pm 1\%$, $I\%_{con} = 16 \pm 2\%$

Discussion

In a previous report (Barilà et al. 2001) we discussed the effects on cerebellar granule $GABA_A$ receptors of a trivalent cation such as La^{3+}. Here, we extend the investigation of the effects of di/trivalent cations on such $GABA_A$ receptors to Zn^{2+} and Cd^{2+}, two divalent cations from the transition metals of Group IIb. These two ions share the same electrical charge but have different sizes, which results in different interactions with proteins (see e.g. Palumaa et al. 2002).

The effects of Zn^{2+} on $GABA_A$ receptors have already received wide attention (Smart and Constanti 1990; Celentano et al. 1991; Smart et al. 1991; Kilic et al. 1993; White and Gurley 1995; Frye et al. 1996; Martina et al. 1996; Saxena and Macdonald 1996; Trombley and Shepherd 1996; Wooltorton et al. 1997; Fisher and Macdonald 1998; Gingrich and Burkat 1998; Horenstein and Akabas 1998; Krishek et al. 1998; Strecker et al. 1999; Barberis et al. 2000; Sharonova et al. 2000), whereas relatively fewer studies have been devoted to the effects of Cd^{2+} (Smart and Constanti 1990; Schwartz et al. 1994; Kumamoto and Murata 1995; Fisher and Macdonald 1998). An interesting observation about the effects of Zn^{2+} ions on $GABA_A$ receptor function was noted from the start, namely that the sensitivity to zinc of those receptors was related to their subunit composition (Draguhn et al. 1990; Smart et al. 1991). In particular, whereas recombinant $\alpha_1\beta_1$ $GABA_A$ receptors are highly sensitive to Zn^{2+}, the coexpression of the γ_2 subunit results in receptors with a lower zinc sensitivity (Drag-

uhn et al. 1990; Smart et al. 1991). The same trend was found coexpressing γ_{2S} with $\alpha_1\beta_1$ (Krishek et al. 1998). However, sensitivity to zinc is also determined by α subunit variants. For instance, $\alpha_1\beta_3\gamma_2$ is much less sensitive to Zn^{2+} than $\alpha_2\beta_3\gamma_2$ and $\alpha_3\beta_3\gamma_2$ (White and Gurley 1995). The β subunit is also of importance in recombinant $GABA_A$ receptors in forming a Zn^{2+} binding site (Wooltorton et al. 1997). Referring to the influence of the different α subunits, studies have been performed about the position of a histidine residue which is critical in determining their different contributions to zinc sensitivity of the recombinant $\alpha_n\beta_3\gamma_{2L}$ (Fisher and Macdonald 1998). Referring to subunit combinations likely to occur in cerebellar granules, it has been shown that the $\alpha_6\beta_3\gamma_{2L}$ subunit combination expressed in mouse fibroblast cells is more potently inhibited by Zn^{2+} than $\alpha_1\beta_3\gamma_{2L}$ (IC_{50} of 47 versus 245 μM). Moreover, the $\alpha_6\beta_3\delta$ is even more potently inhibited by zinc, with an IC_{50} of 4.8 μM (Saxena and Macdonald 1996). These results were confirmed later in the *Xenopus* oocytes expression system, with an IC_{50} for zinc inhibition of 16.3, 615 and 639 μM respectively for the following subunit compositions: $\alpha_1\beta_1\delta$, $\alpha_1\beta_1\gamma_{2S}\delta$ and $\alpha_1\beta_1\gamma_{2S}$ (Krishek et al. 1998). To the best of our knowledge, no information about the effects of Zn^{2+} on a possible $\alpha_1\alpha_6\beta_n\gamma_2$ type (Zhu et al. 1998; Barilà et al. 2001) are available in the relevant literature.

In our experiments, zinc inhibits both the rapidly (peak component) and slowly desensitizing (steady-state) chloride currents elicited by GABA in the cerebellar granules registered in the whole-cell configuration. The effect is due only to extracellular cations and seems completely reversible. The effect on the steady-state component is more potent (IC_{50} of 5 μM versus 13 μM) than that on the peak component.

Previous studies by our group concluded that the rapidly desensitizing component of the GABA-activated chloride current was due to receptors of the $\alpha_1\beta_n\gamma_2$, $\alpha_6\beta_n\gamma_2$ or $\alpha_1\alpha_6\beta_n\gamma_2$ compositions, with possibly a preponderance of the $\alpha_6\beta_n\gamma_2$ type (Robello et al. 1999; Barilà et al. 2001). These receptors represent the dendritic synaptic ones (Cupello and Robello 2000). On the other hand, the slowly desensitizing component was suggested to be due to extrasynaptic receptors with a subunit composition of the $\alpha_6\beta_n\delta$ type (Robello et al. 1999; Cupello and Robello 2000). The present results seem to agree with this interpretation. In fact, the effect of Zn^{2+} is greater and more potent on the component which in our interpretation has a $\alpha_6\beta_n\delta$ subunit composition, as it should be according to previous reports about recombinant receptors (Saxena and Macdonald 1996; Krishek et al. 1998).

That Zn^{2+} is more active in inhibiting extrasynaptic somatic $GABA_A$ receptors in murine cerebellar granule cells has been recently reported by another group (Mellor et al. 2001), whose authors describe a 82% reduction of the chloride current activated by 1 mM GABA on cell bodies by 150 μM zinc. This corresponds rather well to the 75% residual inhibition of the steady-state component by 50 μM zinc at high GABA concentrations we illustrate in Fig. 2B.

Also, the mechanism of inhibition appears to differ in the two cases, the inhibition by Zn^{2+} of the peak component being merely competitive whereas the one of the steady-state component is of the mixed type. In addition, both for the peak and the steady-state component, n for the Zn^{2+} effect is below 1, which could be due either to heterogeneity of binding sites on different supramolecular complexes, or to negative cooperativity of sites on the same supramolecular complex, or both (Dahlquist 1979). The first possibility would correspond to interaction of Zn^{2+} with different receptor types contributing to both the peak and the steady state. The second one would correspond to the interaction of zinc with different but interacting binding sites on the very same receptor type. Of course, the third possibility would be a combination of the previous two possibilities.

Referring to the effect of cadmium, this is an inhibition also on both the peak and the steady-state currents elicited by 10 μM GABA. The affinity of cadmium for the site involved in the inhibition of the peak is quite high, with an IC_{50} of 0.03 μM (Fig. 4A). The inhibitory effect is reversible. The mechanism of cadmium inhibition of the peak current appears to be competitive, the effect of 1 μM cadmium being gradually overcome by GABA concentrations higher than 10 μM (Fig. 4B).

Cd^{2+} inhibits the steady-state chloride current elicited by 10 μM GABA by 20%, regardless of its concentration in the whole 10^{-2}–10^{2} μM range. This result may indicate a potent allosteric effect starting from a Cd^{2+} concentration of $< 10^{-2}$ μM. However, the data in Fig. 4B indicate that, for GABA < 10 μM and with 1 μM Cd^{2+}, part of the inhibition of the steady-state current is due to a competitive mechanism. The presence of a partial competition with GABA in this case seems to rule out the suspicion of aspecifity for the Cd^{2+} effect on the steady-state component. Previous experiments by Fisher and Macdonald (1998) have shown that the recombinant $GABA_A$ receptor of the composition $\alpha_6\beta_3\gamma_{2L}$ is inhibited by 100 μM Cd^{2+} to the same extent as $\alpha_1\beta_3\gamma_{2L}$ (around 40%) in the presence of a GABA concentration giving 50% of its maximal effect. Both these types of $GABA_A$ receptors are likely to participate in the peak component of GABA-activated chloride currents of cerebellar granules (Barilà et al. 2001). With some exceptions (Schwartz et al. 1994), in general, Cd^{2+} has shown negative effects on native $GABA_A$ receptors from different neurons (Smart and Constanti 1990; Mellor et al. 2001). This evidently applies also to the $GABA_A$ receptor populations of granule cells, both to the one which mediates the rapidly desensitizing chloride current activated by GABA and to the one which mediates the slowly desensitizing current. Cd^{2+} appears to inhibit both components very potently (starting from nanomolar concentrations), although the extent of inhibition is quite limited in both cases. Also, in this case the mechanism of inhibition is different for the two components,

further indicating a difference in the two receptor populations.

Overall, the present results appear to be consistent with the previously suggested compositions of the $GABA_A$ receptor populations of cerebellar granules (Robello et al. 1999; Cupello and Robello 2000; Barilà et al. 2001). In this view, the receptor population at the basis of the peak component of the GABA-activated current is made of rapidly desensitizing synaptic receptors of the compositions $\alpha_1\beta_x\gamma_2$, $\alpha6\beta_x\gamma_2$ and $\alpha_1\alpha6\beta_x\gamma_2$. The receptor population at the basis of the steady-state current is instead made of slowly desensitizing extrasynaptic receptors of the composition $\alpha6\beta_x\delta$.

On the other hand, we are aware that definitive conclusions on this matter can be reached only by means of combined approaches, such as, for instance, RT-PCR in conjunction with the patch-clamp approach (Santi et al. 1994; Alsbo et al. 2001). In addition, immunocytochemistry at the EM level, such as the approach applied by Nusser et al. (1998) in situ, can give further valuable information about the $GABA_A$ receptor populations present at dendritic and somatic sites in cerebellar granules in culture, especially in terms of receptor subunits coexistence. Another possibility, which our group is presently exploring with granule cells in culture, is the localized application of photoactivable derivatives of GABA to dendritic sites in conjunction with whole-cell recordings, in order to definitively assign the current peak component to dendritic synaptic receptors.

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