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Loreclezole and La^{3+} differentiate cerebellar granule cell $GABA_A$ receptor subtypes

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Abstract

The effects of loreclezole and La^{3+} on native cerebellar $GABA_A$ receptors were compared between $GABA_A$ receptor $\alpha 6$ subunit-deficient ($\alpha 6^{-/-}$) and wildtype mouse lines, produced through homologous recombination, using *t*-[^{35}S]butylbicyclophosphorothionate ([^{35}S]TBPS) autoradiography in brain sections. In the $\alpha 6$ subunit-deficient mice, the GABA receptor antagonistic ability of La^{3+} was abolished in the cerebellar granule cell layer, consistent with its opposite actions on $\alpha 6$- and $\alpha 1$ subunit-containing receptors. La^{3+} significantly potentiated the action of GABA in the molecular layer of the $\alpha 6^{-/-}$ mice, but not in that of the wildtype mice. The potentiation of agonistic GABA inhibition of [^{35}S]TBPS binding by loreclezole in $\alpha 6^{-/-}$ granule cells was reduced, suggesting an emergence of low-affinity $GABA_A$ receptors. The present results thus identified two ligands that may be useful in studying functional roles of cerebellar $\alpha 1$ and $\alpha 6$ subunit-containing $GABA_A$ receptor subtypes.

Keywords: $GABA_A$ receptor; Cerebellum; Knockout mouse line; Loreclezole; La^{3+}

1. Introduction

$GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the mammalian brain. They are composed of homologous subunits belonging to different groups: $\alpha 1$–$\alpha 6$, $\beta 1$–$\beta 3$, $\gamma 1$–$\gamma 3$, δ and ε, and assemble to form pentameric GABA-gated chloride ion channels (Macdonald and Olsen, 1994; Sieghart, 1995; McKernan and Whiting, 1996; Davies et al., 1997). Although $GABA_A$ receptor subunits are widely and heterogenously expressed in the brain, the necessity for receptor subtypes is not known.

The pharmacological features of the various $GABA_A$ receptor subtypes are determined by the subunit composition of each receptor. Among the known $GABA_A$ receptor subunits, the $\alpha 6$ subunit displays the most unique features: cerebellar and cochlear nucleus granule cell-restricted expression (Lüddens et al., 1990; Varecka et al., 1994; Wisden et al., 1996), benzodiazepine agonist-insensitive pharmacology (Malminiemi and Korpi, 1989; Wieland et al., 1992; due to the critical arginine residue at position 100), high GABA sensitivity (Korpi and Lüddens, 1993) and selective furosemide sensitivity (Korpi et al., 1995). These characteristics are missing in the granule cell layer of '$\alpha 6$ gene knockout' mouse lines, produced by disrupting the $\alpha 6$ subunit gene through homologous recombination (Jones et al., 1997; Homanics et al., 1997; Mäkelä et al., 1997). A connection between benzodiazepine-induced ataxia and the lack of $\alpha 6$-containing $GABA_A$ receptors in $\alpha 6^{-/-}$ mouse line has also been established (Korpi et al., 1998). In addition to the striking pharmacological alterations in the $\alpha 6^{-/-}$ cerebella, other minor alterations have been detected. In the presence of low GABA concentrations that fail to activate $\alpha 1$ subunit-containing receptors, methyl-6,7-dimethoxy-4-ethyl-β-carboline (DMCM), allopregnanolone and Zn^{2+} were less efficacious in modulating the *t*-[^{35}S]butylbicyclophosphorothionate [^{35}S]TBPS binding in the granule cell layer of the $\alpha 6^{-/-}$ than $\alpha 6^{+/+}$

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animals (Mäkelä et al., 1997). These findings suggest alterations in the receptor subtypes in the granule cell layer in response to the loss of the α6 subunit (Jones et al., 1997; Mäkelä et al., 1997). In fact, removal of the α6 protein leads to a large reduction in granule cell $GABA_A$ receptors in $\alpha6^{-/-}$ cerebellum: the δ subunit disappears from the granule cell plasma membrane and β2, β3 and γ2 protein levels decrease by 53%, 15% and 41%, respectively (Nusser et al., 1998a). However, no up-regulation of the α1 protein is detected in the Golgi cell to granule cell synapses, although our autoradiography reveals increased [^{35}S]TBPS binding component with low sensitivity to GABA and other agonists in the granule cell layer of the $\alpha6^{-/-}$ mice (Mäkelä et al., 1997).

In this report, we describe the extension of the pharmacological profiling of the native cerebellar cortical $GABA_A$ receptors in the $\alpha6^{-/-}$ mice. The trivalent cation lanthanum (La^{3+}) inhibits recombinant α6β3γ2L and α6β3δ receptor currents, whereas it potentiates α1β3γ2L subunit-containing receptor current (Saxena et al., 1997). The anticonvulsant and antiepileptic loreclezole [(*Z*)-1-[2-chloro-2-(2,4-dichlorophenyl) ethenyl]-1,2,4-triazole or R72063] (Wauquier et al., 1990; Ashton et al., 1992) discriminates between β1 and β2 or β3 subunit-containing receptors, β2/3 containing receptors being >300 times more sensitive than β1 containing receptors (Wafford et al., 1994; Wingrove et al., 1994). Here, we examine the modulation of [^{35}S]TBPS binding in the absence and presence of a low exogenous GABA concentration by loreclezole and La^{3+} to cerebellar $GABA_A$ receptors in $\alpha6^{-/-}$ mice.

2. Materials and methods

2.1. Animals

A 129Sv × C57BL/6 mouse line, in which the exon 8 of the α6 subunit gene was disrupted (Jones et al., 1997), was maintained as a colony in Turku. The brains of 6 homozygous wild type ($\alpha6^{+/+}$) and 6 homozygous mutant ($\alpha6^{-/-}$) adult mice of the F_3 and F_4 generations were used.

The animals were maintained under a 12/12-h light/dark cycle (lights on at 0600 h) at ambient temperature of 22 ± 2°C and a relative humidity of 55 ± 5%. The animals had free access to R3 rodent pellet feed (Ewos, Södertälje, Sweden) and tap water.

2.2. Preparation of cryostat sections

All animals were killed by decapitation, and the whole brains were rapidly dissected out and frozen on dry ice. For autoradiography, 14-μm horizontal serial sections were cut from six $\alpha6^{+/+}$ and six $\alpha6^{-/-}$ mouse whole brains using a Leitz 1720 cryostat, thaw-mounted onto gelatin-coated object glasses, and stored frozen under desiccant at −20°.

2.3. Ligand autoradiography

The autoradiographic procedure for regional localization of [^{35}S]TPBS (Dupont-New England Nuclear, Dreieich, Germany) binding was as in Mäkelä et al., 1997. Briefly, serial cryostat sections were preincubated in an

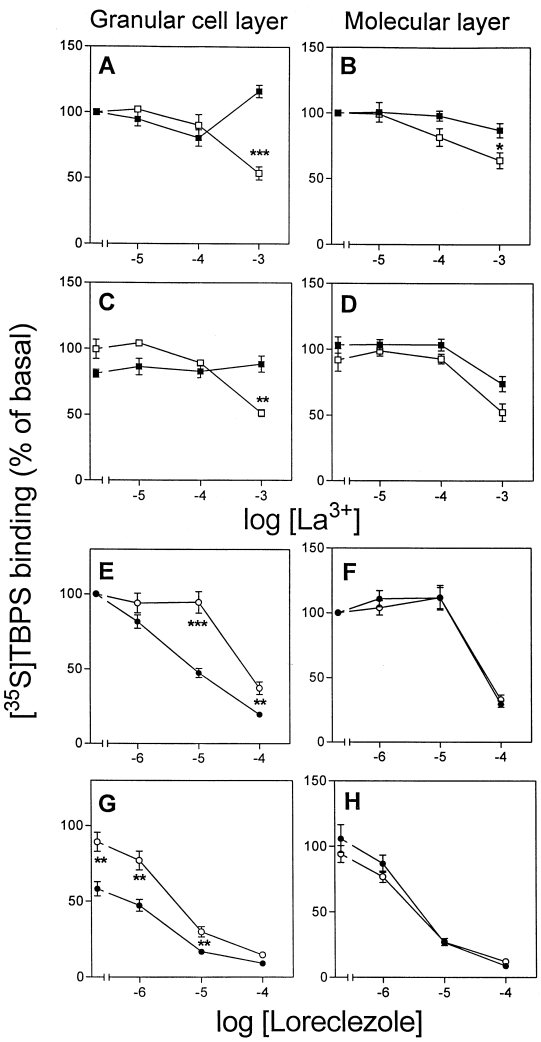

Fig. 1. Effect of La^{3+} (■,□) and loreclezole (●,○) on the picrotoxin-sensitive [^{35}S]TBPS binding in cerebellar sections of $\alpha6^{+/+}$ (■,●) and $\alpha6^{-/-}$ (□,○) mice in the absence (A, B, E, F) and presence (C, D, G, H) of exogenous 0.5 μM GABA. The autoradiographic results are expressed as percentages (mean ± S.E.M. of three or six mice in each group) of basal [^{35}S]TBPS binding (100%) determined in the absence of added La^{3+}, loreclezole or GABA. The results are shown for the granule cell layer (A, C, E, G) and for the molecular layer (B, D, F, H). Values to the left of the gaps were obtained in the absence of added drugs. Statistical significance of differences between the mouse lines (Student's *t*-test): $***P<0.001$, $**P<0.01$, $*P<0.05$.

ice–water bath three times for 10 min in 50 mM Tris–HCl supplemented with 1 mM EDTA, pH 7.4. Incubation with [^{35}S]TBPS (~200 dpm/μl, adjusted to 6 nM with cold TBPS) was performed for 90 min at 22°C in 50 mM Tris–HCl supplemented with 120 mM NaCl buffer, pH 7.4, using 750 μl liquid bubbles over sections on object glasses in a humid chamber. Effects of loreclezole (Janssen Pharmaceutica, Beerse, Belgium) and La^{3+} (Sigma, St. Louis, MO) in the presence or absence of 0.5 μM GABA (Sigma) were tested on [^{35}S]TBPS binding. After the incubations, the sections were washed three times for 15 s in an ice-cold 10 mM Tris–HCl buffer, pH 7.4, dipped into distilled water, air-dried at room temperature, and exposed with plastic [^{14}C]-standards to Hyperfilm-β_{max} (Amersham, Buckinghamshire, UK) for 5 days.

2.4. Data analysis

Regional labeling intensities were quantitated from the autoradiography films by using MCID M4 image analysis devices and programs (Imaging Research, St. Catharines, Canada) as described in Korpi et al., 1995. Binding densities for each brain area were averaged from measurements of one to three sections. The [^{14}C]-standards (Amersham, UK) exposed simultaneously with the brain sections were used as a reference with resulting binding values converting to radioactivity levels estimated for gray matter areas (nCi/g).

Statistical significance of the differences between the mouse lines were assessed using two-way analysis of variance with Tukey post test and between two group means using Student's *t*-test with Prism 2 (GraphPAD Software, San Diego, CA).

3. Results

3.1. Decreased $GABA_A$ receptor antagonism by lanthanum in the $\alpha6^{-/-}$ mice

The basal [^{35}S]TBPS binding to the granule cell layer of $\alpha6^{-/-}$ mice (236 ± 60 nCi/g, mean ± S.D., $n = 3$) was significantly ($P < 0.05$) lower than the binding to granule cells of $\alpha6^{+/+}$ mice (364 ± 51 nCi/g, $n = 3$). There was no difference in the binding to the molecular layer (248 ± 32 vs. 281 ± 30 nCi/g for $\alpha6^{-/-}$ and $\alpha6^{+/+}$ mice, respectively). La^{3+} (1 mM) elevated basal [^{35}S]TBPS binding at the absence of exogenous GABA in the granule cell layer of $\alpha6^{+/+}$ mice, whereas it decreased the binding in the $\alpha6^{-/-}$ mice (Fig. 1A, Fig. 2). La^{3+} (1 mM) decreased [^{35}S]TBPS binding to the molecular layer of both mouse strains, being significantly more efficient in the $\alpha6^{-/-}$ mice (Fig. 1B, Fig. 2). In the presence of 0.5 μM exogenous GABA, La^{3+} at 10 μM–1 mM concentrations had little effect on the binding, except that 1 mM La^{3+} significantly decreased the binding in the $\alpha6^{-/-}$ mice (Fig. 1C, Fig. 2).

3.2. Reduced inhibition of [^{35}S]TBPS binding by loreclezole in the $\alpha6^{-/-}$ mice

Loreclezole (1–100 μM) inhibited [^{35}S]TBPS binding to the granule cells of $\alpha6^{+/+}$ mice (Fig. 1E, Fig. 3) in the absence of exogenous GABA, whereas the [^{35}S]TBPS binding to the granule cell layer of $\alpha6^{-/-}$ mice was inhibited only in the presence of 100 μM loreclezole. In the presence of added 0.5 μM GABA, loreclezole potentiated the inhibition of [^{35}S]TBPS binding by GABA similarly to the granule cell layer of both $\alpha6^{+/+}$ and $\alpha6^{-/-}$ mice [$F(1,40) = 0.89$, $P > 0.05$] (Fig. 1G, Fig. 3). There

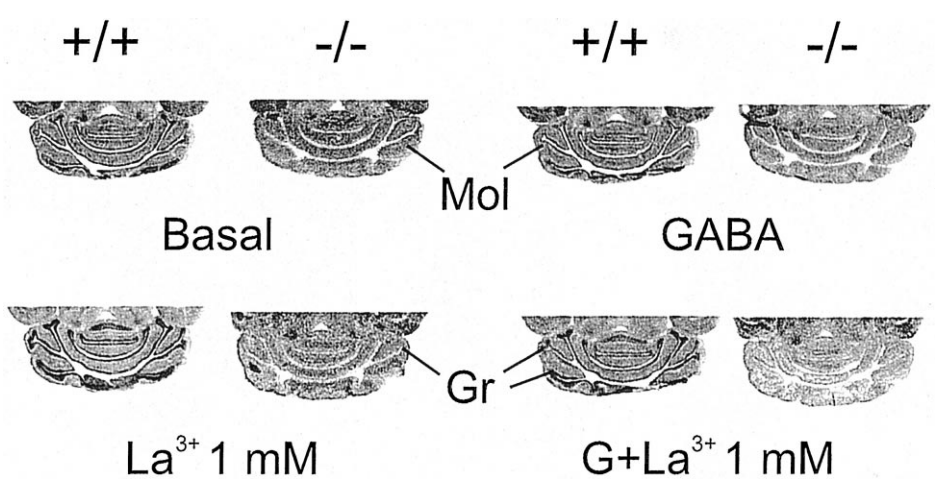

Fig. 2. Action of La^{3+} in the absence and presence of low GABA concentration (0.5 μM) on picrotoxin-sensitive [^{35}S]TBPS binding in serial $\alpha6^{-/-}$ and $\alpha6^{+/+}$ mouse cerebellar sections as revealed by autoradiography. Brain sections were washed extensively before incubation to remove endogenous GABA as described in Materials and methods. Concentration of lanthanum was 1 mM. Gr, granule cell layer; Mol, molecular layer.

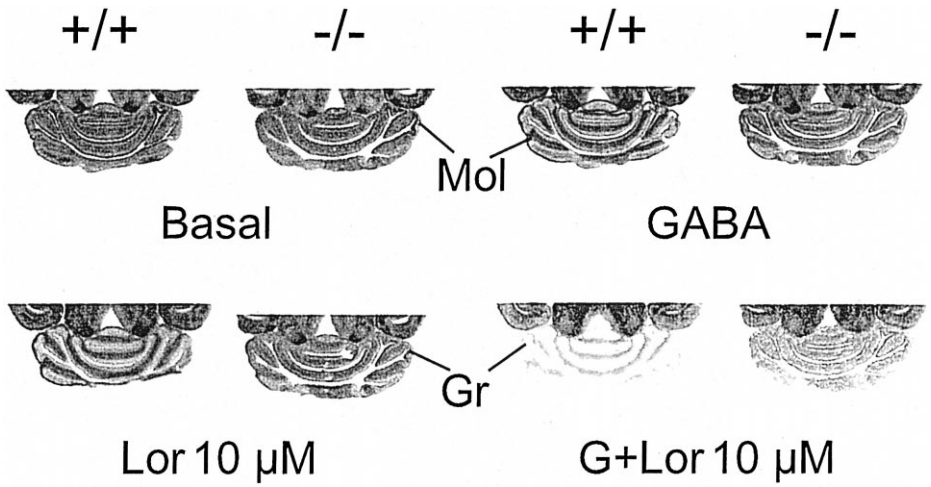

Fig. 3. Action of loreclezole in the absence and presence of low GABA concentration (0.5 μM) on picrotoxin-sensitive [^{35}S]TBPS binding in serial $\alpha6^{-/-}$ and $\alpha6^{+/+}$ mouse cerebellar sections as revealed by autoradiography. Brain sections were washed extensively before incubation to remove endogenous GABA as described in Materials and methods. Concentration of loreclezole was 10 μM. Gr, granule cell layer; Mol, molecular layer.

was no difference in the effect by loreclezole between the molecular layers of $\alpha6^{+/+}$ and $\alpha6^{-/-}$ mice.

4. Discussion

Normal cerebellar granule cells express abundantly the $GABA_A$ receptor α1, α6, β2, β3, γ2 and δ subunits (Laurie et al., 1992; Wisden et al., 1996; Nusser et al., 1996, 1998b). In $\alpha6^{-/-}$ granule cells, in addition to the loss of the α6 subunits, also the peptide levels of β2, β3, γ2 and δ subunits are reduced both synaptically and extrasynaptically, whereas the level of the α1 subunit is reduced only extrasynaptically (Nusser et al., 1998a). In spite of the normal δ subunit mRNA levels in the granule cells of the $\alpha6^{-/-}$ mice, immunostaining and immunoprecipitation revealed a great loss of the δ subunit protein, indicating the coassembly of α6 and δ subunits in normal native $GABA_A$ receptors (Jones et al., 1997). Therefore, the $\alpha6^{-/-}$ mice have effectively lost two subunits, the pharmacological significance of which has now been demonstrated with two ligands with $GABA_A$ receptor subtype-differentiating properties, loreclezole and La^{3+}.

In the absence of exogenous GABA, loreclezole (1–100 μM) clearly inhibited [^{35}S]TBPS binding in the $\alpha6^{+/+}$ cerebella, whereas in the $\alpha6^{-/-}$ cerebella inhibition was detected only with 100 μM loreclezole. The same kind of binding profile as in $\alpha6^{-/-}$ granule cells was detected in the molecular layer of both mouse lines. This could indeed mean that in normal cerebella, the pharmacology of $\alpha1\beta2\gamma2$ receptors is masked by the highly GABA-sensitive α6 subunit-containing receptors (Korpi and Lüddens, 1993). Loreclezole has also been shown to activate $GABA_A$ receptors directly (Sanna et al., 1996), but in our case, this would mean that α6-containing receptors are more sensitive to loreclezole's direct action than the α1-containing receptors. However, Wafford et al. (1994) have reported a slightly less efficient potentiation of $\alpha6\beta2/3\gamma2$ than $\alpha1\beta2/3\gamma2$ receptor currents by loreclezole. In the presence of 0.5 μM exogenous GABA, there was no difference in the effects of loreclezole between the mice; only GABA itself was less efficient in $\alpha6^{-/-}$ mice, as expected due to the lack of highly GABA-sensitive α6 subunit (Korpi and Lüddens, 1993; Mäkelä et al., 1997). Actually, the low-affinity GABA sites were more abundant in the granule cell layer of the $\alpha6^{-/-}$ than $\alpha6^{+/+}$ mice, as illustrated by less efficient loreclezole action in Fig. 3 (panels Lor 10 μM and G + Lor 10 μM). This confirms the apparent emergence of low-affinity sites in the absence of α6 and δ subunits (see Mäkelä et al., 1997).

Recombinant $GABA_A$ receptor isoforms, expressed in L929 fibroblasts, have been shown with the whole cell recording technique to display differential sensitivity to La^{3+} in an α subunit variant-dependent manner. La^{3+} potentiates $\alpha1\beta3\gamma2L$ receptor currents, whereas $\alpha6\beta3\gamma L$ and $\alpha6\beta3\delta$ currents are inhibited, the latter being more sensitive to La^{3+} (Saxena et al., 1997).

In the cerebellar granule cell layer of $\alpha6^{+/+}$ and $\alpha6^{-/-}$ mice, an effect of the α6 subunits on the properties of La^{3+} was clearly demonstrated. A high La^{3+} (1 mM) concentration increased the basal [^{35}S]TBPS binding in $\alpha6^{+/+}$ mice, but decreased it in $\alpha6^{-/-}$ mice. With a low GABA (0.5 μM) concentration, the same difference was detected. Saxena et al. (1997) have suggested, that in the presence of native $\alpha1\beta2/3\gamma2L$, $\alpha6\beta2/3\gamma2L$ and $\alpha6\beta2/3\delta$ receptor subtypes, it is likely that at lower concentrations of La^{3+} (1–100 μM), the enhancing effect of La^{3+} could dominate, whereas at higher concentration of La^{3+} (1 mM and higher), loss of potentiation of the $\alpha1\beta2/3\gamma2L$ receptor by La^{3+} could result from unmasking of the inhibitory effect of La^{3+} on the $\alpha6\beta2/3\delta$ receptor.

Unexpectedly, La^{3+} was more efficient in displacing [^{35}S]TBPS in the molecular layer of the $\alpha6^{-/-}$ than $\alpha6^{+/+}$ mice, suggesting that also this cerebellar cortical layer has altered $GABA_A$ receptor population in the mutant mice. Similar results were recently obtained with diazepam (Mäkelä et al., 1997). $GABA_A$ receptors in the molecular layer might have undergone adaptive alterations due to limited inhibition at the Golgi neuron–granule cell synapses or at the granule cell soma in the $\alpha6^{-/-}$ mice.

In conclusion, La^{3+} and loreclezole revealed specific features that $\alpha6$ subunit-containing native $GABA_A$ receptors are playing in native cerebellar pharmacology, confirming the results on recombinant receptors. Since these compounds differentiate the $\alpha6$ and $\alpha1$ subunit-containing receptors, which have at least partly varying localizations in the granule cell soma and dendrites (Nusser et al., 1998b), they might be utilized in functional tests to gain understanding of the inhibitory processes in the cerebellar cortex.

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References

Ashton, D., Fransen, J.F., Heeres, J., Clincke, G.H.C., Janssen, P.A.J., 1992. In vivo studies on the mechanism of action of the broad spectrum anticonvulsant loreclezole. Epilepsy Res. 11, 27–36.

Davies, P.A., Hanna, M.C., Hales, T.G., Kirkness, E.F., 1997. Insensitivity to anaesthetic agents conferred by a class of $GABA_A$ receptor subunit. Nature 385, 820–823.

Jones, A., Korpi, E.R., McKernan, R.M., Pelz, R., Nusser, Z., Mäkelä, R., Mellor, J.R., Pollard, S., Bahn, S., Stephenson, F.A., Randall, D., Sieghart, W., Somogyi, P., Smith, A.J.H., Wisden, W., 1997. Ligand-gated ion channel subunit partnerships: $GABA_A$ receptor $\alpha6$ subunit gene inactivation inhibits δ subunit expression. J. Neurosci. 17, 1350–1362.

Homanics, G.E., Ferguson, C., Quinlan, J.J., Dagget, J., Snyder, K., Lagenaur, C., Mi, Z.-P., Wang, X.-H., Grayson, D.R., Firestone, L.L., 1997. Gene knockout of the $\alpha6$ subunit of the γ-aminobutyric acid type A receptor: lack of effect on responses to ethanol, pentobarbital, and general anesthetics. Mol. Pharmacol. 51, 588–596.

Korpi, E.R., Lüddens, H., 1993. Regional γ-aminobutyric acid sensitivity of *t*-butylbicyclophosphoro[^{35}S]thionate binding depends on γ-aminobutyric $acid_A$ receptor α subunit. Mol. Pharmacol. 44, 87–92.

Korpi, E.R., Kuner, T., Seeburg, P.E., Lüddens, H., 1995. Selective antagonist for the cerebellar granule cell-specific γ-aminobutyric acid type A receptor. Mol. Pharmacol. 47, 283–289.

Korpi, E.R., Koikkalainen, P., Vekovischeva, O.Y., Mäkelä, R., Kleinz, R., Uusi-Oukari, M., Wisden, W., 1998. Cerebellar granule cell-specific $GABA_A$ receptors attenuate benzodiazepine-induced ataxia: evidence from $\alpha6$ subunit-deficient mice, Eur. J. Neurosci., in press.

Laurie, D.J., Seeburg, P.H., Wisden, W., 1992. The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. Olfactory bulb and cerebellum. J. Neurosci. 12, 1063–1076.

Lüddens, H., Pritchett, D.B., Kohler, M., Killisch, I., Keinänen, K., Monyer, H., Sprengel, R., Seeburg, P.H., 1990. Cerebellar $GABA_A$ receptor selective for a behavioural alcohol antagonist. Nature 346, 648–651.

Macdonald, R.L., Olsen, R.W., 1994. $GABA_A$ receptor channels. Annu. Rev. Neurosci. 17, 569–602.

Malminiemi, O., Korpi, E.R., 1989. Diazepam-insensitive [^{3}H]Ro 15-4513 binding in intact cultured cerebellar granule cells. Eur. J. Pharmacol. 169, 53–60.

McKernan, R.M., Whiting, P.J., 1996. Which $GABA_A$ receptor subtypes really occur in the brain?. Trends Neurosci. 19, 139–143.

Mäkelä, R., Uusi-Oukari, M., Homanics, G.E., Quinlan, J.J., Firestone, L.L., Wisden, W., Korpi, E.R., 1997. Cerebellar γ-aminobutyric acid type A receptors: pharmacological subtypes as revealed by mutant mouse lines. Mol. Pharmacol. 52, 380–388.

Nusser, Z., Sieghart, W., Stephenson, F.A., Somogyi, P., 1996. The $\alpha6$ subunit of the $GABA_A$ receptor is concentrated in both inhibitory and excitatory synapses on cerebellar granule cells. J. Neurosci. 16, 103–114.

Nusser, Z., Ahmad, Z., Tretter, V., Fuchs, K., Wisden, W., Sieghart, W., Somogyi, P., 1998a. Alterations in the expression of $GABA_A$ receptor subunits in cerebellar granule cells after disruption of the $\alpha6$ subunit gene. Eur. J. Neurosci., in press.

Nusser, Z., Sieghart, W., Somogyi, P., 1998b. Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J. Neurosci. 18, 1693–1703.

Sanna, E., Murgia, A., Casula, A., Usala, M., Maciocco, E., Tuligi, G., Biggio, G., 1996. Direct activation of $GABA_A$ receptors by loreclezole, an anticonvulsant drug with selectivity for the beta-subunit. Neuropharmacology 35, 1753–1760.

Saxena, N.C., Neelands, T.R., Macdonald, R.L., 1997. Contrasting actions of La^{3+} on different recombinant γ-aminobutyric acid receptor isoforms expressed in L929 fibroblasts. Mol. Pharmacol. 51, 328–335.

Sieghart, W., 1995. Structure and pharmacology of γ-aminobutyric $acid_A$ receptor subtypes. Pharmacol. Rev. 47, 181–234.

Varecka, L., Wu, C.-H., Rotter, A., Frostholm, A., 1994. $GABA_A$/benzodiazepine receptor $\alpha6$ subunit mRNA in granule cells of the cerebellar cortex and cochlear nuclei: expression in developing and mutant mice. J. Comp. Neurol. 339, 341–352.

Wafford, K.A., Bain, C.J., Quirk, K., McKernan, R.M., Wingrove, P.B., Whiting, P.J., Kemp, J.A., 1994. A novel allosteric modulatory site on the $GABA_A$ receptor β subunit. Neuron 12, 775–782.

Wauquier, A., Fransen, J., Melis, W., Ashton, D., Gillardin, J.M., Lewi, P.J., 1990. Loreclezole (R72063): an anticonvulsant chemically unrelated to prototype antiepileptic drugs. Drug. Dev. Res. 19, 375–392.

Wieland, H.A., Lüddens, H., Seeburg, P.H., 1992. A single histidine in $GABA_A$ receptors is essential for benzodiazepine agonist binding. J. Biol. Chem. 267, 1426–1429.

Wingrove, P.B., Wafford, K.A., Bain, C., Whiting, P.J., 1994. The modulatory action of loreclezole at the γ-aminobutyric acid type A receptor is determined by a single amino acid in the $\beta2$ and $\beta3$ subunit. Proc. Natl. Acad. Sci. USA 91, 4569–4573.

Wisden, W., Korpi, E.R., Bahn, S., 1996. The cerebellum: a model system for studying $GABA_A$ receptor diversity. Neuropharmacology 35, 1139–1160.