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Functional pharmacology of $GABA_A$ receptors containing the chicken brain γ4 subunit

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Abstract

The functional pharmacology of receptors composed of the chicken brain $GABA_A$ receptor γ4 subunit and the mammalian $GABA_A$ receptor α3 and β2 subunits was studied by heterologous expression in *Xenopus laevis* oocytes using the two electrode voltage-clamp technique. GABA-evoked currents had an EC_{50} of 180 ± 30 μM. Responses were blocked by the competitive and non-competitive $GABA_A$ receptor antagonists, bicuculline methochloride and picrotoxin. Sodium pentobarbital reversibly potentiated the current several-fold, and Zn^{2+} ions blocked the current with high potency ($IC_{50} = 20$ μM). GABA-evoked currents were potentiated by the benzodiazepine site full agonists flunitrazepam and triazolam and less by the partial agonists abecarnil and bretazenil. The inverse agonists methyl-β-carboline-3-carboxylate (β-CCM) and methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) reduced the current. However, the imidazobenzodiazepine Ro 15-4513, which acts as an inverse agonist at mammalian $\alpha x\beta y\gamma 2$ $GABA_A$ receptors (where $x = 1$, 2, 3 or 5, and $y = 1$, 2 or 3), acted as a positive agonist at the γ4 subunit-containing receptors.

Keywords: Benzodiazepine pharmacology; Electrophysiology; $GABA_A$ receptor; *Xenopus* oocyte; Zn^{2+} inhibition

1. Introduction

Mammalian $GABA_A$ receptors, which mediate rapid inhibitory neurotransmission in the central nervous system, are pentameric agonist-gated chloride channels. They are assembled from different combinations of homologous subunits named α1–α6, β1–β3, γ1–γ3, δ, ε, π and θ (Darlison and Albrecht, 1995; Sieghart, 1995; Davies et al., 1997; Hedblom and Kirkness, 1997; Bonnert et al., 1999; Mehta and Ticku, 1999). Although the total number of $GABA_A$ receptor subtypes and their compositions are currently unknown, it is generally accepted that most receptors contain at least one α, one β and either one γ or one δ subunit. For receptors composed of α, β and γ subunits, the stoichiometry is probably $2\alpha 2\beta 1\gamma$ (Chang et al., 1996).

$GABA_A$ receptors are the site of action of a variety of clinically important compounds, such as benzodiazepines, barbiturates and general anaesthetics (Hevers and Lüddens, 1998), and the different receptor subunits make distinct contributions to the various binding pockets. Thus, it is the γ subunit that confers benzodiazepine sensitivity to $GABA_A$ receptors (Pritchett et al., 1989; Ymer et al., 1990; Herb et al., 1992), with the binding site being formed by residues present in both α (i.e. the α1, α2, α3 and α5 subunits; receptors containing either the α4 or the α6 subunit are insensitive to benzodiazepine agonists) and γ subunits (for review, see Sigel and Buhr, 1997). However, the three mammalian γ polypeptides impart quite different benzodiazepine pharmacologies to recombinant receptors. For example, whereas the γ2 subunit confers sensitivity to a wide range of benzodiazepines (agonists, antagonists and inverse agonists; Pritchett et al., 1989), γ1 subunit-containing receptors have a lower affinity for antagonists, such as flumazenil (8-fluoro-3-carboethoxy-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5*a*]1,4-benzodiaze-

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pine; Ro 15-1788), and inverse agonists such as methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM; Ymer et al., 1990; Wafford et al., 1993; Benke et al., 1996). In contrast, the affinity for benzodiazepine agonists is markedly decreased in receptors incorporating the γ3 subunit compared to γ2 subunit-containing receptors (Knoflach et al., 1991; Herb et al., 1992; Hadingham et al., 1995; Benke et al., 1996). The presence of a γ subunit also changes the sensitivity of recombinant $GABA_A$ receptors to Zn^{2+}; receptors comprising only α and β subunits are potently blocked by this cation, while αβγ subunit receptors are only weakly sensitive (Draguhn et al., 1990; Smart et al., 1994).

A fourth type of $GABA_A$ receptor γ subunit, named γ4, which exhibits 70% or less identity to the mammalian γ1, γ2 and γ3 subunits, has been described in the chicken (Harvey et al., 1993). This animal appears to lack a γ3 subunit and, thus, like other vertebrates, is thought to possess three γ subunits (γ1, γ2 and γ4; Darlison and Albrecht, 1995). To date, however, no pharmacological data have been published on recombinant $GABA_A$ receptors that contain the γ4 polypeptide. This subunit is of interest not only from a functional point of view, but also because of its striking expression pattern; the corresponding mRNA is found at high levels, in the 1-day-old chick brain, in structures that are either part of, or receive inputs from, visual and auditory pathways (Harvey et al., 1998). Furthermore, the level of the γ4 subunit transcript has been shown to be dramatically down-regulated, in a brain region-specific manner, as a result of imprinting training on a visual stimulus (Harvey et al., 1998; Darlison et al., manuscript in preparation). This observation is entirely consistent with studies on imprinting in chicks, which have demonstrated that injection of either the $GABA_A$ receptor agonist muscimol or the benzodiazepine full agonist diazepam into the brain causes amnesia, whereas injection of the antagonist bicuculline or the benzodiazepine site inverse agonist methyl-β-carboline-3-carboxylate (β-CCM) improves memory performance (Venault et al., 1986, 1987; Clements and Bourne, 1996).

To investigate the consequences of inclusion of the avian γ4 subunit on the pharmacological properties of $GABA_A$ receptors, we have expressed this polypeptide with an α and a β subunit in *Xenopus laevis* oocytes. Our data reveal that γ4 subunit-containing receptors have functional characteristics that are quite distinct from those of receptors that harbour the γ1, γ2 or γ3 subunit.

2. Materials and methods

2.1. Subcloning of the chicken γ4 subunit complementary DNA (cDNA)

The chicken $GABA_A$ receptor γ4 subunit cDNA (Harvey et al., 1993) was subcloned into the vector pBC12/CMV (Benson et al., 1998) for functional expression in *Xenopus* oocytes. For this, the cDNA insert was excised from pBluescript SK + (Stratagene, Amsterdam Zuidoost, The Netherlands) as a 1.6-kb *Eco*RI fragment, while pBC12/CMV was digested with *Bam*HI. The ends of both the cDNA and the linearized vector were polished using T4 DNA polymerase (Boehringer Mannheim, Mannheim, Germany), in the presence of 250 μM of each deoxynucleotide triphosphate, and then joined using T4 DNA ligase (Boehringer Mannheim). A correctly orientated construct was selected by restriction mapping of miniprep DNAs and confirmed by automated DNA sequencing. The rat $GABA_A$ receptor α3 and β2 subunit cDNAs used here have been described previously (reviewed by Barnard et al., 1998).

2.2. Oocyte preparation and receptor expression

X. laevis oocytes were prepared according to a standard procedure (Bertrand et al., 1994) and nuclear injected with 10 nl each of plasmids (100 ng/μl) containing cDNAs for the α3, β2 and γ4 subunits. The oocytes were then incubated at 14–18°C in standard OR2 + Ca^{2+} solution, supplemented with antibiotics (50 μg/ml penicillin, 50 μg/ml streptomycin and 20 μg/ml kanamycin). The composition of the OR2 + Ca^{2+} solution (in mM) was: NaCl 82.5, KCl 2.5, Na_2HPO_4 1, $MgCl_2$ 1, $CaCl_2$ 2.5, HEPES 15, pH adjusted to 7.4 using NaOH. All reagents were obtained from either Fluka (Buchs, Switzerland) or Sigma (Buchs, Switzerland). Recordings were made 3–4 days after injection.

2.3. Electrophysiology

Oocytes were voltage-clamped with a laboratory-built two electrode voltage-clamp which included series resistance compensation to counteract voltage-clamp errors arising from the finite resistance of the bath electrode when measuring currents in the μA range. The bath electrode incorporated an agar bridge filled with 3 M KCl, and the voltage and current microelectrodes were pulled from borosilicate glass (Hilgenberg, Malsfeld, Germany), with resistances of approximately 2–3 MΩ, and also filled with 3 M KCl. The oocytes were clamped at −50 mV. Currents were filtered at 20 Hz using an eight-pole Bessel filter (Frequency Devices, Model 902, Haverhill, MA, USA) and sampled at more than twice the cutoff frequency using a PC-based data acquisition system (Bertrand and Bader, 1986). All experiments were performed at room temperature (20–24°C).

2.4. Drug application

All experiments were performed on intact oocytes placed in a small chamber (0.2 ml volume) and continuously superfused with control solution (OR2 + Ca^{2+}) at a rate of 7 ml/min. Computer-controlled valves allowed fast and reproducible solution changes (< 30 ms).

2.5. Preparation of test substances

Stock solutions of test compounds were prepared in 100% dimethyl sulphoxide (DMSO) and diluted 1000-fold before use. During experiments, all bath solutions contained 0.1% (v/v) DMSO, which by itself had no detectable effect on GABA responses. The benzodiazepine ligands flunitrazepam, bretazenil and ethyl 8-azido-6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5*a*][1,4]benzodiazepine-3-carboxylate (Ro 15-4513) were kindly provided by Hoffmann-La Roche (Basel, Switzerland), abecarnil by Schering (Berlin, Germany) and triazolam by Upjohn (Milton Keynes, UK). GABA, sodium pentobarbital, picrotoxin and bicuculline methochloride were purchased from Sigma, $ZnSO_4$ from Fluka, and DMCM and β-CCM from Research Biochemicals International (Zürich, Switzerland).

2.6. Data analysis

The maximum current amplitudes from individual cells were first fitted separately using the equation:

$$I/I_{max} = 1/\left(1 + \left(EC_{50}/[GABA]\right)^{Hill}\right) \quad (1)$$

where I is GABA-evoked current; I_{max} is the maximum of the fit; EC_{50} is the GABA concentration evoking the half-maximal response; and Hill is the Hill coefficient.

The individual dose–response curves were then normalized to I_{max}, and the means and standard errors (S.E.), calculated from the normalized data for each concentration, were plotted and fitted with a sigmoidal curve. The EC_{50} and Hill values are the means ± S.E. of the data obtained from the individual fits.

3. Results

3.1. GABA-evoked currents

Application of GABA pulses to injected *Xenopus* oocytes evoked a dose-dependent inward current in cells voltage-clamped at −50 mV (Fig. 1A), which indicated that expression of functional receptors had taken place. When Eq. (1) was fit to these data, the following parameters were obtained (see Materials and methods): EC_{50} = 180 ± 30 μM, Hill coefficient = 1.32 ± 0.28 (mean ± S.E., n = 5). Thus, the expressed receptors had a low GABA sensitivity, with a threshold of activation of about 10 μM and an EC_{50} value characteristic of α3βxγ2 subunit receptors (where x = 1, 2 or 3), which are the least sensitive γ2 subunit-containing αβγ receptors (Sigel et al., 1990; Ebert et al., 1994). This low GABA sensitivity strongly suggests that the γ4 subunit is actually incorporated into the expressed receptor, since αβ subunit receptors are characteristically highly sensitive to agonist (EC_{50} values are usually below 20 μM, Sigel et al., 1990; Ducic et al., 1995).

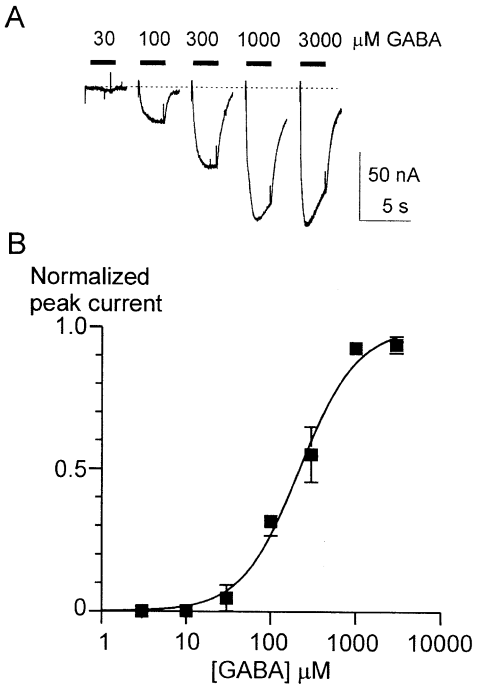

Fig. 1. GABA dose-dependence of responses mediated by recombinant α3β2γ4 subunit receptors. (A) Representative responses evoked by application of increasing concentrations of GABA to *Xenopus* oocytes nuclear injected with cDNAs coding for the rat α3 and β2 subunits and the chicken γ4 subunit. (B) GABA dose–response curve obtained from *Xenopus* oocytes expressing α3β2γ4 subunit receptors. The maximum evoked current amplitudes were normalized and plotted as described in Materials and methods. In (B), values represent the mean ± standard error (n = 3–5).

3.2. Confirmation of the basic pharmacological profile for $GABA_A$ receptors

We first confirmed the basic pharmacological profile of the recombinant α3β2γ4 subunit receptors by determining the response of oocytes to a number of standard $GABA_A$ receptor modulators. In all experiments, we chose a GABA concentration of 100 μM that was close to the EC_{25} value (Fig. 1B). This allowed the possibility of several-fold drug-induced potentiation of the GABA-evoked response. The barbiturate sodium pentobarbital potentiates the responses mediated by almost all $GABA_A$ receptor subtypes, whereas the classical competitive and non-competitive blockers of $GABA_A$ receptors are bicuculline and picrotoxin, respectively. In *Xenopus* oocytes expressing the α3β2γ4 subunit receptor, the GABA-evoked current was reversibly enhanced several-fold by 100 μM sodium pentobarbital (Fig. 2A) and reversibly blocked by bicuculline methochloride (10 μM, Fig. 2B) and picrotoxin (10 μM, Fig. 2C). These findings estab-

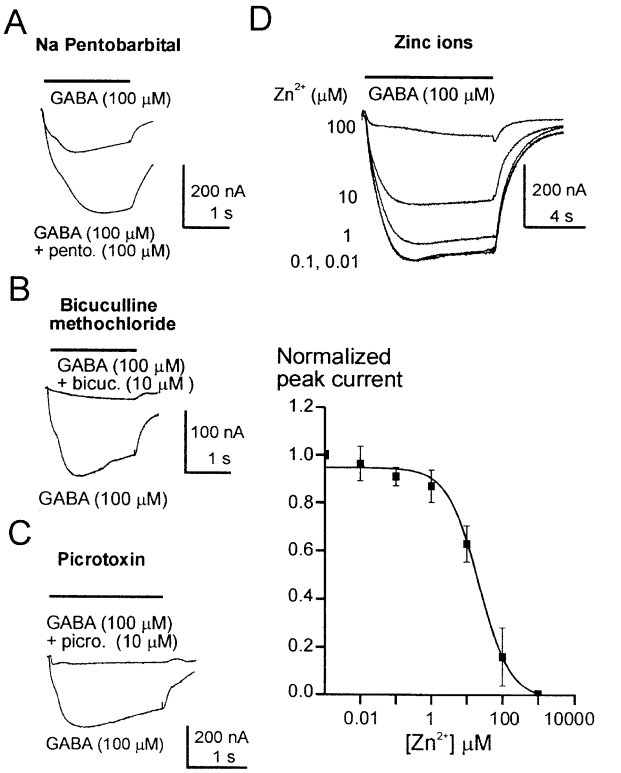

Fig. 2. $GABA_A$ receptor-specific drug effects on the currents evoked by application of GABA (100 μM) to injected *Xenopus* oocytes expressing recombinant α3β2γ4 subunit receptors. (A) Barbiturate, sodium pentobarbital (potentiation), (B) competitive antagonist, bicuculline methochloride (inhibition), and (C) non-competitive channel blocker, picrotoxin (inhibition). (D) Sample records showing the degree of block at different Zn^{2+} concentrations and the dose–response curve illustrating the average concentration-dependence of the inhibitory effect of Zn^{2+} ions.

lished that the GABA-induced current was produced by $GABA_A$ receptors.

3.3. Effect of Zn^{2+} ions

Zn^{2+} ions potently block GABA-evoked currents mediated by $GABA_A$ receptors that lack a mammalian γ subunit (i.e. γ1, γ2 and γ3); such αβ subunit receptors are usually completely inhibited by 5–10 μM Zn^{2+} ions (Smart et al., 1994). In contrast, most receptors that contain a γ2 subunit are only slightly blocked by 100 μM Zn^{2+} (Smart et al., 1994); α4β2γ2 and α6β2γ2 subunit receptors are more sensitive than other γ2 subunit-containing combinations, with half-maximal block at about 100 μM (Knoflach et al., 1996). Zn^{2+} ions blocked the GABA-evoked response at α3β2γ4 subunit receptors in a dose-dependent manner with a half-maximal effect at a concentration of about 20 μM (Fig. 2D). The threshold for Zn^{2+} inhibition was between 0.1 and 1 μM, and the GABA-induced current was totally blocked by 300 μM Zn^{2+}. These values indicate an unusually high sensitivity to Zn^{2+} for a γ subunit-containing receptor, being within the range observed for $GABA_A$ receptors lacking a γ subunit, and show that the avian γ4 polypeptide differs from mammalian γ subunits with regard to the Zn^{2+} sensitivity it confers on the receptor into which it is incorporated.

3.4. Modulators of the GABA-evoked current

Drugs acting at the benzodiazepine binding site of $GABA_A$ receptors can allosterically potentiate (full agonists and partial agonists) or diminish (inverse agonists) the GABA-evoked current. The classical full agonists are diazepam and flunitrazepam. We chose flunitrazepam for our experiments and, at a concentration of 1 μM, it potentiated the GABA-evoked response by 87 ± 37% (n = 8, Fig. 3). Triazolam has been reported to be a "super agonist" at α1β1γ2 subunit receptors, potentiating beyond the maximal full agonist level, but is less efficacious than diazepam on α3β1γ2 subunit receptors (Ducic et al., 1993). Triazolam was equal in efficacy to flunitrazepam when tested on the α3β2γ4 subunit receptor at 1 μM (Fig. 3). Abecarnil and bretazenil, which are partial agonists at many receptors containing the γ2 subunit (Knoflach et al., 1993), are also partial agonists at the α3β2γ4 subunit receptor; at a concentration of 1 μM, their potentiating efficacy was less than that of flunitrazepam (Fig. 3). Conversely, the inverse agonists β-CCM and DMCM, which are both β-carbolines, reduced the amplitude of the GABA-evoked response when applied at 1 μM (Figs. 3 and 4A). These results show that drugs belonging to the

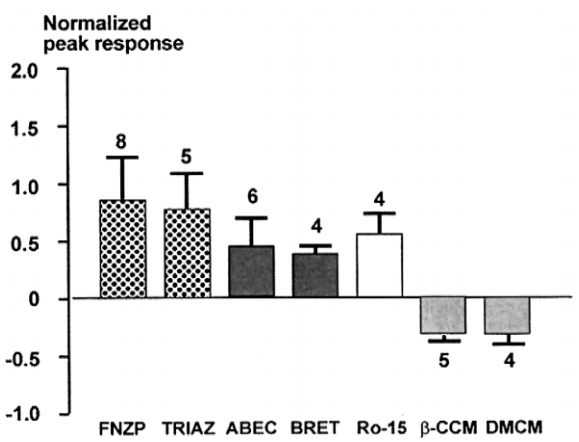

Fig. 3. Modulation by benzodiazepine binding site ligands of GABA-evoked currents recorded from *Xenopus* oocytes expressing recombinant α3β2γ4 subunit receptors. Modulation is represented relative to the normalized control GABA response (i.e. 1.0). Error bars represent the standard error, with the numbers of oocytes tested for each compound being indicated. The ligands tested were: flunitrazepam, FNZP; triazolam, TRIAZ; abecarnil, ABEC; bretazenil, BRET; Ro 15-4513, Ro-15; β-CCM and DMCM, all at 1 μM. In each case, the GABA concentration was 100 μM. Bar fills indicate type of agonist—dotted: full agonist; dark grey: partial agonist; light grey: inverse agonist.

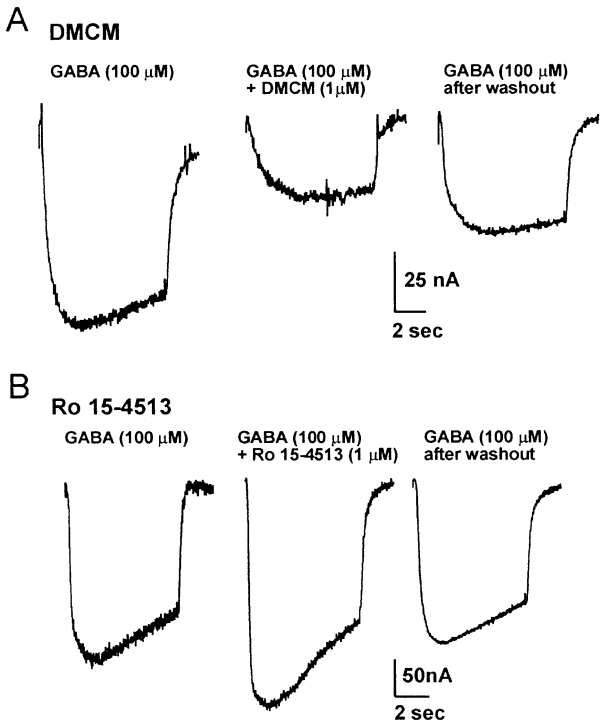

Fig. 4. Representative current traces illustrating modulation by benzodiazepine binding site inverse agonists of GABA-evoked currents recorded from *Xenopus* oocytes expressing recombinant $\alpha 3\beta 2\gamma 4$ subunit receptors. (A) DMCM (1 μM) reduces the GABA-induced current. (B) Ro 15-4513 (1 μM), an inverse agonist at many $GABA_A$ receptors (see text), acts as a positive agonist at the $\alpha 3\beta 2\gamma 4$ subunit receptor.

three main classes of benzodiazepine binding site modulators have effects on the γ4 subunit-containing receptor that are similar to those seen at γ2 subunit-containing receptors (reviewed by Hevers and Lüddens, 1998).

However, surprisingly, the imidazobenzodiazepine Ro 15-4513, an inverse agonist that reduces the amplitude of GABA-evoked responses at non-α4, non-α6 $\alpha\beta\gamma$ subunit receptors, including the $\alpha 3\beta 1\gamma 2$ and $\alpha 3\beta 3\gamma 2$ subunit receptors (Hadingham et al., 1993; Benson et al., 1998), enhanced the current mediated by the $\alpha 3\beta 2\gamma 4$ subunit receptor by (on average) more than 50% (Figs. 3 and 4B). Ro 15-4513 is characteristically sensitive to amino acid substitutions in the benzodiazepine binding site and putative allosteric pathway (Wieland et al., 1992; Benson et al., 1998). The "diazepam-insensitive" α4 and α6 subunit-containing receptors, for example, are potentiated by Ro 15-4513 (Knoflach et al., 1996) and the γ1 subunit can confer a positive modulatory response to this compound (Wafford et al., 1993). However, the pharmacological profile of the $\alpha 3\beta 2\gamma 4$ subunit receptor seems to differ from those of known γ1 subunit-containing receptors since DMCM is also a positive agonist at the $\alpha 2\beta 1\gamma 1$ and $\alpha 3\beta 1\gamma 1$ subunit receptors (Puia et al., 1991; Wafford et al., 1993); in contrast, DMCM acts as an inverse agonist at $\alpha 3\beta 2\gamma 4$ subunit receptors (Figs. 3 and 4A).

4. Discussion

We have described here the functional expression and preliminary pharmacological characterization of the chicken $GABA_A$ receptor γ4 subunit in a recombinant receptor that contained, in addition, the α3 and β2 subunits. Inclusion of the γ4 subunit resulted in clear and reliable modulation of the GABA response by a variety of benzodiazepine binding site ligands. Since αβ subunit receptors are insensitive to benzodiazepines, these observations demonstrate not only that the γ4 subunit is synthesized and incorporated into $GABA_A$ receptor complexes, but also that it possesses determinants that (together with those in the α3 subunit) form a benzodiazepine binding site. As expected, GABA-induced responses at $\alpha 3\beta 2\gamma 4$ subunit receptors were potentiated by sodium pentobarbital, and blocked by both bicuculline methochloride and picrotoxin.

The mammalian $GABA_A$ receptor γ subunits impart quite distinct benzodiazepine pharmacologies to recombinant $\alpha\beta\gamma$ subunit receptors (Pritchett et al., 1989; Ymer et al., 1990; Herb et al., 1992), reflecting in part the sequence differences between the γ1, γ2 and γ3 subunits. It is, therefore, surprising to find that the chicken γ4 polypeptide, which exhibits only 69% identity to the rat γ2 subunit (Harvey et al., 1993), assembles with the α3 and β2 subunits to yield receptors that have a very similar benzodiazepine pharmacology to those containing the γ2 subunit (Pritchett et al., 1989; Knoflach et al., 1993; Sieghart, 1995; Hevers and Lüddens, 1998). Whereas flunitrazepam and triazolam acted as full agonists, abecarnil and bretazenil behaved as partial agonists, and β-CCM and DMCM functioned as inverse agonists, Ro 15-4513, which generally behaves as a partial inverse agonist (Barnard et al., 1998), potentiated GABA responses at $\alpha 3\beta 2\gamma 4$ subunit receptors. It is intriguing that the same compound (Ro 15-4513) can act as an inverse agonist at γ2 subunit-containing receptors, where it presumably reduces the frequency of channel opening and as a positive agonist at $\alpha 3\beta 2\gamma 4$ subunit receptors, where it likely increases the frequency of channel opening. One interpretation of these observations is that one or more of the residues that mediate the binding of Ro 15-4513, and/or are involved in the transduction of this signal, are either also part of the gating mechanism of the chloride-selective channel or are directly linked to it. The consequence of Ro 15-4513 binding would then be an alteration in the conformation of γ2 and γ4 subunit-containing receptors so as to make the channel either more or less resistant to opening, respectively, when GABA is bound.

Although a number of studies have defined residues on $GABA_A$ receptor α and γ subunits that mediate the binding of benzodiazepine site ligands (see Sigel and Buhr, 1997), to our knowledge, mutation of only one γ subunit residue has been shown to change the pharmacological profile of Ro 15-4513. While this compound acts as a

weak inverse agonist at recombinant human receptors that contain a wild-type γ2 subunit, it behaves as a partial agonist when the threonine residue at position 142 (T142) of the γ2 subunit is changed to a serine (S142; Mihic et al., 1994). However, alignment of the human γ2 and chicken γ4 subunits (not shown) reveals that the avian polypeptide has a threonine at the position corresponding to T142 in the human sequence. Thus, this residue cannot be responsible for the partial agonistic properties reported here for Ro 15-4513 at γ4 subunit-containing $GABA_A$ receptors.

A feature of $GABA_A$ receptors that possess a mammalian γ subunit is their relative insensitivity to Zn^{2+} compared to αβ subunit receptors (Smart et al., 1994; Knoflach et al., 1996; Krishek et al., 1998). For example, α1β1 and α1β1γ2 subunit receptors have IC_{50} concentrations for Zn^{2+}, at pH 7.4, of 1.2 and 639 μM, respectively (Krishek et al., 1998); α4β2γ2 and α6β2γ2 subunit receptors are somewhat more sensitive than α1β1γ2 subunit receptors, having IC_{50} values of approximately 100 μM (Knoflach et al., 1996). In contrast, we found that Zn^{2+} blocked the GABA-evoked response at α3β2γ4 subunit receptors with an IC_{50} value of only about 20 μM. Since the GABA responses that we have recorded are sensitive to a wide variety of benzodiazepine binding site ligands, it is clear that we have been studying α3β2γ4 subunit receptors rather than heterodimeric α3β2 subunit receptors. We can also likely rule out contamination from β2γ4 subunit receptors since βγ subunit receptors have been reported to give significantly smaller current amplitudes than αβγ subunit receptors (Sigel et al., 1990). Thus, $GABA_A$ receptors containing the avian γ4 polypeptide are quite different, in their Zn^{2+} sensitivity, to those harbouring instead either the mammalian γ1, γ2 or γ3 subunit.

A number of investigations have defined residues on $GABA_A$ receptor α and β subunits that mediate the inhibition by Zn^{2+}. These are located either within the pore-forming second membrane-spanning domain (M2) or within the short extracellular loop between the second and third (M3) membrane-spanning segments. Thus, mutation of a histidine residue within β subunits, at the carboxy-terminal end of M2, has been shown to be associated with a dramatic decrease in Zn^{2+} potency (Wooltorton et al., 1997; Horenstein and Akabas, 1998). In contrast, based on the observation that α6β3γ2 subunit receptors are more sensitive to Zn^{2+} than receptors comprising α1β3γ2 subunits (IC_{50} values of 25 and 190 μM, respectively), Fisher and Macdonald (1998) have implicated a histidine residue in the α6 subunit that is located between M2 and M3. Although no data have been published on determinants within γ subunits that might play a role in Zn^{2+} blockade, presumably because the presence of the γ2 subunit abolishes inhibition (Draguhn et al., 1990; Smart et al., 1994), it is noteworthy that the chicken γ4 subunit has a histidine residue in the extracellular loop region between M2 and M3 (H287) and that this is replaced by serine in the γ1, γ2 and γ3 subunits (see Harvey et al., 1993). The possible role played by this histidine in the relatively high sensitivity of α3β2γ4 subunit receptors to Zn^{2+} is currently under investigation.

In summary, we have shown here that recombinant $GABA_A$ receptors that contain the chicken γ4 subunit have novel benzodiazepine pharmacology (Ro 15-4513 functions as a positive agonist rather than as an inverse agonist) and are highly sensitive to Zn^{2+} ions. Future experiments are aimed at elucidating the molecular bases for these two interesting properties by the construction of chimeric γ subunits and the subsequent generation and analysis of point mutants.

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