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Chicken $GABA_A$ receptor β4 subunits form robust homomeric GABA-gated channels in *Xenopus* oocytes

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Abstract

Chicken $GABA_A$ receptor β4L and β4S subunits were expressed in *Xenopus* oocytes by cRNA injection. Oocytes expressing either β4 subunit alone or in combination with the chicken α1 subunit were studied using the two-electrode voltage-clamp technique. Both the β4L and β4S subunits form homomeric GABA-gated Cl^- channels with similar efficiencies. In comparison, oocytes expressing either the chicken α1 or β2S polypeptide show no or barely detectable GABA responses, as reported by others for most single-subunit vertebrate $GABA_A$ receptors. The GABA-gated currents due to the β4L-subunit homomer were not affected by the presence of actinomycin D during cRNA expression, indicating that nascent oocyte polypeptides are not required for channel formation. The homomeric β4L-subunit receptors show high affinity for GABA with an EC_{50} value of 4.3 ± 0.4 μM and a Hill coefficient of 1.1 ± 0.1 ($n = 6$). In response to GABA application at the EC_{25} value, currents elicited from the β4L-subunit receptor are enhanced by 50 μM pentobarbital ($110 \pm 10\%$, $n = 3$) and 10 μM loreclezole ($60 \pm 3\%$, $n = 3$), inhibited by 10 μM picrotoxinin ($93 \pm 3\%$, $n = 3$), but not affected by 1 μM diazepam. These properties are similar to those found for oocytes expressing heteromeric chicken α1β4L and α1β2S receptors. Since the β subunits of $GABA_A$ receptors provide essential determinants for receptor assembly and subcellular localization, homomeric β4-subunit receptors are a useful model system for further study of the structure and function of $GABA_A$ receptors.

Keywords: Allosteric modulation; β4 subunit, chicken; $GABA_A$ receptor; Homomeric receptor; *Xenopus* oocyte

1. Introduction

GABA is the predominant inhibitory neurotransmitter in the vertebrate brain. Most of the rapid actions of GABA occur, through $GABA_A$ receptors on the postsynaptic membrane, by the gated opening of a Cl^- channel integral to the receptor complex. The resulting increase in Cl^- permeability of the postsynaptic membrane produces neuronal inhibition. $GABA_A$ receptors are composed of subunits from six families (α, β, γ, δ, ε and π). There are multiple isoforms within three of these subunit families (α1-6, β1-4 and γ1-4) and additional diversity is produced by variations in the splicing of certain RNAs (reviewed by Darlison and Albrecht, 1995; Rabow et al., 1995). The function of $GABA_A$ receptors is allosterically modulated by a number of therapeutically important agents including benzodiazepines, barbiturates, and neurosteroids (Macdonald and Olsen, 1994). The contribution of different subunits to some of the modulatory sites on $GABA_A$ receptors has been elucidated. The benzodiazepine-binding site of the $GABA_A$ receptor is determined by the type of α and γ subunit present, with the γ polypeptide being essential for benzodiazepine binding (Pritchett et al., 1989; Pritchett and Seeburg, 1990). Loreclezole, a broad-spectrum anticonvulsant compound, shows selective affinity for recombinant $GABA_A$ receptors containing certain β subunits (Wingrove et al., 1994). More recently, it has been shown that reduced sensitivity of recombinant $GABA_A$

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receptors to general anaesthetic agents and pregnanolone is conferred by the ε and π subunits, respectively (Davies et al., 1997a; Hedblom and Kirkness, 1997).

As model systems, homomeric $GABA_A$ receptors with properties that are comparable to native, heteromeric $GABA_A$ receptors could be useful in the further investigation of the pharmacology, assembly, subcellular localization, and structure of this ion channel. Homomeric $GABA_A$ receptors showing a limited number of native receptor properties have been reported after heterologous expression of certain subunits in *Xenopus* oocytes (Blair et al., 1988; Sigel et al., 1989, 1990; Sanna et al., 1995; Cestari et al., 1996; Krishek et al., 1996), human embryonic kidney 293 (HEK) cells (Pritchett et al., 1988; Po et al., 1990; Slany et al., 1995; Krishek et al., 1996; Davies et al., 1997b), and insect cell lines (Atkinson et al., 1992; Joyce et al., 1993). The GABA-gated currents elicited by homomeric receptors are typically much smaller than those produced by heteromeric receptors. Most of the functional homomeric $GABA_A$ receptors reported are formed from β subunits, which have been shown to be a key component for robust expression of recombinant receptors (Sigel et al., 1990; Verdoorn et al., 1990). Homomeric β1-subunit $GABA_A$ receptors exhibit species-dependent differences in functional properties. Human and bovine β1-subunit receptors yielded small GABA-gated Cl^- currents (10–50 nA oocyte whole-cell currents elicited by 100 μM agonist) that were potentiated by pentobarbital and inhibited by picrotoxin (Blair et al., 1988; Pritchett et al., 1988; Joyce et al., 1993; Sanna et al., 1995; Krishek et al., 1996), whereas murine and rat β1-subunit receptors were not gated by GABA but showed spontaneous openings that were blocked by picrotoxin (Sigel et al., 1989, 1990; Krishek et al., 1996). On the other hand, rat β3-subunit $GABA_A$ receptors expressed in HEK cells showed neither spontaneous activity nor sensitivity to GABA, but they could be activated by pentobarbital (Davies et al., 1997b). There is no report of a functional homomeric β2-subunit $GABA_A$ receptor, in accord with the observation that individual murine α1, β2 and γ2L subunits were not transported to the surface membrane, but were retained within the endoplasmic reticulum of transfected HEK cells (Connolly et al., 1996a,b). However, Cestari et al. (1996) recorded pentobarbital-induced currents from murine β2- and β3-subunit homomers, which were unresponsive to GABA. Some studies demonstrated that $GABA_A$ receptor β subunits are able to form receptors without incorporating endogenous polypeptides of either oocytes or HEK cells (Slany et al., 1995; Krishek et al., 1996), but other experiments show possible involvement of endogenous HEK polypeptides, including the $GABA_A$ receptor β3 subunit which may normally be present in HEK cells (Kirkness and Fraser, 1993; Fuchs et al., 1995; Davies et al., 1996; Ueno et al., 1996).

The $GABA_A$ receptor β4 subunit has, to date, been identified only in chick brain. There are two alternatively spliced variants (β4L and β4S), with the β4L subunit containing a 4 amino acid insert of unknown function (Bateson et al., 1991b). It is intriguing that neither a mammalian homologue of the β4 subunit nor a chicken homologue of the β1 polypeptide has been reported. Baumgartner et al. (1994) have shown that the β4-subunit mRNA accumulates more rapidly than those encoding the α1 and β2 subunits during maturation of cultured neurons from the chick embryonic cerebral cortex. Immunoprecipitation and immunoblotting experiments (Tehrani et al., 1995) suggest that the β4 subunit is a major component of $GABA_A$ receptors in the chick cerebral cortex. However, the $GABA_A$ receptor β4 polypeptide has not been functionally expressed, either alone or in combination with other subunits. To investigate the properties of the β4 subunit, *Xenopus* oocytes expressing homomeric and heteromeric $GABA_A$ receptors were examined by the two-electrode voltage-clamp technique.

2. Materials and methods

2.1. Generation of full-length complementary DNAs (cDNAs)

A full-length β2S-subunit cDNA was constructed by ligating part of the previously-reported chicken $GABA_A$ receptor β2L-subunit partial cDNA (~1.5 kb; Harvey et al., 1994), which lacks sequences that encode the carboxy-terminal part of the large intracellular loop and the fourth membrane-spanning domain (M4), to a polymerase chain reaction (PCR)-derived cDNA fragment. This latter fragment was generated using primers 5′-AGTAAGCTTT GGTCAACTACATCTTCTTTG-3′ and 5′-TAACTCGAGTTCTTGCTTCCTGTGTGGCTT-3′; the former recognizes the sequence that specifies the amino acids Glu–Tyr–Ala–Leu–Val–Asn–Tyr–Ile–Phe–Phe which span the end of the third membrane-spanning domain (M3), while the latter is complementary to part of the 3′-untranslated region. Amplification of random-nonamer primed embryonic day-18 chick brain first-strand cDNA was for 35 cycles of 94°C for 1 min, 65°C for 1 min and 72°C for 1 min. The resultant products (497 bp; β2S-subunit cDNA, and 548 bp; β2L-subunit cDNA) were digested with *Hin*cII and *Xho*I, at unique restriction endonuclease recognition sites (underlined) that were incorporated into the PCR primers, and cloned into *Hin*cII- and *Xho*I-restricted pBluescript SK + yielding plasmids pcGRβ2S.3′end and pcGRβ2L.3′end, respectively. Finally, the pBluescript II SK + plasmid containing the ~1.5 kb β2L-subunit partial cDNA (Harvey et al., 1994) was digested with *Eco*RI (which cuts at the 5′ end of the insert) and *Hin*cII (which cuts at a sequence that encodes the end of M3), and the released cDNA fragment was ligated into *Eco*RI- and *Hin*cII-restricted pcGRβ2S.3′end. The resultant construct was completely sequenced.

A full-length cDNA encoding the β4L subunit (previously called the β4′ subunit) was constructed by ligating a previously-reported chicken $GABA_A$ receptor β4L-subunit partial cDNA (Bateson et al., 1991b), which lacks sequences that encode the carboxy-terminal part of the large intracellular loop and M4, to a PCR-derived cDNA fragment. This latter fragment was generated using primers 5′-GGCTCAGGAATTCAGTTCCGCAAGCCACTG-3′ and 5′-CTATCAAGCTTGGAGGCTGCAGGCATCA-3′; the former recognizes the sequence that specifies the amino acids Gly–Ser–Gly–Ile–Gln–Phe–Arg–Lys–Pro–Leu in the large intracellular loop, while the latter is complementary to part of the 3′-untranslated region. Amplification of random-nonamer primed 1-day-old chick brain first-strand cDNA was for 30 cycles of 94°C for 1 min, 65°C for 1 min and 72°C for 1 min. The resultant product (289 bp) was digested with *Eco*RI and *Hin*dIII, at unique restriction endonuclease recognition sites (underlined) that were incorporated into the PCR primers. Next, the pBluescript plasmid containing the β4L-subunit partial cDNA (Bateson et al., 1991b) was completely digested with *Hin*dIII (which recognizes a unique site within the vector polylinker, 3′ of the cDNA sequence), and then partially digested with *Eco*RI. The resultant mixture of products was ligated together with the *Eco*RI- and *Hin*dIII-digested PCR-generated fragment, and a construct containing a full-length β4L-subunit cDNA (pcGRβ4L) was selected by restriction mapping and fully sequenced.

A full-length cDNA encoding the β4S subunit (previously called the β4 subunit; Bateson et al., 1991b), which lacks the amino acid sequence Val–Arg–Glu–Gln in the large intracellular loop, was generated by in vitro mutagenesis of plasmid pcGRβ4L using the method of Kunkel (Sambrook et al., 1989). Single-stranded DNA was mutagenized using the antisense oligonucleotide 5′-ACCGTAAGGGTCGACCCTCTTTTCTTCATA-3′, which is complementary to the sequences that flank the 12-bp insertion in the β4L-subunit cDNA. Note that this primer contains a single mismatch to the published sequence (Bateson et al., 1991b) which generates a *Sal*I site (underlined); this permitted the rapid detection of mutated cDNAs but does not change the encoded amino-acid sequence. Fourteen mutated plasmids were identified. One of these was fully sequenced to confirm that the 12 nucleotides had been deleted and to check the integrity of the complete β4S-subunit coding sequence.

The chicken $GABA_A$ receptor full-length α1-subunit cDNA used in these studies was that described by Bateson et al. (1991a).

2.2. cRNA synthesis

cRNAs were synthesized using the T3 and T7 mMESSAGE mMACHINE kits (Ambion) following the protocol recommended by the manufacturer. cRNAs were ethanol precipitated, resuspended in 0.1 M KCl at a final concentration of 1 μg/μl, and stored at −80°C until use.

2.3. Oocyte preparation and microinjection

Xenopus oocytes were prepared and injected following routine protocols (Parent and Gopalakrishnan, 1995). Defolliculated oocytes were injected with 50 nl of solution containing a single $GABA_A$ receptor subunit cRNA, or combinations of α1- and β-subunit cRNAs each at a concentration of approximately 600 ng/μl. In experiments differentiating chicken $GABA_A$ receptor subunit expression from that of the host cell, oocytes were incubated in the presence of actinomycin D (50 μg/ml) following cRNA injection to inactivate transcription of *Xenopus* genes.

2.4. Electrophysiological recording

Whole-cell inward currents in response to GABA were measured by two-electrode voltage-clamp (Oocyte clamp OC-725B, Warner Instrument) 4–8 days after cRNA injection. During electrophysiological recording, oocytes were voltage-clamped between −60 and −70 mV and perfused with modified Barth's medium (in mM: 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 10 HEPES, 0.82 $MgSO_4$, 0.33 $Ca(NO_3)_2$, 0.91 $CaCl_2$, pH 7.4) at 6 ml/min throughout the experiment. $GABA_A$ receptor modulators were applied in the perfusate for 30 s before GABA addition. GABA was applied for 30–45 s or until the peak of the response was observed. Intervals of 10–15 min were allowed between applications to ensure full recovery from desensitization. Each batch of oocytes expressing $GABA_A$ receptors was tested for their ability to produce consistent GABA-induced currents by repeatedly applying 100 μM GABA during a period of at least 90 min. $GABA_A$ receptor modulators were dissolved in dimethylsulphoxide (DMSO) prior to dilution into the perfusate. The resulting levels of DMSO ($\leq 0.1\%$) had no effect on either non-injected, sham-injected or $GABA_A$ receptor subunit cRNA-injected oocytes. Loreclezole was a gift from Janssen Laboratories, diazepam was donated by Hoffmann-La Roche, and picrotoxinin and pentobarbital were purchased from Sigma.

2.5. Data analysis

The computer software CLAMPEX 6.0 from pCLAMP (Axon Instruments) was used for on-line data acquisition and analysis. Membrane currents were digitally sampled at 5 kHz (200 μs/data point) and simultaneously traced by chart recorder. To study the current–voltage relationship of the GABA-induced current, discontinuous voltage pulses (−70 to −10 mV) of 200 ms duration were applied from a holding potential of −70 mV before and after application of 100 μM GABA. The peak amplitude of the GABA-evoked currents at various potentials was normalized by assigning 100% to the value at −70 mV. All measurements for the GABA dose–response curves were standardized by assigning 100% to the current amplitude

elicited by 1 mM GABA. All measurements of the effects of $GABA_A$ receptor modulators were standardized by assigning 100% to the current amplitude evoked by an approximately EC_{25} concentration of GABA (predetermined for each individual oocyte based on the maximum current response induced by 2 mM GABA). Dose–response curves were calculated using a nonlinear regression fit (Marquardt–Levenberg algorithm, SigmaPlot v. 2.0, Jandel Scientific) of the equation: $I_C/I_{max} = 1/(1 + (EC_{50}/C)^n)$, where C is the GABA concentration, I_C is the current elicited by GABA at concentration C, I_{max} is the current elicited by 1 mM GABA, and n is the Hill coefficient.

3. Results

Co-expression of the chicken $GABA_A$ receptor α1 and β4L subunits in *Xenopus* oocytes resulted in a GABA-gated inward current (Fig. 1A). The peak response to 100 μM GABA (1.9 ± 0.3 μA, mean ± S.E.; $n = 4$ oocytes) was comparable to that of oocytes co-expressing the chicken α1 and β2S subunits (2.5 ± 0.3 μA, $n = 4$).

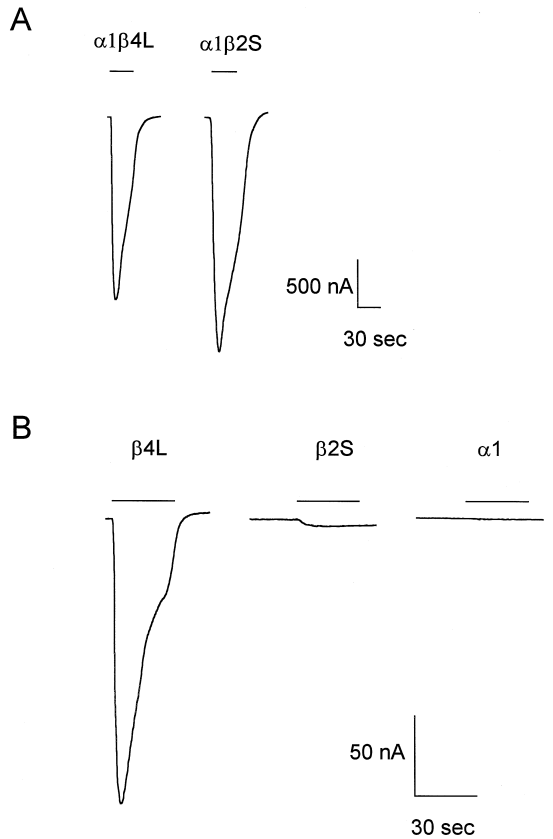

Fig. 1. Membrane currents in response to the application of GABA. (A) Oocytes expressing either α1β4L- or α1β2S-subunit receptors. (B) Oocytes expressing either the β4L, β2S or α1 subunit alone. GABA (100 μM) was applied for the times indicated by the horizontal bars above the current traces.

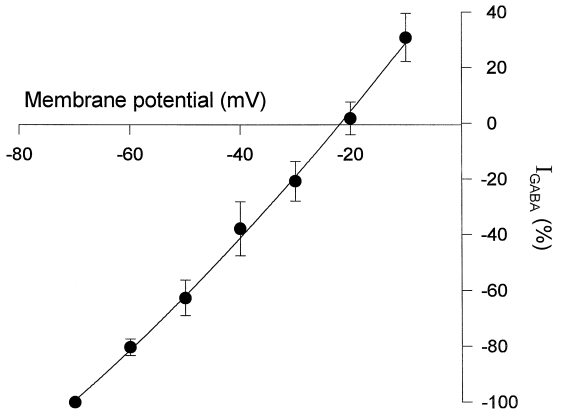

Fig. 2. Current–voltage relationship of the GABA-induced current from oocytes expressing homomeric β4L-subunit $GABA_A$ receptors. Before application of 100 μM GABA and during the peak current, discontinuous voltage pulses (−70 to −10 mV) of 200 ms duration were applied from a holding potential of −70 mV. The amplitudes of the GABA-induced currents at various potentials were normalized by assigning 100% to the value obtained at −70 mV and are expressed as the mean ± S.E. ($n = 3$ oocytes).

Injection of the β4L-subunit cRNA alone (Fig. 1B) produced receptors that also yielded a GABA-gated inward current with a peak response of 110 ± 10 nA ($n = 8$). Similar currents were obtained with β4S-subunit channels (not shown). In paired experiments similar to those shown in Fig. 1B, the peak currents evoked by 100 μM GABA from β4S-subunit receptors were not significantly different from those of β4L-subunit channels when analyzed by *t*-test ($t = 0.498$, df = 3, P-value = 0.653). However, expression of the chicken $GABA_A$ receptor α1 subunit or β2S subunit alone resulted in no or a barely detectable response to the application of 100 μM GABA (Fig. 1B). GABA had no effect on either non-injected or sham-injected oocytes (not shown).

Sigel et al. (1990) have suggested that currents associated with homomeric β1-subunit $GABA_A$ receptors may be due to incorporation of endogenous oocyte polypeptides. In order to investigate this possibility, oocytes injected with the β4L-subunit cRNA were incubated with actinomycin D (50 μg/ml) to inactivate *Xenopus* gene transcription. Similar responses to 100 μM GABA were observed after incubation either with (130 ± 14 nA, $n = 3$) or without (110 ± 16 nA, $n = 3$) actinomycin D. To characterize the GABA-gated current attributed to the β4-subunit homomer, the current–voltage relationship of the inward current induced by 100 μM GABA was examined (Fig. 2). The reversal potential was −20 mV, consistent with the equilibrium potential for Cl^- in oocytes (Barish, 1983).

The relationship of the peak current amplitudes to the concentration of applied GABA is shown in Fig. 3. The curves represent the best fit by Marquardt–Levenberg algorithm for the least-square solution of the parameters (EC_{50} value and Hill coefficient). It is apparent that the

homomeric β4L-subunit receptor shows a high affinity for GABA. The arithmetic mean of individual EC_{50} values from 6 oocytes injected with β4L-subunit cRNA was 4.3 ± 0.4 μM and the Hill coefficient was 1.1 ± 0.1. When the $GABA_A$ receptor β4L subunit was co-expressed in oocytes with the α1 subunit, a lower affinity for GABA was observed ($EC_{50} = 16 \pm 3$ μM, Hill coefficient = 1.3 ± 0.2, $n = 3$). A similar dose–response curve (Fig. 3) was obtained for heteromeric α1β2S receptors ($EC_{50} = 14 \pm 2$ μM, Hill coefficient = 1.1 ± 0.2, $n = 3$). At the lowest GABA concentration tested (1 μM), the data for both heteromeric receptors show a small deviation from the fitted curves. However, this is unlikely to significantly affect the resulting EC_{50} values.

The allosteric modulation of GABA-elicited currents from the β4L-subunit $GABA_A$ receptor was also investigated (Fig. 4). At GABA concentrations representing the EC_{25} value, the peak currents were enhanced by 50 μM pentobarbital ($110 \pm 10\%$, $n = 3$) and by 10 μM loreclezole ($60 \pm 3\%$, $n = 3$). In the presence of 10 μM picrotoxinin, the responses were inhibited by $93 \pm 3\%$ ($n = 3$), while 1 μM diazepam had no significant effect. Similar modulatory effects were also observed for heteromeric α1β4L and α1β2S receptors (Fig. 4) with 50 μM pentobarbital enhancing ($110 \pm 17\%$ and $150 \pm 23\%$, respectively), 10 μM loreclezole enhancing ($65 \pm 7\%$ and $87 \pm 2\%$, respectively), and 10 μM picrotoxinin inhibiting ($87 \pm 2\%$ and $89 \pm 3\%$, respectively) the GABA-gated currents. Higher concentrations of pentobarbital, which presumably could directly gate channels in the absence of GABA, were not tested. Under conditions similar to those used to generate the data shown in Fig. 4, β4L-subunit receptors did not elicit detectable currents during application of either pentobarbital, loreclezole or picrotoxinin in the absence of GABA (deflections < 3 nA; $n = 4$; not shown). Likewise, no response to the application of any of these modulators ($n \geq 4$) was observed with oocytes injected with either the α1- or β2S-subunit cRNA alone or with non-injected or sham-injected oocytes.

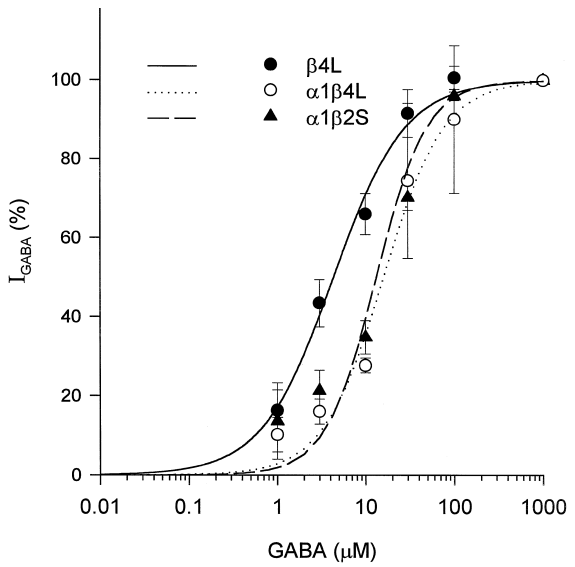

Fig. 3. GABA dose–response curves for homomeric β4L-subunit and heteromeric α1β4L- and α1β2S-subunit $GABA_A$ receptors. The peak currents were normalized to the values obtained with 1 mM GABA and represent the mean ± S.E. The values for n (number of oocytes), the Hill coefficients, and the EC_{50} values are given in the text. Individual dose–response curves were fitted as described in Section 2.5.

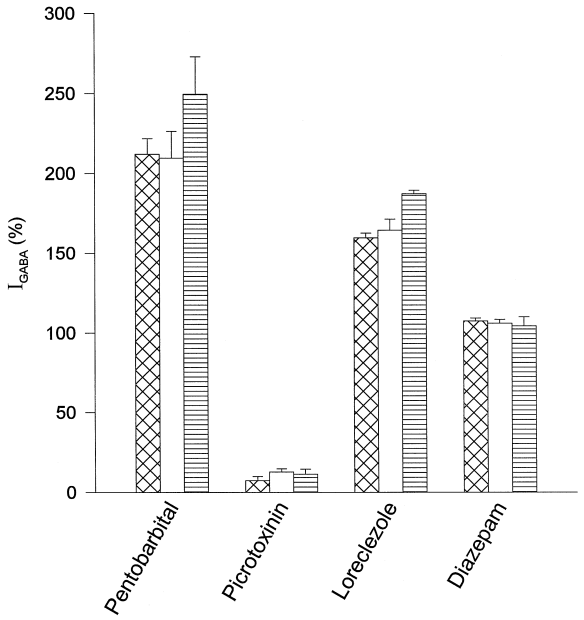

Fig. 4. Effect of 50 μM pentobarbital, 10 μM picrotoxinin, 10 μM loreclezole and 1 μM diazepam on homomeric β4L-subunit and heteromeric α1β4L- and α1β2S-subunit $GABA_A$ receptors. After application of the indicated modulator for 30 s, GABA at the appropriate EC_{25} value, determined from Fig. 3, was introduced into the perfusate. Values for the peak GABA-gated currents are expressed as a percentage of controls (100%) in which the modulator was omitted and represent the mean ± S.E. ($n = 3$).

4. Discussion

These experiments provide the first description of $GABA_A$ receptor β4-subunit function. A combination of chicken $GABA_A$ receptor β4L and α1 subunits co-expressed in *Xenopus* oocytes yielded GABA-gated currents of a magnitude similar to those recorded from an α1β2S-subunit combination. Oocytes injected with the β4L-subunit cRNA alone also showed GABA-evoked currents which were approximately 10% of those obtained with the α1β4L-subunit receptor. Since both the β4S and the β4L homomer gave similar sized GABA-gated currents, it seems unlikely that the additional sequence of 4 amino acids in the predicted large intracellular loop region of the β4L subunit has a substantial effect on receptor assembly. The failure of actinomycin D to affect the expression of homomeric β4-subunit currents appears to rule out potential

contributions of nascent oocyte polypeptides in the assembly of these channels. For the formation of $GABA_A$ receptor β1-subunit channels, such endogenous polypeptides have been suggested to be required (Sigel et al., 1990).

The β4L-subunit $GABA_A$ receptor has a much higher apparent affinity for GABA than other homomeric $GABA_A$ receptors reported previously (cf. Blair et al., 1988). Interestingly, the GABA EC_{50} value for the homomeric β4L-subunit channel is also lower than that of the heteromeric α1β4L- and α1β2S-subunit receptors (Fig. 3). It has been suggested that the GABA binding site in $GABA_A$ receptors is formed by the interface between α and β subunits (Smith and Olsen, 1995). The proposed locus of GABA binding includes Phe^{64} and flanking residues of the α1 subunit (Sigel et al., 1992; Smith and Olsen, 1994) and two motifs in the β2 subunit, one surrounding Tyr^{157} and the other at Tyr^{205} (Amin and Weiss, 1993). The $GABA_A$ receptor β4-subunit sequence (Bateson et al., 1991b) shows conservation of these β2-subunit motifs, but lacks that in the α1 subunit. Nevertheless, our data imply that the interface between two β4 subunits is capable of forming a site whose affinity for GABA gating is similar to that of native $GABA_A$ receptors.

At GABA concentrations near the EC_{25} value, pentobarbital and picrotoxinin give similar potentiation and inhibition, respectively, with homomeric β4L- and heteromeric α1β4L- and α1β2S-subunit $GABA_A$ receptors. In the absence of GABA, application of either pentobarbital or picrotoxinin has been reported to modulate the currents from misassembled $GABA_A$ receptors (Krishek et al., 1996). However, in our studies when applied alone, neither compound had an effect on either non-injected oocytes or sham-injected oocytes, or on oocytes expressing chicken $GABA_A$ receptor subunits. Therefore, the GABA-gated currents from homomeric β4L-subunit channels are not artifacts of improperly assembled receptors.

The affinity for loreclezole is more than 300-fold higher with $GABA_A$ receptors containing either a β2 or a β3 subunit than those having a β1 subunit. This selectivity is determined by a single amino-acid residue at the distal end of M2, Asn^{289} and Asn^{290} in human $GABA_A$ receptor β2 and β3 subunits, respectively, and Ser^{290} in the β1 subunit (numbering from the amino-terminal methionine residue; Wingrove et al., 1994). The predicted amino acid sequences of the chicken β4 and β2 subunits (Bateson et al., 1991b; Harvey et al., 1994) show that both contain Asn at this position (Asn^{289}). Co-application of 10 μM loreclezole together with a GABA concentration at the EC_{25} value enhanced the currents from homomeric β4L- and heteromeric α1β4L- and α1β2S-subunit receptors to a similar degree (60–87%). Since 10 μM loreclezole produced much smaller enhancements at $GABA_A$ receptors containing a β1 subunit (Wingrove et al., 1994), it is apparent that the β4 subunit functionally resembles the β2 and β3 subunits. This is consistent with the conservation of Asn, in M2, in the β2, β3, and β4 polypeptides.

In the putative intracellular loop region between M3 and M4, the chicken $GABA_A$ receptor β4 subunit shows substantial divergence from the mammalian β1- and chicken β2- and β3-subunit sequences. This points to possible novel properties for the β4 polypeptide. One of these is the ability to form robust homomeric agonist-gated channels, a property not found among other vertebrate $GABA_A$ receptor β subunits. Furthermore, investigation reveals that homomeric β4-subunit receptors possess a very high affinity site for GABA. Although such homomeric receptors are unlikely to occur in vivo, we believe that the β4-subunit homomer will prove useful for examining receptor assembly and trafficking as well as the relationship between molecular structure and receptor function.

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