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The rat β_1-subunit of the $GABA_A$ receptor forms a picrotoxin-sensitive anion channel open in the absence of GABA

Erwin Sigel, Roland Baur, Pari Malherbe[+] and Hanns Möhler[°]

Institute of Pharmacology, University of Bern, 3010 Bern, [+] Pharmaceutical Research Department, Hoffmann-La Roche, 4051 Basel and [°]Institute of Pharmacology, University of Zürich, 8006 Zürich, Switzerland

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The structural basis of GABA-gated chloride channels in mammalian brain is presently explored by the functonal expression of cDNAs coding for the α, β or γ-subunits of the receptor and their isoforms. In this context, we expressed the cloned cDNA coding for the rat β_1-subunit of the $GABA_A$ receptor in the *Xenopus* oocyte. Surprisingly, efficient expression of a functional ion channel was found. The channel was anion-selective, and able to open in the absence of GABA. Since this channel could be shut by the GABA-channel blocker picrotoxin, we conclude that the β_1-subunit of the $GABA_A$ receptor is sufficient to form binding sites for picrotoxin.

Aminobutyric acid, γ-; Aminobutyric acid receptor, γ-; Ion channel; Picrotoxin; Expression; (*Xenopus* oocyte)

1. INTRODUCTION

The major inhibitory neurotransmitter of mammalian brain, γ-aminobutyric acid (GABA), exerts its action through the $GABA_A$-receptor channel, which also binds benzodiazepines. A $GABA_A$-receptor complex has recently been purified to homogeneity from bovine brain [1,2]. Subsequently, its subunits have been cloned from this [3–5], and other species [5–7]. Cloning work also revealed a large number of subunit isoforms. The pertinent questions are now (i) what is the composition of a functional GABA receptor-channel complex in a given cell, and (ii) what are the functional and pharmacological properties of the channel isoforms. Different combinations of cloned $GABA_A$-receptor subunits have been functionally expressed [3–9]. Expression of combinations of α- and β-subunit isoforms leads to efficient formation of GABA-gated anion channels [3,4,7–9]. Co-expression of the γ-subunit is essential for the bidirectional modulation of the channel by drugs acting at the benzodiazepine-binding site [5]. Only very inefficient functional expression of single bovine subunits has been observed [8]. We report here, that the rat β_1-subunit alone is sufficient for the efficient expression of a picrotoxin-sensitive, anion-selective channel in the *Xenopus* oocyte. However, this channel lacks the GABA-gating properties.

Correspondence address: E. Sigel, Pharmakologisches Institut der Universität Bern, Friedbühlstrasse 49, CH-3010 Bern, Switzerland

2. MATERIALS AND METHODS

The cDNA coding for the subunits of the rat $GABA_A$ receptor was transcribed, capped and poly(A)-tailed [7]. The resulting RNA was injected into *Xenopus* follicles (7 fmol of each subunit per cell). After three days in culture, the oocytes were freed from the surrounding follicle cells as described in detail elsewhere [10]. Ion currents in the denuded oocytes were recorded at room temperature (21–27°C), using the two electrode voltage clamp technique. The membrane potential was held at −80 mV. Drugs were applied by bath perfusion as previously reported [10,11]. The composition of the perfusion medium was 90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM Hepes-NaOH (pH 7.4).

3. RESULTS AND DISCUSSION

Four days after injection with RNA coding for subunits of the rat $GABA_A$-receptor channels, the oocytes were screened for newly expressed ion currents. Rat GABA receptor subunit combinations such as $\alpha_1\beta_1$ [7] or $\alpha_1\beta_1\gamma_2$ (not shown) produce large inward currents (several μA) that can be elicited by 100 μM GABA. In contrast, when the α_1-, α_5-, β_1- or γ_2-subunits were injected individually, no such currents could be detected (detection limit 3 nA; see table 1). In some oocytes injected with RNA coding for the α_3-subunit, GABA elicited a small current (<25 nA).

The resting membrane properties for non-injected control oocytes, and for oocytes injected with RNA coding for any of the individual α- and γ-subunits alone, or with $\alpha_1\beta_1$ were similar, with a resting resistance of 0.5–1.0 MΩ. In contrast, oocytes injected with the β_1-subunit only, had a very low membrane resistance amounting to 0.04–0.10 MΩ. No mor-

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Table 1

Currents observed in *Xenopus* oocytes after injection of different subunit RNAs

Subunits injected	Current (nA) induced by	
	GABA	Picrotoxin
Non-injected	n	n
α_1	n (4)	n (4)
α_3	−5 (5)	n (5)
α_5	n (3)	n (3)
β_1	n (4)	390 (37)
γ_2	n (3)	n (3)
$\alpha_1\beta_1$	−3990 (25)	28 (7)

Each of the subunits was injected in stoichiometrical amounts (7 fmol RNA/cell). Average current signals that could be elicited in the late expression phase (at least 3 days after injection) by 100 μM GABA (inward current negative) or 10 μM picrotoxin (outward current positive) are given for the different subunit combinations. Inward currents induced by GABA are negative by definition. The number in brackets indicates the number of cells measured. Conditions that resulted in non-detectable currents (<3 nA) are indicated with n. The cDNAs coding for the rat brain α_1- and β_1-subunits are given in [7], those of the α_3-, α_5-, and γ_2-subunits will be described elsewhere

phological difference between these batches of oocytes could be seen, making a structural damage induced by the β_1-subunit unlikely.

In attempts to find a reason for the extremely low membrane resistance in β_1 injected oocytes, we discovered that addition of low concentrations of the GABA-channel blocker, picrotoxin, restored the normal membrane resistance in these oocytes. Fig.1 compares typical GABA and picrotoxin responses recorded from an oocyte injected with RNAs coding for the α_1- and β_1-subunits with those obtained from a β_1-subunit injected oocyte. In the first case, 100 μM GABA elicited a large inward current with a concomitant decrease in the membrane resistance. In the latter case,

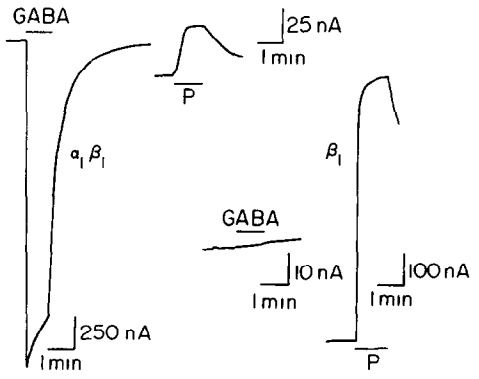

Fig.1. Membrane current responses to 100 μM GABA and 10 μM picrotoxin (P) of an oocyte injected with RNAs coding for the rat brain α_1- and β_1-subunits ($\alpha_1\beta_1$) are compared to the responses of an oocyte injected with the β_1-subunit only (β_1). Continuous perfusion was switched for the periods of time indicated by the bar to the same medium containing either GABA or picrotoxin.

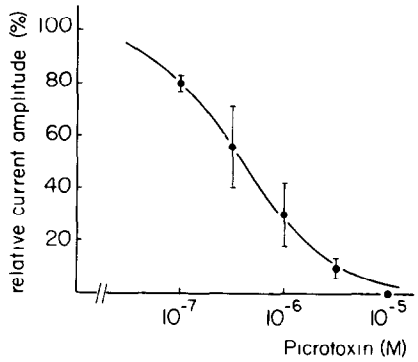

Fig.2. Picrotoxin concentration-dependence of the inhibition of the permanent current. The dose-response curve was obtained three times, from different oocytes, with cells exhibiting different degrees of channel expression. Maximal current was arbitrarily set at 100% and the current amplitudes were expressed as relative values. Each point represents the average of three determinations. The inhibition curve was fitted using a nonlinear least-squares method.

an apparent outward current was induced by 10 μM picrotoxin with a concomitant increase in the membrane resistance to values typical for healthy oocytes. The average picrotoxin inhibited current component amounted to 0.39 ± 0.24 μA (mean ± SD, 37 oocytes from four separate batches of oocytes).

Fig.2 shows a dose-response curve of the inhibition of this current by picrotoxin. Half-maximal inhibition was observed at about 0.4 μM. The apparent Hill slope was 1.0. These values are similar to those for picrotoxin inhibition of the GABA (10 μM)-activated current in oocytes injected with total mRNA isolated from chick forebrain [11].

This unusual ion current was not affected by the positive allosteric modulator of the GABA current diazepam (1 μM) or the negative allosteric modulator

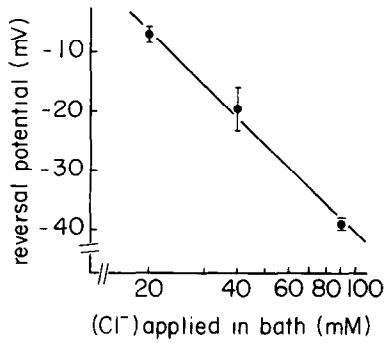

Fig.3. Dependence of the reversal potential of the permanent current on the external chloride concentration. The same oocyte was exposed to standard medium and to media, in which part of the NaCl was replaced by Na-methanesulfonate, maintaining constant ionic strength. In each medium, before and during application of 10 μM picrotoxin, a discontinuous voltage ramp of 100 ms step duration and 10 mV step size was applied from a holding potential of −60 mV, and the reversal potential of the picrotoxin-sensitive component was determined. The reversal potential is plotted against the total chloride concentration in the medium. Averaged data from four experiments were averaged and fitted by linear regression.

methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM; 0.3 μM).

In order to determine the ion selectivity of the permanent current, the reversal potential was measured at different chloride concentrations in the bathing medium (fig.3). A ten-fold increase in the chloride concentration resulted in an average positive shift of the reversal potential by 49 mV, close to the value of about 58 mV expected for an anion-selective channel. The presence of the non-gated anion channel presumably led to a decrease of the intracellular chloride concentration, since the reversal potential in standard medium was shifted from about −22 mV in control cells [11] to about −39 mV (fig.4).

When the β_1-subunit was complemented with the α_1-subunit, only a tiny fraction (<0.5% of the total) was assembled to form a non-gated channel (table 1).

We conclude that injection into *Xenopus* oocytes of the RNA coding for the rat β_1-subunit of the GABA receptor results in efficient expression of a presumably homomeric, anion-selective channel which retains normal affinity for the $GABA_A$ channel blocker picrotoxin. This channel, however, is not gated by GABA, since it is open in its absence. It may be inferred that synthesis or processing of the β_1-subunit in neurones must be under regulatory control, in order to suppress formation of non-gated GABA channels.

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