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European Journal of Pharmacology 408 (2000) R1–R2

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Rapid communication

Loreclezole as a simple functional marker for homomeric ρ type $GABA_C$ receptors

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Received 10 October 2000; accepted 10 November 2000

Abstract

$GABA_C$ receptors are expressed in the whole brain, but predominantly in the retina. They can be identified by their unique pharmacology. The establishment of the entire pharmacology is, however, quite tedious. We show here that loreclezole dose dependently inhibits ionic currents elicited by GABA (γ-aminobutyric acid) with an IC_{50} of about 0.5 μM in homomeric ρ1 $GABA_C$ receptors expressed in *Xenopus oocytes*. Thus, loreclezole may constitute a functional marker for these receptors.

Keywords: GABA (γ-aminobutyric acid); $GABA_C$ receptor; ρ1 subunit; Loreclezole

The GABA receptors consisting of ρ subunits have been termed $GABA_C$ receptors based on their different pharmacology (Shimada et al., 1992), in spite of the fact that they share similarity with subunits of the $GABA_A$ receptors. To our knowledge, a simple, functional signature, identifying these receptors in vivo is lacking. Here, we describe that loreclezole is an inhibitor of homomeric ρ1 channels in electrophysiologigal experiments. Loreclezole is known as a potent allosteric stimulator of $GABA_A$ receptors containing a β2 or β3 subunits, but not a β1 subunit (Wingrove et al., 1994).

Xenopus laevis oocytes were prepared, injected, defolliculated and currents recorded as described (Sigel et al., 1990). Briefly, oocytes were injected with 50 nl of cRNA dissolved in 5 mM K-HEPES (pH 6.8). This solution contained the transcripts coding for the ρ1 subunit at a concentration of 75 nM. RNA transcripts were synthesized from linearized plasmids encoding ρ1 (Cutting et al., 1991) obtained from Dr. G. Cutting, using the message machine kit (Ambion) according to the recommendation of the manufacturers. A poly(A) tail of about 300 residues was added to the transcripts by using yeast poly(A) polymerase (USB or Amersham). Transcripts were quantified on agarose gels after staining with Radiant Red RNA Stain (Bio-Rad) by comparing staining intensities with various amounts of molecular weight markers (RNA-Ladder, Gibco-BRL). Electrophysiological experiments were performed by the two-electrode voltage clamp method at a holding potential of −80 mV. The medium contained 90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$ and 10 mM Na-HEPES (pH 7.4). A concentration of GABA (γ-aminobutyric acid) eliciting about 10% of the maximal current response was applied for 20 s and a washout period of 4 min was allowed to ensure full recovery from desensitization. Subsequently, the same concentration of GABA was co-applied in combination with increasing concentrations of either loreclezole, (+)-ROD188 ((1*R*, 2′*R*)-1-(2,3,4,5-Tetrahydro-5-oxo-2-furyl)-2-*N*-(*p*-toluene-sulfonyl)-1,2,3,4-tetrahydroisoquinoline) (Razet et al., 2000) or pentobarbital. The perfusion system was cleaned between drug applications by washing with dimethyl sulfoxide to avoid contamination. Loreclezole was obtained from Janssen (Switzerland).

In oocytes expressing the homomeric ρ1 receptors, pentobarbital is known to act as a negative allosteric modulator (Shimada et al., 1992; Fig. 1). Interestingly, two other positive allosteric modulators of $GABA_A$ receptors, loreclezole and (+)-ROD188 (Thomet et al., 2000), also acted here in cumulative dose–response curves as negative

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00778-0

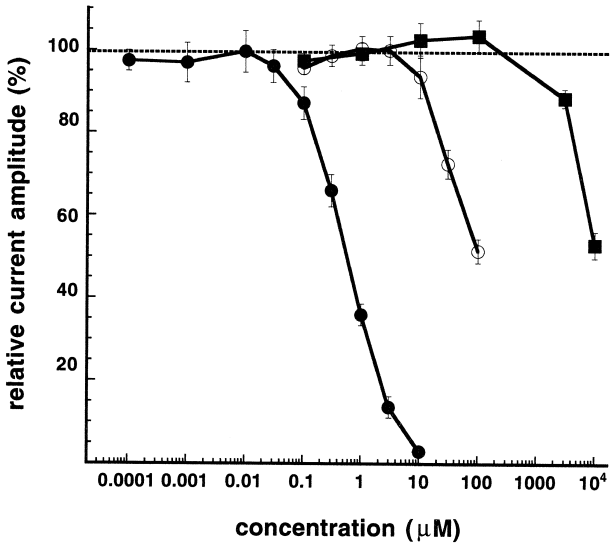

Fig. 1. Concentration–response curves for loreclezole (●), (+)-ROD188 (○) and pentobarbital (■) in homomeric recombinant rat ρ1 receptors. Values are presented as mean ± S.E.M. of four oocytes from two batches.

allosteric modulators. Whereas pentobarbital has a very high IC_{50} of about 10,000 μM, loreclezole inhibited with an IC_{50} of about 0.5 μM and (+)-ROD188 with an intermediate IC_{50} of about 100 μM (Fig. 1).

Pentobarbital, loreclezole and (+)-ROD188 potently stimulate $GABA_A$ receptors and strongly inhibit currents elicited by GABA in ρ1 receptors. The neurosteroid 5β-pregnan-3α-ol-20-one and propofol, which also both greatly enhance $GABA_A$ receptor-mediated responses, have been demonstrated to depress ρ1 receptors as well (Belelli et al., 1999). Most probably, in ρ1 receptors, the binding sites for these modulators inversely influence the effects of GABA on ion conductance as compared to $GABA_A$ receptors. The observed similar properties of pentobarbitone, (+)-ROD188 and loreclezole are most probably due to this general inverse action on homomeric ρ1 receptors of positive modulators of $GABA_A$ receptors.

Acknowledgements

This study was supported by the European Union grant BIO4-CT96-0585 (BBW 96.0010) and grant 3100-053599.98/1 from the Swiss National Science Foundation.

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