

# Differential expression of GABA<sub>A</sub> receptor $\alpha$ -subunits in rat brain during development

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Received 23 April 1991

Unique cytoplasmic loop regions of the  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$  and  $\alpha_5$  subunits of the GABA<sub>A</sub> receptor have been expressed in *E. coli* and used to generate polyclonal antisera specific for these subunits. The antibodies identify proteins by SDS-polyacrylamide gel electrophoresis and western blotting of molecular size 51 kDa, 53 kDa, 59 kDa and 55 kDa, respectively, which show differential patterns of expression during development. Whereas the  $\alpha_2$  and  $\alpha_3$  subunits are present at early stages, the expression of  $\alpha_1$  and  $\alpha_5$  subunits is low at birth and increases with age. This differential expression could be correlated with previous studies examining the developmental expression of BZ1 and BZ2 benzodiazepine binding sites.

GABA<sub>A</sub> receptor;  $\alpha$ -Subunit; Polyclonal antiserum; Ontogeny; Rat brain

## 1. INTRODUCTION

The GABA<sub>A</sub> receptor is a multimeric ligand gated ion channel which possesses binding sites for the endogenous agonist GABA, as well as allosteric binding sites for benzodiazepines, barbiturates and steroids (for reviews see [1,2]). The combination of subunits forming the GABA<sub>A</sub> receptor has yet to be clearly defined. Molecular cloning studies have so far revealed 6 types of  $\alpha$ , 3 types of  $\beta$ , 2 types of  $\gamma$  and one  $\delta$  subunit [2–4]. It has been demonstrated that at least one  $\alpha$ , one  $\beta$  and one  $\gamma$  subunit are required to reconstruct a GABA<sub>A</sub> receptor whose pharmacological and electrophysiological properties approach those of native receptors in vivo [5–7]. The  $\alpha$  subunit is of particular interest since it contains the primary binding site for benzodiazepines [8] and the type of  $\alpha$  subunit present appears to define the pharmacology of receptors expressed in transfected cells [9–11].

In the present study we have demonstrated that there is differential expression of  $\alpha$  subunits at different stages of development in the rat brain and that it is possible to correlate this with the developmental expression of [<sup>3</sup>H]benzodiazepine binding sites.

## 2. MATERIALS AND METHODS

Polyclonal antisera were raised against the unique cytoplasmic loop regions of the  $\alpha_1$  (V<sub>322</sub>–P<sub>382</sub>),  $\alpha_2$  (V<sub>322</sub>–A<sub>382</sub>),  $\alpha_3$  (P<sub>347</sub>–T<sub>421</sub>) and  $\alpha_5$  (A<sub>325</sub>–S<sub>386</sub>) subunits of the GABA<sub>A</sub> receptor as described elsewhere

[12]. Membranes were prepared from cortex, hippocampus, cerebellum and striatum of rat brain according to the method of Olsen et al. [13]. Tissue was taken from rats of the following ages: newborn (1 day), 7 days, 14 days, 3 months, 12 months and 18 months. Membranes were resuspended at a protein concentration of 2 mg/ml, 20  $\mu$ g of membrane protein was loaded per lane for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting, and 50  $\mu$ g was used per tube for radioligand binding assays. Western blots were carried out using a 1:500 dilution of antiserum [12] and radioligand assays were conducted with [<sup>3</sup>H]Ro 15-1788 (2.5 nM), or [<sup>3</sup>H]flunitrazepam (2 nM) in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5, for 1 h at 4°C as previously described [12].

## 3. RESULTS AND DISCUSSION

In agreement with other studies using antipeptide antibodies [14–16] or polyclonal antisera raised against bacterially expressed GABA<sub>A</sub> protein [12], the antiserum detected  $\alpha$  subunit proteins of the appropriate sizes ( $\alpha_1$  50 kDa,  $\alpha_2$  53 kDa,  $\alpha_3$  59 kDa,  $\alpha_5$  55 kDa) upon SDS-PAGE and Western blot analysis. The  $\alpha_1$  antiserum identified two bands in very young animals only (Fig. 1). The additional lower band may represent an immature form of the subunit with incomplete post-translational modification, e.g. incomplete glycosylation. In tissue prepared from older rats this species is not observed. The  $\alpha_3$  antiserum identified a broad band at 59 kDa which could be resolved into a doublet in all regions studied. A lower molecular weight species of 53 kDa was also inconsistently observed and this may well represent a proteolytic fragment. The most striking developmental pattern is observed with the  $\alpha_1$  and  $\alpha_5$  subunits. Both are almost below detection level at birth and increase dramatically with maximum levels of expression being reached by 3 months. This same pattern of expression for  $\alpha_1$  was also observed in other brain

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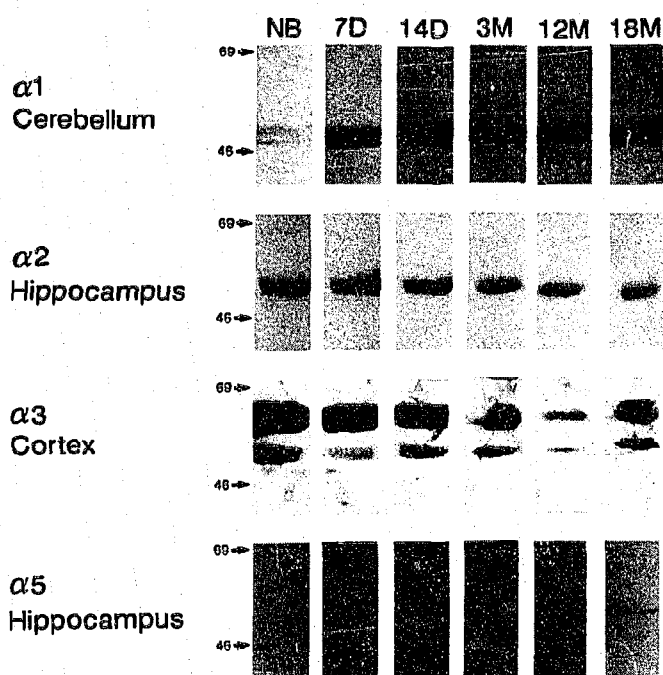
Developmental Expression of  $\alpha$ -Subunits

Fig. 1. Developmental expression of  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$  and  $\alpha_5$  subunits of the GABA<sub>A</sub> receptor. Membranes were prepared from various brain regions of rats of the following ages. Newborn (NB), 7 day, 14 day, 3 month, 12 month and 18 month, as indicated. 20  $\mu$ g of membrane protein was applied per lane, subjected to SDS/12% PAGE and Western blotting. Blots were probed with a 1:500 dilution of antiserum and bands visualised with a peroxidase/ethylcarbazole reaction [12]. Gels were calibrated with prestained molecular wt. markers (Amersham) and the positions of the 69 kDa and 46 kDa markers are indicated. Data shown are representative of 2 or 3 experiments.

regions such as the cortex (data not shown) and is consistent with immunochemical localisation of  $\alpha_1$  subunits on cerebellar granule cells only after several days of maturation [17] and the steady increase in  $\alpha_1$  mRNA in whole rat brain up to 25 days after birth [18,23]. The low level of expression of the  $\alpha_5$  subunit in brain areas other than the hippocampus precluded the detection of a consistent pattern of developmental expression.

The pattern of developmental expression for  $\alpha_2$  and  $\alpha_3$  subunits was quite different from that seen with  $\alpha_1$  and  $\alpha_5$  subunits. Both subunits were present at high levels at birth and both showed a tendency to decline in aged rats (greater than 12 months old). This is in accord with photoaffinity labelling experiments which reveal two bands labelled with [ $^3$ H]flunitrazepam in young animals of 53 kDa and 59 kDa presumably the  $\alpha_2$  and  $\alpha_3$  subunits, respectively [16,19,22], and a much heavier labelling of a 51 kDa protein (the  $\alpha_1$  subunit) in mature rats [19]. In newborn animals the band detected by the  $\alpha_3$  antiserum appeared to be a doublet, and like the  $\alpha_1$

subunit this may represent different post-translational modifications.

Early studies investigating the development of binding sites for [ $^3$ H]benzodiazepines showed there to be no change in the kDa for any of the ligands with age [20,21]. We therefore used one concentration of ligand to measure the number of benzodiazepine binding sites in the same membranes that were used for Western blot analysis. As shown in Fig. 2, the number of [ $^3$ H]flunitrazepam and [ $^3$ H]Ro 15-1788 binding sites increased from newborn to adult levels at 14 days, after which time they remained fairly constant. A similar pattern has previously been observed using the ligand [ $^3$ H]diazepam [20]. It is currently held that the ligands [ $^3$ H]flunitrazepam, [ $^3$ H]Ro 15-1788 and [ $^3$ H]diazepam bind with high affinity to the same sites, i.e. receptors composed of either  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$  or  $\alpha_5$  together with  $\beta$  and  $\gamma_2$  [12]. Therefore the increase in expression of receptors containing  $\alpha_1$  and  $\alpha_5$  must more than compensate for the decrease in receptors containing  $\alpha_2$  and  $\alpha_3$  subunits to result in a net increase in benzodiazepine binding. The  $\alpha_1$  subunit has been shown in transfected cells to correlate well with GABA<sub>A</sub> receptors exhibiting a BZ1 pharmacology [10] whereas BZ2 type receptors may contain  $\alpha_2$ ,  $\alpha_3$  or  $\alpha_5$  subunits [9,11]. The increase in the developmental expression of the  $\alpha_1$  subunit parallels earlier studies demonstrating an increase in the expression of BZ1 receptors with age as measured by CL218,872 displacement of [ $^3$ H]flunitrazepam binding [21]. Similarly, the abundance of  $\alpha_2$  and  $\alpha_3$  subunits early on in development is consistent with the observation that BZ2 receptor binding sites are already present

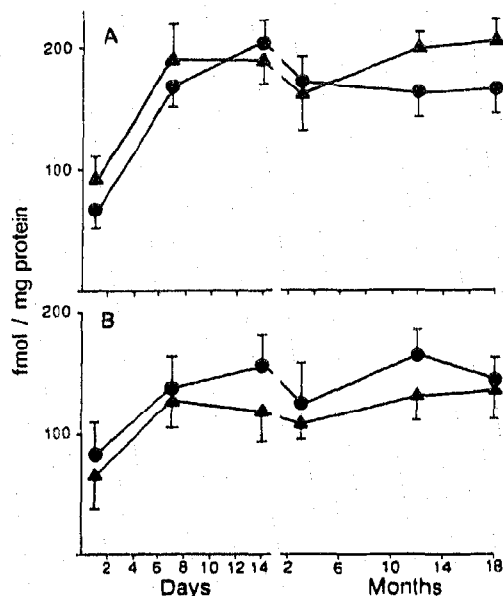


Fig. 2. Binding of [ $^3$ H]flunitrazepam (2 nM; ●—●), and [ $^3$ H]Ro 15-1788 (2.5 nM, ▲—▲) to cortical (a) and cerebellar (b) membranes prepared from rats of various ages. Data shown are mean  $\pm$  SE of 4 experiments.

at high levels in newborn rats [21]. GABA<sub>A</sub> receptors containing  $\alpha_4$  or  $\alpha_6$  subunits have not been considered because mRNA for the  $\alpha_4$  subunit is very rare in rat brain [24] and expressed receptors containing the  $\alpha_6$  subunit are reported not to bind classical benzodiazepines with high affinity [9].

We have clearly shown here differential expression of GABA<sub>A</sub> receptor  $\alpha$  subunits during development. Whether other subunits ( $\beta$ ,  $\gamma$  and  $\delta$ ) are also differentially expressed during development remains to be determined. Characterization of the developmental expression of GABA<sub>A</sub> receptor subtypes may yield some clues towards understanding their role in nerve transmission.

*Acknowledgements:* We thank Jan Baynes for technical assistance and Andy Butler and Roy Hammans for photography.

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