Volume 286, number 1,2, 44–46 FEBS 09909 July 1991

ADONIS 0014579391006195

Differential expression of $GABA_A$ receptor α-subunits in rat brain during development

R.M. McKernan, P. Cox, N.P. Gillard and P. Whiting

Department of Biochemistry, Neuroscience Research Centre, Merck Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR, UK

Received 23 April 1991

Unique cytoplasmic loop regions of the α_1, α_2, α_3 and α_5 subunits of the $GABA_A$ 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 α_2 and α_3 subunits are present at early stages, the expression of α_1 and α_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.

$GABA_A$ receptor; α-Subunit; Polyclonal antiserum; Ontogeny; Rat brain

1. INTRODUCTION

The $GABA_A$ 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 $GABA_A$ receptor has yet to be clearly defined. Molecular cloning studies have so far revealed 6 types of α, 3 types of β, 2 types of γ and one δ subunit [2–4]. It has been demonstrated that at least one α, one β and one γ subunit are required to reconstruct a $GABA_A$ receptor whose pharmacological and electrophysiological properties approach those of native receptors in vivo [5–7]. The α subunit is of particular interest since it contains the primary binding site for benzodiazepines [8] and the type of α 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 α subunits at different stages of development in the rat brain and that it is possible to correlate this with the developmental expression of [^{3}H]benzodiazepine binding sites.

2. MATERIALS AND METHODS

Polyclonal antisera were raised against the unique cytoplasmic loop regions of the α_1 (V_{322}–P_{382}), α_2 (V_{322}–A_{382}), α_3 (P_{347}–T_{421}) and α_5 (A_{325}–S_{386}) subunits of the $GABA_A$ 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 μg of membrane protein was loaded per lane for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting, and 50 μg 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 [^{3}H]Ro 15-1788 (2.5 nM), or [^{3}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].

Correspondence address: R.M. McKernan, Department of Biochemistry, Neuroscience Research Centre, Merck Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR, UK. Fax: (44) (279) 440390

3. RESULTS AND DISCUSSION

In agreement with other studies using antipeptide antibodies [14–16] or polyclonal antisera raised against bacterially expressed $GABA_A$ protein [12], the antiserum detected α subunit proteins of the appropriate sizes (α_1 50 kDa, α_2 53 kDa, α_3 59 kDa, α_5 55 kDa) upon SDS-PAGE and Western blot analysis. The α_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 α_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 α_1 and α_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 α_1 was also observed in other brain

Published by Elsevier Science Publishers B.V.

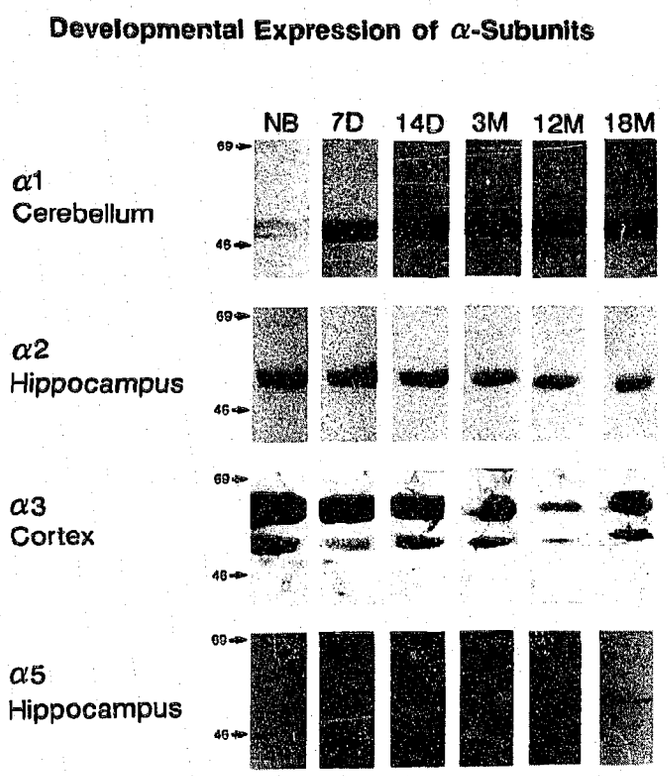

Fig. 1. Developmental expression of α_1, α_2, α_3 and α_5 subunits of the $GABA_A$ receptor. Membranes were prepared from various brain regions of rats of the following ages. Newborn (NB), 7 day, 14 day, 3 month, 12 month and 18 month, as indicated. 20 μg of membrane protein was applied per lane, subjected 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 α_1 subunits on cerebellar granule cells only after several days of maturation [17] and the steady increase in α_1 mRNA in whole rat brain up to 25 days after birth [18,23]. The low level of expression of the α_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 α_2 and α_3 subunits was quite different from that seen with α_1 and α_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 α_2 and α_3 subunits, respectively [16,19,22], and a much heavier labelling of a 51 kDa protein (the α_1 subunit) in mature rats [19]. In newborn animals the band detected by the α_3 antiserum appeared to be a doublet, and like the α_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 α_1, α_2, α_3 or α_5 together with β and γ_2 [121. Therefore the increase in expression of receptors containing α_1 and α_5 must more than compensate for the decrease in receptors containing α_2 and α_3 subunits to result in a net increase in benzodiazepine binding. The α_1 subunit has been shown in transfected cells to correlate well with $GABA_A$ receptors exhibiting a BZ1 pharmacology [10] whereas BZ2 type receptors may contain α_2, α_3 or α_5 subunits [9,11]. The increase in the developmental expression of the α_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 α_2 and α_3 subunits early on in development is consistent with the observation that BZ2 receptor binding sites are already present

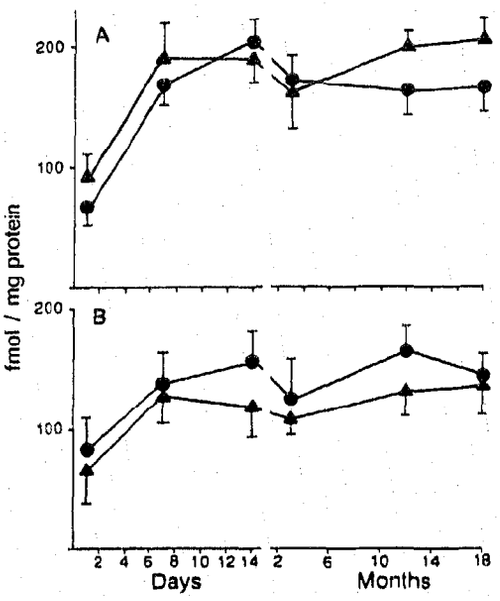

Fig. 2. Binding of [^{3}H]flunitrazepam (2 nM; ●—●), and [^{3}H]Ro 15-1788 (2.5 nM, ▲—▲) to cortical (a) and cerebellar (b) membranes prepared from rats of various ages. Data shown are mean ± SE of 4 experiments.

at high levels in newborn rats [21]. $GABA_A$ receptors containing α_4 or α_6 subunits have not been considered because mRNA for the α_4 subunit is very rare in rat brain [24] and expressed receptors containing the α_6 subunit are reported not to bind classical benzodiazepines with high affinity [9].

We have clearly shown here differential expression of $GABA_A$ receptor α subunits during development. Whether other subunits (β, γ and δ) are also differentially expressed during development remains to be determined. Characterization of the developmental expression of $GABA_A$ 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.

REFERENCES

[1] Stephenson, F.A. (1988) Biochem. J. 249, 21–32.
[2] Olsen, R.W. and Tobin, A.J. (1990) FASEB J. 4, 1469–1480.
[3] Sieghart, W. (1989) Trends Pharmacol. Sci. 10, 407–411.
[4] Schofield, P.R. (1989) Trends Pharmacol. Sci. 10, 476–478.
[5] Pritchett, D.B., Sontheimer, H., Shirers, B.D., Ymer, S., Kettenmann, H., Schofield, P.R. and Seeberg, P.H. (1989) Nature 338, 582–585.
[6] Malherbe, P., Sigel, E., Baur, R., Persohn, E., Richards, J.G. and Mohler, H. (1990) J. Neurosci. 10, 2330–2337.
[7] Sigel, E., Baur, R., Trube, G., Mohler, H. and Malherbe, P. (1990) Neuron 5, 7033–7110.
[8] Stephenson, F.A., Duggan, M.J. and Pollard, S. (1990) J. Biol. Chem. 265, 21160–21165.
[9] Luddens, H., Pritchett, D.B., Kohler, M., Killisch, I., Keinanen, K., Monyer, H., Sprengel, R. and Seeberg, P.H. (1990) Nature 346, 648–651.
[10] Pritchett, D.B., Luddens, M. and Seeberg, P.H. (1989) Science 245, 1389–1392.
[11] Pritchett, D.B. and Seeberg, P.H. (1990) J. Neurochem. 54, 1802–1804.
[12] McKernan, R.M., Quirk, K., Prince, R., Cox, P.A., Ragan, C.I. and Whiting, P. (1991) (submitted).
[13] Olsen, R.W., Bergman, M.O., Van Ness, P.C., Lummis, S.C., Watkins, A.E., Napiss, C. and Greenlee, D.V. (1981) Mol. Pharmacol. 19, 217–227.
[14] Stephenson, F.A., Duggan, M.J. and Casalotti, S.O. (1989) FEBS Lett. 243, 358–362.
[15] Duggan, M.J. and Stephenson, F.A. (1989) J. Neurochem. 53, 132–139.
[16] Fuchs, K., Adamikes, D. and Sieghart, W. (1990) FEBS Lett. 261, 52–54.
[17] Meinecke, D.L. and Rakic, P. (1990) Dev. Brain Res. 73–86.
[18] Garrett, K.M., Saito, N., Duman, R.S., Abel, M.S., Ashton, R.A., Fujimosi, S., Beer, B., Tallman, J.F., Vitek, M.P. and Blume, A.J. (1990) Mol. Pharmacol. 37, 652–657.
[19] Fuchs, K. and Sieghart, W. (1989) Neurosci. Lett. 97, 329–333.
[20] Candy, J.M. and Martin, I.L. (1979) J. Neurochem. 32, 655–658.
[21] Lippa, A.S., Beer, B., Sano, M.C., Vogel, R.A. and Meyerson, L.R. (1981) Life Sci. 28, 2343–2347.
[22] Sato, T.N. and Neale, J.H. (1989) J. Neurochem. 52, 1114–1122.
[23] Gambarana, C., Pittman, R. and Siegel, R.E. (1990) J. Neurobiol. 21, 1169–1179.
[24] Ymer, S., Dragulin, A., Hohler, M., Schofield, P.R. and Seeberg, P.H. (1989) FEBS Lett. 258, 119–122.
[25] Ymer, S., Dragulin, A., Wisden, W., Werner, P., Keinanen, K., Schofield, P.R., Sprengel, R., Pritchett, D.B. and Seeberg, P.H. (1990) EMBO J. 9, 3261–3267.