Development of GABAA Receptors in the Central Visual Structures of Rat Brain. Effect of Visual Pattern Deprivation

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Abstract. The postnatal development of high-affinity ^{3}H-muscimol binding to GABAA receptors was studied in the lateral geniculate nucleus, superior colliculus, frontal and visual cortex of the rat brain. In the lateral geniculate nucleus ^{3}H-muscimol binding rises from day 10 through day 37 reaching the highest value during the entire development followed by a slight decrease until adulthood. In the superior colliculus ^{3}H-muscimol binding increased continuously from day 10 through day 37, and then decreased until day 50 reaching the adult value. In the visual and frontal cortex, binding reached the highest levels on days 14 and 25, respectively, persisted until day 37 followed by a slight decrease until adulthood. The ontogeny of ^{3}H-muscimol binding sites in the visual regions does not essentially differ from that in other brain regions, suggesting that the appearance of ^{3}H-muscimol binding sites in the visual system is not correlated with the functional maturation of the visual system. Unilateral eyelid closure from day 11 until day 25 did not affect the development of GABAA receptors in any of the central visual regions examined, indicating the lack of environmentally controlled mechanism.

Key words: GABAA receptor — Development — Visual system — Rat brain — Monocular deprivation

Introduction

γ-Aminobutyric acid (GABA) has been established to be the major inhibitory transmitter also in the central visual structures of the brain. High GABA levels and high activities of the glutamic acid decarboxylase (GAD), the synthesizing

Abbreviations used: FC, frontal cortex; GABA, γ-aminobutyric acid; GAD, L-glutamic acid decarboxylase; LGN, lateral geniculate nucleus; SC, superior colliculus; VC, visual cortex.

enzyme for GABA, were found in both visual subcortical (lateral geniculate nucleus — LGN; superior colliculus — SC) and cortical regions of the rat (Kunert et al. 1978; McDonald et al. 1981; Ohara et al. 1983; Okada 1974; Okada et al. 1971). Iontophoretically applied GABA showed an inhibitory action on neurons of both LGN (Kayama et al. 1981) and SC (Kayama et al. 1980). In SC, GABA is mainly localized in interneurons (Houser et al. 1983; Okada 1974) or in GABAergic fibres from substantia nigra to SC (DiChiara et al. 1979). LGN receives a strong inhibitory GABAergic input from the thalamic reticular nucleus (Houser et al. 1980; Montero and Scott 1981; Ohara et al. 1983). Measurements of GAD activity in individual layers of rat visual cortex (VC) indicated a fairly even distribution throughout the cortex. Further, GAD was shown to be localized in somata, dendrites and axon terminals of nonpyramidal cells of rat VC (for review, see Parnavelas and McDonald 1983).

The visual system in the rat undergoes postnatal changes which are expected to be correlated with the development of retinal function and the maturation of the system to respond to light stimulation. In a previous study it was shown that the onset of retinal pattern vision seems to trigger the development of the glutamate binding sites in rat central visual regions (Schliebs et al. 1986a). GABA plays an important role as the major inhibitory transmitter in the visual nuclei; therefore, the formation of GABAergic synapses should also be tuned and/or controlled by the onset of pattern vision as was seen for the excitatory glutamatergic system in the rat visual system. Therefore, we studied the normal development of GABAA receptors in rat LGN, SC, and VC to see if the onset of pattern recognition on postnatal day (PD) 14 has consequences on the further expression of GABA binding sites. To elucidate whether the normal ontogeny of GABA receptors is dependent on adequate visual input, eyelids of some animals were unilaterally sutured (monocular deprivation).

Materials and Methods

Materials

[Methylamine-^{3}H]muscimol, specific activity 296 GBq/mmol, was obtained from Radiochemical Centre Amersham, U.K., and used without further purification. Analytical grade γ-aminobutyric acid (GABA) was purchased from Reanal, Hungary. All other chemicals used were commercial products of the highest purity available.

Animals

Rats (strain BD III) of either sex from the laboratory's own animal stock were studied at the age of 10, 14, 25, 37, 50 and 90 postnatal days.

Animals were kept under normal animal housing conditions on a 12 h light-dark cycle

(controls). In visual deprivation experiments, rats were subjected to unilateral eyelid closure at the age of 11 days (monocular deprivation). Eyelid suture was carried out under anaesthesia (Nembutal sodium solution, Abbott Lab., Chicago, 40 mg/kg body weight). Some control animals received the anaesthetic only; no effects on ^{3}H-muscimol binding compared to untreated control rats could be observed. Litter size was reduced to 8 rats per litter on the day following birth. Visually deprived rats and their control littermates were kept together. All animals received food and water ad libitum.

Tissue preparation

Rats were killed by decapitation at the age indicated. The brains were rapidly removed, placed on ice and the visual cortex (VC), the frontal cortex (FC), the lateral geniculate nucleus (LGN) and the superior colliculus (SC) were isolated under a stereo-microscope as described previously (Bigl et al. 1974). In the case of control animals, visual structures from either side of the brain were pooled. In the case of monocularly deprived animals, the corresponding visual nuclei from either side of brains of two rats were pooled. Tissue samples were homogenized in 1 ml 50 mmol/l Tris-citrate buffer, pH 7.1 (containing 3—15 mg wet weight of tissue), and stored at −20 °C until used for binding assay (not longer than two weeks). For the binding assay, samples were rehomogenized and centrifuged at 48,000 × *g* for 20 min. The resulting pellets were dispersed in 1 ml distilled water by ultrasonication and again centrifuged at 48,000 × *g* for 20 min. Finally, the pellets were resuspended in 1 ml 50 mmol/l Tris-citrate buffer, pH 7.1, and frozen at −20 °C overnight.

For removing endogenous GABA the tissue samples were extensively washed by centrifugation at 48,000 × *g* for 20 min and the pellet was resuspended in 50 mmol/l Tris-citrate buffer, pH 7.1. The resuspension and centrifugation steps were repeated three times and the final tissue suspensions were stored at −20 °C overnight. For the binding assay, tissue suspensions were thawed, again centrifuged at 48,000 × *g* for 20 min, the pellets were resuspended in 1 ml fresh buffer, and immediately used for the binding assay.

Binding assay

High-affinity ^{3}H-muscimol binding to GABA$_A$ receptors was measured using the centrifugation method as described by Olson et al. (1981). One hundred µl of the tissue preparation containing about 100—150 µg protein were added to 300 µl 50 mmol/l Tris-citrate buffer, pH 7.1. The reaction was initiated by adding 100 µl ^{3}H-muscimol at a final concentration of 2 nmol/l. The incubation was continued for 30 min at 0 °C followed by centrifugation at 70,000 × *g* for 10 min. The supernatant was discarded and the pellet was twice rinsed superficially with 2 ml of ice-cold buffer to remove unbound ligand. The rinsed pellet was solubilized in 200 µl 1 N Hyamine hydroxide, and the radioactivity was determined by liquid scintillation counting. Each assay was run in duplicate. Non-specific binding was assayed by including 0.1 mmol/l non-radioactive GABA in the incubation medium. Specific binding was calculated by subtracting from the total bound radioactivity the amount not displaced by 0.1 mmol/l GABA; it represented about 30 % of total binding.

Data were expressed as specifically bound ^{3}H-muscimol per gram protein or per gram wet weight of tissue. Protein content was measured according to the method of Lowry et al. (1951). In the developmental studies specific binding was also expressed as binding per tissue region.

In preliminary experiments, it was shown that specific ^{3}H-muscimol binding under the conditions mentioned above, was saturable up to 16 nmol/l and was linearly dependent on protein content up to 800 µg protein/ml. Raising the pH of the 50 mmol/l Tris-citrate buffer up to 8.0 resulted in a continuous decrease of total ^{3}H-muscimol binding without a significant change in non-specific binding. Specific binding was shown to reach equilibrium during an incubation time of 30 min at 0 °C.

For evaluating binding parameters such as equilibrium dissociation constant K_D and maximum receptor number B_{max} the experimental data (bound radioligand (b_{sp}) versus free ligand concentration (f)) were fitted by non-linear least squares regression analysis to the saturation function

$$b_{sp} = \frac{B_{max}\,f}{K_D + f}$$

For these experiments the ligand concentration in the assay was varied between 1 and 17 nmol/l ^{3}H--muscimol.

Results

Table 1 summarizes the binding parameters of specific high-affinity ^{3}H-muscimol binding to GABAA receptors obtained in VC and SC at two different age levels. At both ages examined no differences in K_D-value could be observed. Therefore, the postnatal development of specific high-affinity ^{3}H-muscimol binding in VC, SC, and LGN was measured at a constant ligand concentration (2 nmol/l ^{3}H-muscimol).

Table 1. Summary of binding parameters of ^{3}H-muscimol binding in rat visual cortex and superior colliculus at different age

Region	K_D (nmol/l)	B_{max} (fmol/mg prot.)
	Postnatal day 12	
Visual cortex	76 ± 1	5511 ± 58
Superior colliculus	60 ± 2	2938 ± 117
	Postnatal day 90	
Visual cortex	70 ± 1	6578 ± 90
Superior colliculus	65 ± 2	2080 ± 57

Binding parameters K_D (equilibrium dissociation constant) and B_{max} (maximum receptor number) were obtained by fitting experimental data to the saturation curve using non-linear least squares regression analysis. Means ± S.E.M. are shown.

The binding data were calculated in terms of total binding (i.e. fmol specifically bound ^{3}H-muscimol per whole tissue region) as well as in terms of density (i.e. pmol specifically bound ^{3}H-muscimol per gram protein or per gram wet weight of tissue), thus taking into account the developmental changes of the reference system itself.

^{3}H-muscimol binding in VC rises sharply from PD 10 through PD 14 (by a factor of about 4), it remains unchanged until PD 37, and continues decreasing by 43 % up to day 50 when the adult value is reached (Fig. 1). A qualitatively similar developmental profile was seen when binding data were expressed in

pmol/g wet weight of tissue. Considering the development of ^{3}H-muscimol binding sites per VC, binding increased sharply from PD 10 to PD 14 (by a factor of ten) and then remained constant until adulthood (Fig. 1).

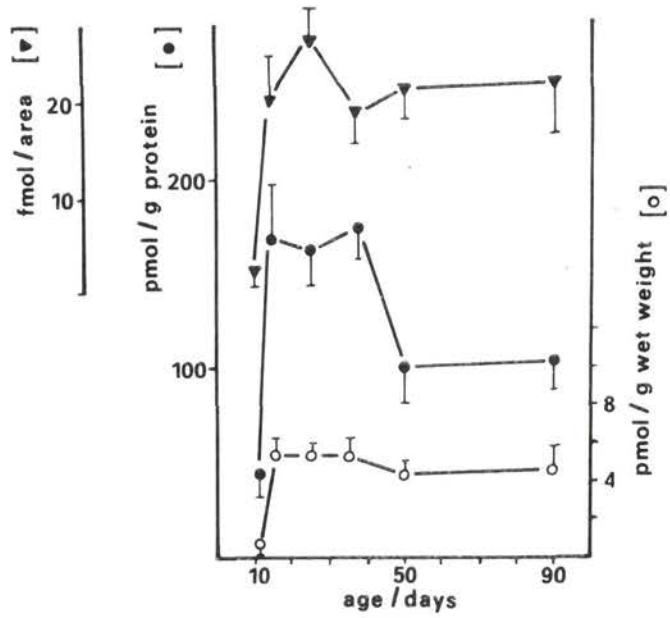

Fig. 1. Postnatal development of high-affinity ^{3}H-muscimol binding sites in the visual cortex of the rat. ^{3}H-muscimol binding data were expressed in terms of both pmol bound per g protein (●) and per g wet weight of tissue (○) as well as in terms of fmol bound per whole tissue region (▼). Points represent mean ± S.E.M. of 3 to 8 separate experiments each performed in duplicate. ^{3}H-muscimol concentration in the assay was 2 nmol/l. For details of the assay conditions, see MATERIALS AND METHODS.

The ontogenic profile of the GABAA receptor in FC, a non-visual area, looks rather similar (Fig. 2). Regardless of the reference system used, the highest binding was observed between PD 25 and PD 50, followed by a decrease by about 40 % until adulthood.

When binding data were related to the protein content, ^{3}H-muscimol binding in SC rises from PD 10 until PD 37 (by a factor of about two) reaching the highest binding level during the development (Fig. 3). From PD 37 to PD 50 binding decreases by about 54 % to reach the adult level. Whether expressed in terms of pmol/g wet weight or fmol per SC, the ontogeny of ^{3}H-muscimol binding follows a qualitatively similar pattern.

Regardless of the reference system used ^{3}H-muscimol binding in LGN rises

markedly from PD 10 until PD 37 followed by a slight decrease until adulthood (Fig. 4).

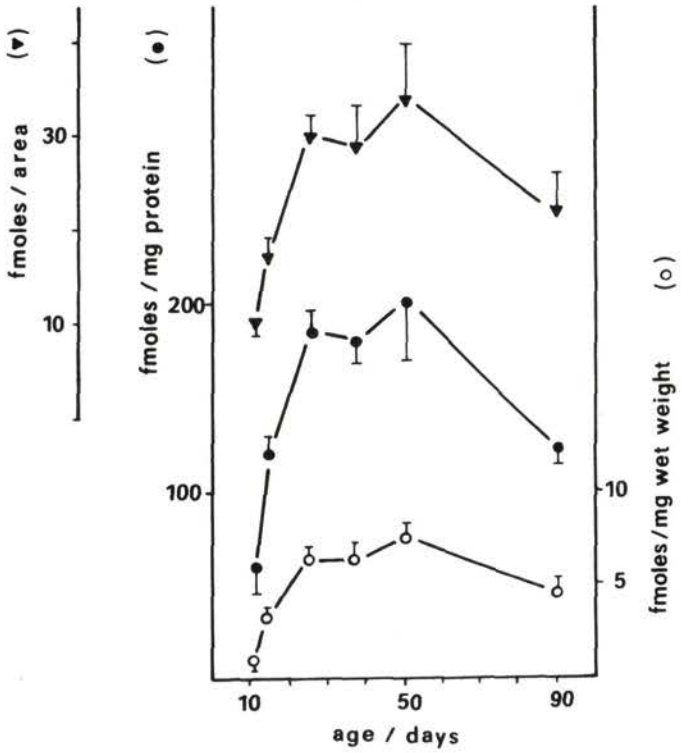

Fig. 2. Postnatal development of high-affinity ^{3}H-muscimol binding sites in the rat frontal cortex. For symbols, see legend to Fig. 1.

To elucidate whether the development of GABAA receptors in the visual structures needs adequate visual stimulation during the early postnatal life, unilateral eyelid suture was carried out on PD 11 just before the natural eyeopening. Monocular deprivation until PD 25 did not affect ^{3}H-muscimol binding in any of the central visual regions examined (Table 2).

Discussion

The main problem in describing developmental profiles of different constituents of small brain areas arises from the fact that the reference system itself undergoes certain developmental changes. Therefore, the binding data were expressed

both in terms of total binding (fmol bound per tissue region) and in terms of density (pmol bound per gram protein or per gram wet weight of tissue). Both these reference systems showed qualitatively similar developmental patterns.

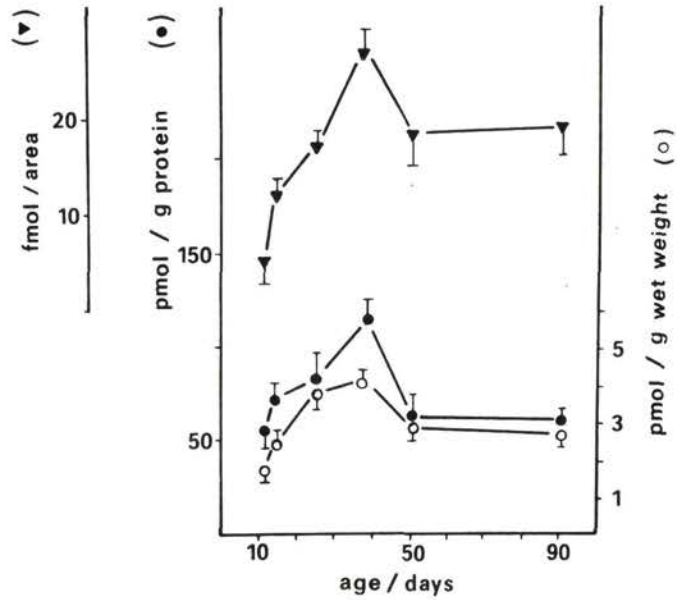

Fig. 3. Postnatal development of high-affinity ^{3}H-muscimol binding sites in the rat superior colliculus. For symbols, see legend to Fig. 1.

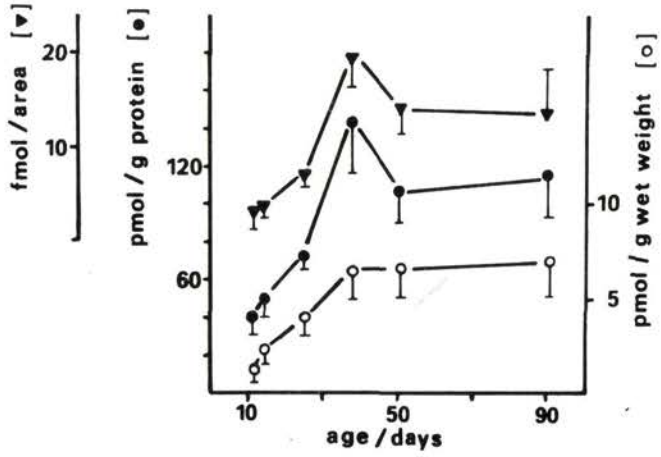

Fig. 4. Postnatal development of high-affinity ^{3}H-muscimol binding sites in the rat lateral geniculate nucleus. For symbols, see legend to Fig. 1.

Table 2. Effect of unilateral eyelid closure from postnatal day 11 until the age of 25 days on high-affinity ^{3}H-muscimol binding to GABAA receptors in the central visual structures of the rat brain

Region	Control	Monocular deprivation	
		contralateral to the closed eye	ipsilateral
	^{3}H-muscimol specifically bound (pmol/g prot.)		
Visual cortex	162 ± 14 (8)	154 ± 12 (8)	176 ± 20 (7)
Lateral geniculate nucleus	74 ± 7 (7)	77 ± 12 (7)	80 ± 10 (7)
Superior colliculus	84 ± 7 (6)	84 ± 7 (4)	81 ± 17 (4)

Means ± S.E.M. are shown. Figures in the parentheses indicate numbers of determinations. Each assay was run in duplicate using a constant ligand concentration of 2 nmol/l ^{3}H-muscimol in the assay. For details of the binding assay, see MATERIALS AND METHODS.

The present report analyzes the ontogenic development of ^{3}H-muscimol binding in the central visual structures assayed at a single ligand concentration (2 nmol/l). This kind of studies do not allow distinguishing between changes due to altered binding affinity and those due to changed receptor numbers. However, our data obtained from saturation experiments did not reveal any alteration in binding affinity during two selected age levels. This is in agreement with other studies which also showed that the affinities of GABA receptors do not change markedly with the age or region (Brooksbank et al. 1981; Coyle and Enna 1976; Patel et al. 1980; Shaw et al. 1984; Skerritt and Johnston 1982), suggesting that the age-related changes in ^{3}H-muscimol binding in the visual structures and FC reported herein may predominantly reflect changes in the number of receptor sites.

In both cortical regions, ^{3}H-muscimol binding reached the highest level between days 14 and 25, similarly as reported previously for rat cerebral cortex (Aldinio et al. 1980; Patel et al. 1980).

Regardless of small temporal variations in the developmental profiles, the ontogeny of ^{3}H-muscimol binding sites in the visual regions reported herein, does not essentially differ from that in other non-visual brain regions. This might indicate that the development of GABA binding sites in the visual system is not correlated with the development of retinal function and the functional maturation of the visual system with regard to the response to light stimuli. However, it is interesting to note that the development of GABA receptors in VC seems to precede that in the subcortical visual regions.

The slight decrease in binding after reaching the highest binding level in nearly all brain regions examined might support the view concerning selective

stabilization of developing synapses as a mechanism for the specification of neuronal networks (Changeux and Danchin 1976).

The temporal pattern of the development of ^{3}H-flunitrazepam binding to benzodiazepine receptors in rat SC correlates rather well with that of ^{3}H-muscimol binding in SC, whereas in VC age-related increase in ^{3}H-flunitrazepam binding is delayed compared to that of ^{3}H-muscimol binding (Schliebs et al. 1986b). In LGN, a continuous decrease of benzodiazepine binding expressed in terms of binding per protein content, was observed (Schliebs et al. 1986b). This contrasts with the development of ^{3}H-muscimol binding in this region. GABA receptors appear to be associated with benzodiazepine receptors in various parts of the brain (Tallman and Gallager 1985). A comparison of the temporal pattern of the ontogeny of benzodiazepine and GABA binding sites, however, does not evidence whether this is true also for the visual regions in the rat.

In the forebrain the development of GABA receptors was found to precede that of GAD activity (Coyle and Enna 1976), the latter being considered to be a marker of GABAergic presynaptic terminals. In rat LGN, GAD activity reached adult levels on PD 15, whereas in VC and SC the activity was not fully developed after 30 days (Kvale et al. 1983). In all the three regions the high-affinity uptake of GABA showed a marked peak on day 15; this contrasts with the development of GAD activity (Kvale et al. 1983).

In SC and VC, the development of ^{3}H-muscimol binding sites also seems to precede that of GAD activity. In contrast, the ontogeny of GABA binding sites in LGN is delayed compared to the development of GAD activity in this region, similarly as observed in the cerebellum (Brooksbank et al. 1981; Patel et al. 1980). Similar indications for the receptor formation not needing to be induced by functional synapse formation were found also in ontogenetic studies of other transmitter systems (Bylund 1979; Coyle and Yamamura 1976).

Monocular deprivation did not affect ^{3}H-muscimol binding in LGN, SC, and VC indicating that the development of GABA receptors in these brain regions is independent on adequate visual stimuli, at least until the age of 25 days. Similarly, ^{3}H-flunitrazepam binding to benzodiazepine receptors in the central visual regions of rat was not affected by monocular deprivation until PD 25 (Rothe et al. 1985). This compares well with a recent study showing that in cats monocular deprivation did not affect ^{3}H-muscimol binding in the visual cortex either (Mower et al. 1986).

Monocular deprivation led to permanent changes in β-adrenergic (Schliebs et al. 1982), serotoninergic (Aurich et al. 1985) and glutamatergic (Schliebs et al. 1986a; Schliebs et al. 1984) receptor binding, in particular in LGN. Obviously, the development of various neurotransmitter receptors in the visual regions is differently affected by inadequate visual input during the early life indicating

that the receptors are involved in different mechanisms of modulating visual information.

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