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ENHANCEMENT OF HIGH AFFINITY γ-AMINOBUTYRIC ACID RECEPTOR BINDING IN CEREBELLUM OF PYRIDOXINE-DEFICIENT RAT

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The high-affinity of [^{3}H]γ-aminobutyric acid (GABA) to $GABA_A$ receptors and [^{3}H]baclofen to $GABA_B$ receptors were studied in the cerebellum of pyridoxine-deficient rats and compared to pyridoxine-supplemented controls. There was a significant increase in the maximal binding (B_{max}) of both $GABA_A$ and $GABA_B$ receptors with no significant difference in their binding affinities (K_d). The changes observed suggest a supersensitivity of $GABA_A$ and $GABA_B$ receptors which seems to correlate negatively with the concentration of GABA in the cerebellum of pyridoxine-deficient rats.

γ-Aminobutyric acid (GABA) has been established as a post-synaptic inhibitory neurotransmitter in the central nervous system, particularly in the cerebellum [7, 16]. There are several pharmacologically and functionally distinct classes of GABA receptors in the mammalian CNS [8]. On the basis of sensitivity to inhibition by bicuculline, two categories of GABA receptors ($GABA_A$ and $GABA_B$) have been recognized [3, 9]. $GABA_A$ which is labelled with agonists like [^{3}H]GABA and antagonists like bicuculline is a Na^+-independent component of the recognition site of GABA-receptor complex. $GABA_B$ seems to be involved in the modulation of the release of other neurotransmitters [9]. Changes in the kinetic parameters, maximal binding (B_{max}) and binding affinity (K_d), of several neurotransmitters in various disease states [1, 20] as well as during dietary restriction [18] have been reported.

Experimental conditions that increase intrasynaptic neurotransmitter concentration decrease post-synaptic receptor sensitivity and conversely experimental conditions that decrease neurotransmitter concentration lead to enhanced post-synaptic sensitivity [2]. We have reported earlier [24] that GABA concentration in the brain of the pyridoxine-deficient rat is decreased significantly compared to pyridoxine-supplemented controls. In the present study the effect of such chronic deficiency of GABA on $GABA_A$ and $GABA_B$ receptor sensitivity in the cerebellum of rat brain has been investigated.

Sperm-positive Sprague–Dawley rats were housed individually and fed a pyridoxine-supplemented diet containing 100 mg pyridoxine/kg of diet for the period of gestation. After delivery the dams were divided into two groups, one was transferred to the pyridoxine deficient diet [6] and the other was continued on the pyridoxine-supplemented diet. The number of pups per litter was adjusted so that the dams on the pyridoxine-deficient diet had 8 pups per litter and the dams on the pyridoxine-supplemented diet had 16 pups per litter. Thus, the control pups were subjected to a generalized malnutrition. Pups from both the groups were killed when they were 21 days old. The brain regions were dissected according to Glowinski and Iversen [11]. Tissues were frozen immediately in dry ice and stored at −70°C. Cerebellum was used for measuring pyridoxal phosphate, GABA and the kinetic parameters of $GABA_A$ and $GABA_B$ receptors. Pyridoxal phosphate was determined using tyrosine apodecarboxylase, as described previously [6]. GABA content was determined using the radioreceptor assay of Enna and Snyder [10]. [^{3}H]GABA binding to the GABA receptor was assayed in Triton X-100 treated synaptic membranes according to the method of Kurioka et al. [17]. [^{3}H]baclofen binding to $GABA_B$ receptor in synaptic membrane preparations was assayed according to Hill and Bowery [14] and Bowery et al. [4]. The specific binding data for [^{3}H]GABA and [^{3}H]baclofen were analyzed according to Scatchard [22] from which the binding parameters, maximal binding (B_{max}) and dissociation constant (K_d) were derived by linear regression analysis. The data were analyzed statistically by analysis of variance and two-tailed *t*-test. Protein was measured according to Lowry et al. [19]. DNA was estimated according to Schneider [23].

Earlier findings [24] and the present observations clearly show a significant decrease in body and brain weights of the pyridoxine-deficient rats compared to pyridoxine-supplemented controls: body weight: controls, 40 g ± 3.5 g; pyridoxine-deficient, 27 ± 1.1 g, $P<0.001$; brain weight: controls, 1.57 ± 0.02 g; pyridoxine deficient, 1.30 ± 0.02 g, $P<0.001$. The concentrations of pyridoxal phosphate and GABA were reduced significantly in the cerebellum of the deficient rats (Table I). Similar decreases in pyridoxal phosphate content of other brain regions like cerebral cortex (controls, 925 ± 39 ng; pyridoxine deficient, 425 ± 23 ng, $P<0.001$) and cor-

TABLE I

PYRIDOXAL PHOSPHATE AND γ-AMINOBUTYRIC ACID (GABA) CONCENTRATIONS IN THE CEREBELLUM OF 3-WEEK-OLD RAT

*$P<0.01$; **$P<0.001$ with respect to control.

Experimental group	Pyridoxal phosphate[a] (ng/g wet wt.)	GABA[a] (μmol/g wet wt.)
Control	1056 ± 54	2.62 ± 0.42
Pyridoxine-deficient	568** ± 72	1.15* ± 0.32

[a]Mean ± S.E.M. were determined from 6–8 separate experiments each assayed in triplicate.

pus striatum (controls, 860 ± 88 ng; pyridoxine deficient; 247 ± 25 ng, $P < 0.001$) were seen in deficient rats. There were no significant differences between pyridoxine-deficient and control groups in the concentrations of total protein or DNA in the cerebellum. The choice of cerebellum as the tissue for our study was based on the finding that this region contains at least four cell types which utilize GABA as an inhibitory transmitter [7].

Kinetics of [^{3}H]GABA binding to bicuculline-sensitive $GABA_A$ receptors reveal both high and low affinity binding sites. GABA agonists have a higher affinity for the slowly dissociating high-affinity $GABA_A$ sites whereas bicuculline has a higher affinity for rapidly dissociating low-affinity sites [21]. We have investigated the high affinity binding site using [^{3}H]GABA as the ligand. A significant increase in the B_{max} of [^{3}H]GABA binding (Fig. 1 and Table II) to synaptic membrane preparations from pyridoxine-deficient rat cerebellum, as compared to controls, was seen. There

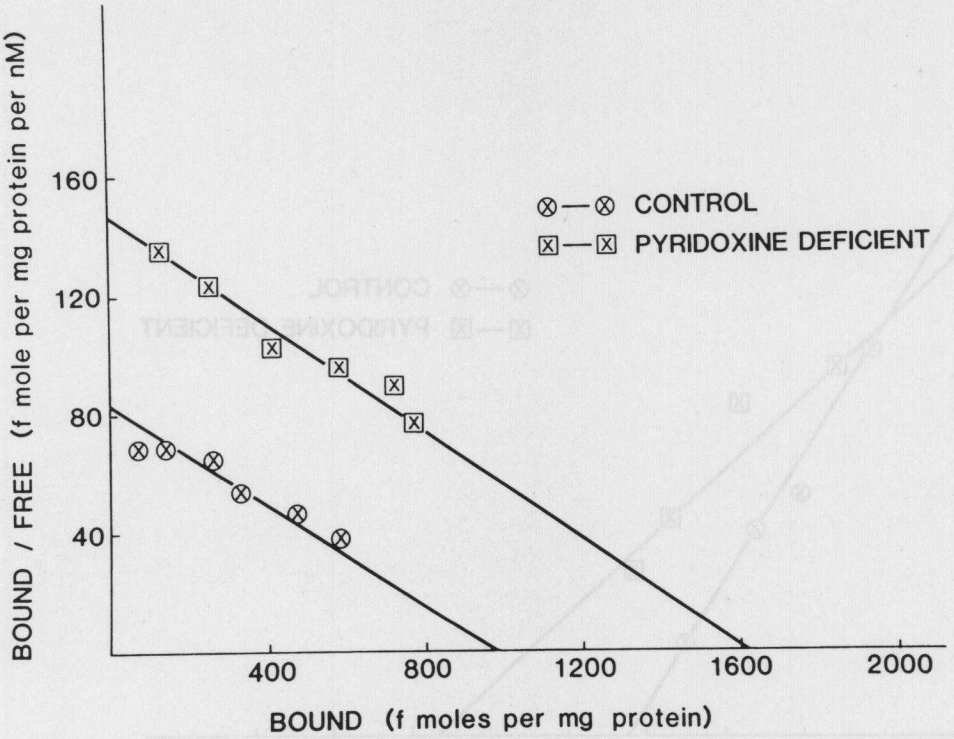

Fig. 1. Scatchard analysis of [^{3}H]GABA binding in crude synaptic membrane preparations from the cerebellum of 21-day-old rat. Crude synaptic membrane was prepared using sodium-free 10 mM Tris buffer (pH 7.4) and 0.3–0.4 mg protein was used in each assay. The incubation mixture containing 1–10 nM [^{3}H]GABA with and without excess of unlabelled GABA (100 μM) was incubated for 20 min at 0–5°C. The binding reaction was terminated by centrifugation at 35,000 *g* for 20 min. [^{3}H]GABA in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting nonspecific binding from total binding.

TABLE II

[^{3}H]γ-AMINOBUTYRIC ACID (GABA) AND [^{3}H]BACLOFEN BINDING IN THE CEREBELLUM OF 3-WEEK-OLD RAT

**$P<0.001$ with respect to control.

Experimental group	[^{3}H]GABA binding[a]		[^{3}H]Baclofen binding[a]	
	B_{max} (fmol/mg protein)	K_d (nM)	B_{max} (pmol/mg protein)	K_d (nM)
Control	961 ± 58	10.01 ± 0.63	2.18 ± 0.13	66.67 ± 7.74
Pyridoxine-deficient	1728** ± 62	10.82 ± 0.83	3.52** ± 0.20	73.73 ± 9.25

[a]Mean ± S.E.M. were determined from 6–8 separate experiments each assayed in triplicate.

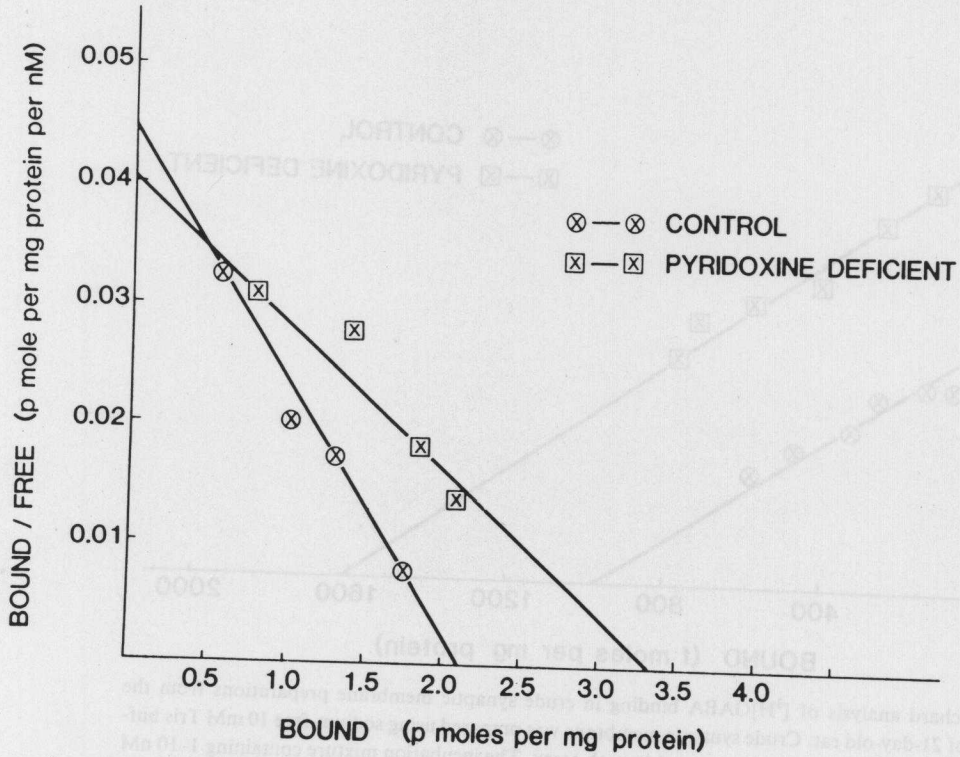

Fig. 2. Scatchard analysis of [^{3}H]baclofen binding in crude synaptic membrane preparations from the cerebellum of 21-day-old rat. Crude synaptic membrane preparation was suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 2.5 mM $CaCl_2$ and 0.3–0.4 mg protein was used in each assay. The incubation mixture containing 10–100 nM [^{3}H]baclofen with and without excess of unlabelled baclofen (100 μM) was incubated for 10 min at 20°C. The binding reaction was terminated by centrifugation at 7000 *g* for 10 min. [^{3}H]baclofen in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting nonspecific binding from total binding.

was no significant difference in the K_d values between these two groups. The bicuculline-insensitive presynaptic $GABA_B$ receptors localized on non-GABAergic terminals are involved in neurotransmitter release mechanisms [15]. These binding sites are most highly concentrated in the rat brain cerebellum [26]. We have, again, studied only the high affinity $GABA_B$ site. The results (Fig. 2 and Table II) indicate a significant increase in the B_{max} of [^{3}H]baclofen binding to synaptic membrane preparations from the deficient rat cerebellum. There were no significant differences in the K_d values between the two groups.

We have earlier established the validity of using chronically malnourished but pyridoxine-sufficient rats as the control group to study the effects of specific pyridoxine deficiency [5]. The binding data for the ad libitum fed control rats were comparable to those obtained for the control, malnourished but pyridoxine-sufficient rats. Thus, in the deficient group cerebellar GABA content correlates with the pyridoxal phosphate content. The physiological implication of the chronic decrease in the cerebellar GABA is the increase in sensitivity of the post-synaptic membrane receptors to this neurotransmitter. Our observations are restricted to the high-affinity receptor types. Supersensitivity to dopaminergic or non-adrenergic agonists is seen under conditions like inhibition of catecholamine synthesis, chemical denervation, depletion of catecholamine stores and blockade of post-synaptic receptors [12]. The increased GABA receptor binding in post-mortem brain tissue of chronic alcoholics has been linked to the chronically reduced levels of brain GABA in these patients [25]. The results presented here indicate the basis for the impairment of the GABA pathway in the pyridoxine-deficient rat cerebellum.

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