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Alterations of $GABA_B$ binding caused by acute and chronic lead administration

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Short communication

Abstract. The effect of lead on $GABA_B$ binding was studied on membranes obtained from acute and chronically lead-treated rats. Acute lead treatment reduced both $GABA_B$ affinity (K_D) by about 30% and density of receptor (B_{max}) by about 15%. On the contrary chronic lead treatment increased receptor capacity by about 20% in spite of decreased receptor affinity by 25%. Both acute and chronic lead treatment shiffted displacement curves toward higher concentration of non-labelled compound(decreased affinity). The results show that lead can affect the $GABA_B$ binding in two ways: by reducing affinity of binding and by altering capacity of binding.

Key words: GABA , gamma-aminobutyric acid, K_D, affinity of receptor, B_{max} , density of receptor

Lead ions are known to be toxic to the nervous system of man and animal (Davis et al. 1993). Both acute and chronic exposures to lead result in behavioural and neurological symptomes. In lead neurotoxicology an important issue relates to how lead interferes with chemical neurotransmission. Numerous investigations have been undertaken in order to explain the possible molecular mechanism of lead toxicity (Silbergeld 1982, Tschudy and Lamon 1990, Zareba and Chmielnicka 1992, Guilarte 1993, Jabońska et al. 1994). It has been postulated that Pb/Ca interactions may play an important role in the process (Silbergeld et al. 1974, Kolton and Yassi 1982).

Gamma-aminobutyric acid (GABA) is known as one of inhibitory neurotransmitters in the mammalian brain that induces the hyperpolarization of membrane potential by activating GABA receptor coupled with chloride ion channel (Olsen 1981). The observation that some effects of GABA in the CNS are not antagonized by the GABA receptor antagonist bicuculline (Hill and Bowery 1981), has led to the discovery of the $GABA_B$ class of GABA receptor, which is insensitive to bicuculline but sensitive to (-)-baclofen(Hill and Bowery 1981). It has been reported that the activation of $GABA_B$ receptor causes reduction of evoked transmitter release (Bowery et al. 1984).

Activation of $GABA_B$ receptors by baclofen or GABA increases membrane K^+ conductance post-synaptically and decreases Ca^{2+} conductance presynaptically to depress transmitter release; these receptors are directly coupled to the K^+ and Ca^{2+} channels through Gi/Go proteins and are modulated by guaninenucleotides (Bowery 1993).

The aim of this work was to assess the sensitivity of Ca-dependent GABA binding to lead toxicity,especially its influence on affinity and capacity of $GABA_B$ binding. As shown previously, Pb^{2+} level in synaptosomal fraction (obtained from P_2 fraction) was more than twice higher than in control fraction (Strużyńska and Rafałowska 1994). Membranes for $GABA_B$ binding were obtained from crude P_2 fraction.

The study was performed on male Wistar rats. Two models of poisoning were used:

1. In the acute model 15 mg $Pb(CH_3COO)_2$/kg b.w. was injected intraperitoneally for 7 days into rats weighting 150 g. The control animals were injected with distilled water. The animals were decapitated and brains were removed.

2. In the chronic model of toxicity , lead acetate (200 mg/l H_2O) was given to 3-week old rats in drinking water for 3 months. Control animals received distilled water without $Pb(CH_3COO)_2$. The mechanism of the gamma-aminobutyric acid receptor ($GABA_B$) binding was examined using crude membrane fraction (P_2) obtained from the rat brain according to Zukin et al. (1974). For the assay pellets (P_2 fraction) which had been frozen for at least 16 h at - 15° C were allowed to thow for 20 min at room temperature before resuspension in Tris-HCl buffer (50 mM pH 7,4) plus $CaCl_2$ (2.5 mM).

The pellet obtained from the equivalent of one rat brain was resuspended in 10 ml Tris-HCl. The suspension was incubated for 45 min at 20°C before centrifugation at 7,000 g for 10 min. This working procedure was repeated three more times allowing 15 min incubation with addition of Tris-HCl plus $CaCl_2$ each time. The final pellet was resuspended in Tris-HCl buffer+$CaCl_2$ (~1 mg protein per 0.8 ml buffer plus 40 µM isoguvacine for the assay. Protein concentrations were determined by the method of Lowry et al. (1951). To each 0.8 ml aliquot of membrane suspension 0.1 ml Tris-HCl plus $CaCl_2$ containing various concentrations of unlabelled GABA and 0.1 ml Tris-HCl containing a fixed concentration(1-3 nM) of [^{3}H]-GABA was added. The range of final concentrations of unlabelled GABA was 1 nM-100 µM. Nonspecific binding was determined by adding 100 µM baclofen. The mixture was incubated for 15 min at 20°C. Each incubation was terminated by filtration under vacuum through Whatman GF/B glass filter and then the filter was rinsed twice with 5 ml of ice-cold distilled water according to Ohmori et al. (1990). The radioactivity trapped on each filter was measured by a liquid scintillation spectrometer at a counting efficiency of 40%.

Acute lead treatment reduced $GABA_B$ receptor affinity (K_D) by about 30% and density of receptor (B_{max}) by about 15% (Table I).

TABLE I

Kinetic parameters for [^{3}H] GABA binding to $GABA_B$ receptors after acute lead treatment

Group	K_D(nM)	B_{max}(fmol/mg protein)
control	105±11	952±93
lead treated	136±14	707±79

The values represent mean ± SEM from 4 experiments; $P<0.05$ (Student's t test).

TABLE II

Kinetic parameters for[^{3}H] GABA binding to the $GABA_B$ receptors after chronic lead treatment

Group	K_D(nM)	B_{max}(fmol/mg protein)
control	99±11	984±91
lead treated	126±12	1210±111

The results represent mean ± SEM from 4 experiments; $P<0,05$ (Student's t test).

Chronic lead treatment decreased receptor affinity (K_D) by about 25% but increases receptor capacity (B_{max}) by about 20% (Table II).

There was no significant difference between receptor affinity in acute and chronic lead treated animals . Decreased receptor affinity in both models of intoxication is illustrated by ligand displacement curves (Fig. 1. A and B), which they shiffted toward higher concentrations of non-labelled compound in both acute and chronic lead-treated material (decreased receptor affinity K_D).

The mechanism through which lead affects behaviour and neurochemistry is unknown. It has been hypothesized that Pb^{2+} and Ca^{2+} may competitively interact at the sites involved in binding of GABA. Two hypotheses are proposed to explain these interactions: (1) competition with [Ca^{2+}] in membrane Ca channels , which would inhibit the depolarization- induced influx of [Ca^{2+}]; and/or (2) on intracellular [Ca^{2+}] rise caused by inhibition of mitochondrial [Ca^{2+}] fluxes (Silbergeld 1983). Our investigation on membrane fractions obtained from acute and chronic lead treatment rats confirm the latter hypothesis. Pb^{2+} treatment of rats was associated with decrease affinity of GABA, binding and changes of GABA receptor density. As previously shown, chronic treatment of rat results in the inhibition of KCl dependent GABA release (Strużyńska

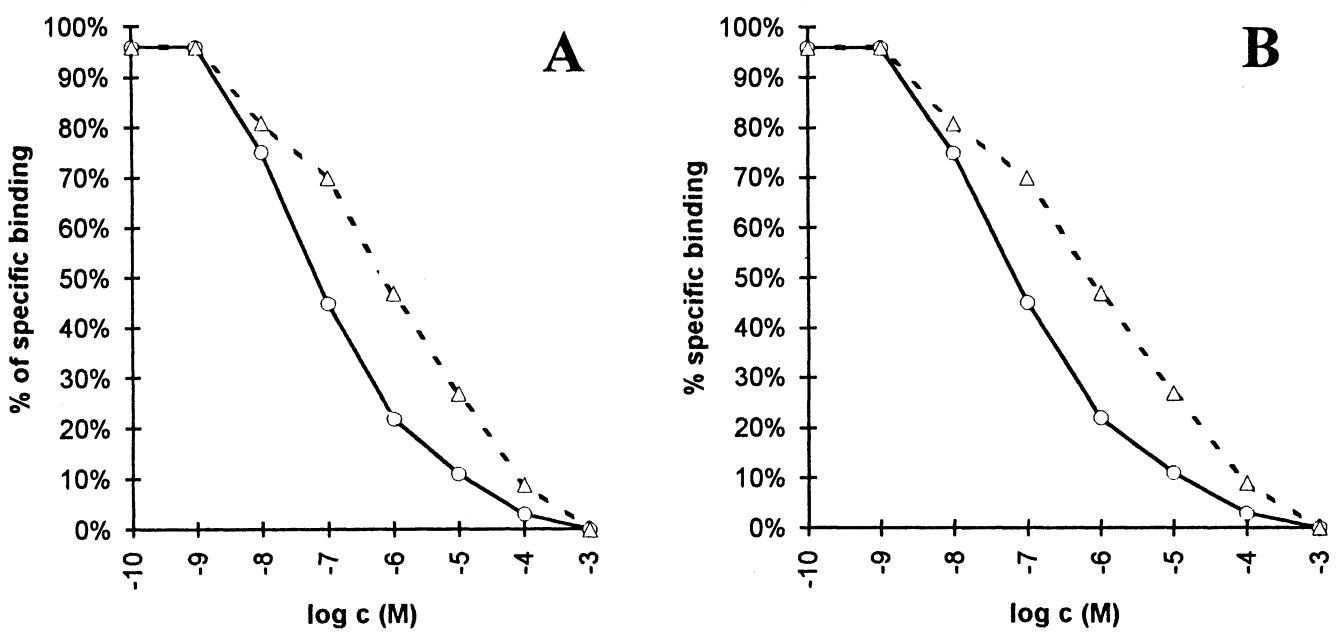

Fig. 1. Inhibition of 3 H-GABA binding by non-labelled GABA after (A)-acute and (B)-chronic treatment of lead . Control-(circles) ; Pb-treted-(triangles). The results are means of five experiments; the SEM was less than 5%.

and Rafałowska 1994). Our results are in agreement with these findings; lead inhibits GABA release and GABA affinity.

Decreased GABA receptor density in calcium dependent GABA binding in acute lead treated animals could be interpreted to manifest a decrease in number of the synapses and would be consistent with histological observations of brain tissue from lead treated animals (Krigman et al. 1974).

The variability in binding to lead treated membranes (changed receptor density) may result from the relative immaturity of this structure. Changes in chronic lead treated animals might be the consequence of changed protein synthesis which has been reported in both chronic and acute (Kennedy et al. 1983) lead studies and is consistent with the suggestion that a reduction in synapse number could result from a direct effect of lead on protein synthesis in chronically treated animals. Depression of protein synthesis and/or effects on lipid production (Krigman et al. 1974) by lead may also explain the decreased receptor affinity shown in acute and chronic lead treatment at calcium-dependent binding, which could result from increased production of endogennous receptor inhibitor rather than structural changes (Johnston 1981). The fact that different effects are observed in different types of neurones and at different times may reflect local differences in the concentration of free lead cations at critical developmental periods resulting from varying local pH and phosphate concentrations (Spence et al. 1985) in the different regions of brain. The solubility of lead complexes is dependent on pH and increases as pH declines (Bradbury 1979).

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