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Changes in γ-aminobutyric acid during different stages of picrotoxin-induced seizure, and the effect of pretreatment with γ-acetylenic GABA and phenobarbital

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Abstract. Changes in GABA content of various brain areas during different stages of picrotoxin-induced seizures and following pretreatment with the anti-convulsants phenobarbital and γ-acetylenic GABA were studied. Picrotoxin (6mg/kg) produced clonic/tonic convulsions associated with a 34% reduction in GABA content of the sensory motor cortex. A reduction of 24% was observed 1 min before the onset of seizure and the reduction in GABA content was reversible 20 min after the convulsion. No significant changes were observed in the cerebellum or spinal cord/medulla oblongata. Pretreatment with phenobarbital (100mg/kg) delayed the onset of convulsion and decreased the mortality rate without causing any change in GABA content at the pre-convulsive, convulsive or post-convulsive stages. γ-Acetylenic GABA (100mg/kg) has elevated GABA levels in different areas of the brain by 2-3-fold after 60 min treatment. This increase was reduced by 44% during the onset of picrotoxin-induced seizures. Picrotoxin convulsion can occur in the presence of normal, reduced or even elevated brain GABA content. The only consistent factor is a one-third reduction in GABA content before the onset of seizure.

Keywords. GABA; γ-acetylenic GABA; picrotoxin; phenobarbital.

Introduction

It is now generally accepted that γ-aminobutyric acid (GABA) is one of the main inhibitory transmitters in the mammalian central nervous system (Curtis and Johnston, 1974; Krnjevic, 1974) and to some extent there is a correlation between brain GABA content and its neuronal activity. Thus drugs which reduce brain GABA content are often convulsants (Killam and Bain, 1957; Bradford, 1976). Cases of reduced GABA content were reported after afferent electrical stimulation of the brachial plexus (Lichtshtein and Dobkin, 1976), high pressure oxygen induced convulsions (Wood *et al.,* 1967; De-Feudis and Elliot, 1968), thiosemicarbazide-induced seizures (Wood *et al.,* 1966) and following picrotoxin-induced seizures (Abdul-Ghani, 1980).

On the other hand, all inhibitors of GABA transaminase, which elevates brain GABA content, have anticonvulsive properties (Collins, 1973; Jung *et al.,* 1977; Schechter *et al.,* 1979; Loscher and Vetter, 1984). Augmentation of GABA transmission could also be brought about by blocking reuptake into presynaptic membranes and glia (Krogsgaard-Larsen *et al.,* 1981; Schousboe *et al.,* 1983; Wood and Geddes, 1983). Previous results in our laboratory have shown the protective effect of γ-acetylenic GABA and phenobarbital against picrotoxin-induced convulsion (Abdul-Ghani *et al.,* 1987).

The aim of the present investigation is to test the pattern of change in GABA content of different areas of the brain during the development of picrotoxin convulsion, and the effect of pretreatment with phenobarbital and γ-acetylenic GABA.

Materials and methods

Materials

γ-Acetylenic GABA [4-amino hex-5-ynoic acid, RMI (71645)] was a gift of Dr J. Wilkins and his colleagues at the Centre de Recherche Merrell International, Strasbourg, France.

Picrotoxin, phenobarbital and gabase were purchased from the Sigma Chemical Co., St Louis, Missouri, USA.

Methods

White female albino mice weighing 25–30 g were used in the experiments. The animals were allowed free access to food and water before treatment. The mice were distributed into 3 groups and were injected intraperitoneally either with phenobarbital (100 mg/kg) or with γ-acetylenic GABA (100mg/kg) 45–60 min before injection of the convulsant Picrotoxin (6mg/kg). Control animals were pretreated with the same volume of 0·9% (w/v) NaCl.

After injection of Picrotoxin the mice were under constant observation for measuring the time to onset of convulsion and the mortality rate. At different pre-convulsive, convulsive and post-convulsive stages, animals were stunned and killed by cervical dislocation, and the brains were rapidly removed and frozen on dry ice. The brains were dissected into 3 main areas, sensory motor cortex, cerebellum and spinal cord/medulla oblongata combined. Each part was quickly weighed and dropped into ice-cold trichloroacetic acid 10% for homogenization and centrifugation. The supernatants were stored frozen (–70°C) until assay for GABA content.

GABA content was measured using the enzymatic method. Gabase (Sigma) contains both GABAtransaminase (5-aminobutyrate; 2-oxoglutarate aminotransferase; EC2·6·1·19) and succinate semialdehyde dehydrogenase [succinate semialdehyde: NAD (P) oxidoreductase; EC 1·2·1·16]. The reaction mixture consisted of 0·1 M potassium pyrophosphate pH 8·6, 0·004 M NADP pH 7, 0·02 M α-ketoglutarate solution pH 7·9 and 0·2 units/ml gabase. Extract equivalent to 25 mg of brain tissue was added to 3 ml of reaction mixture.

Concentrations of GABA are expressed in this paper as μmol per gram of fresh tissue. Where required, statistical analysis was performed using Student's '*t*' test.

Results

Convulsive action of Picrotoxin

Injection of mice with Picrotoxin (6 mg/kg) produced clonic convulsions after a latent period of 8–11 min. These convulsive movements gradually increased and ended in a tonic extensor spasm of the 4 limbs after 12–15 min. Most of the animals died in this phase, and the remainder lay in a flaccid state until the second phase began—a clonic seizure less violent, less severe and of shorter duration. After 3–6 phases of clonic convulsion the convulsive activity vanished.

We have found a relatively large decrease of 20% in GABA brain content associated with picrotoxin-induced seizure. At the onset of convulsion the concentration of GABA in the sensory motor cortex was significantly decreased (by 34%, from 1·4±0·06 µmol/g tissue to 1·09±0·10 μmol/g tissue, $P<0·01$). No significant changes were observed in the cerebellum or spinal cord/medulla oblongata (table 1).

Table 1. Distribution of GABA in different areas of the brain following treatment with Picrotoxin, phenobarbital and γ-acetylenic GABA.

	Sensory motor cortex	Cerebellum	Spinal cord/ medulla oblongata
Control	1·64 ± 0·06 (20)	1·64 ± 0·13 (10)	1·66 ± 0·16 (10)
Picrotoxin convulsion	1·09 ± 0·10* (5)	1·35 ± 0·09 (5)	1·51 ± 0·16 (5)
Phenobarbital	1·59 ± 0·08 (11)	1·60 ± 0·06 (6)	1·65 ± 0·13 (6)
γ-Acetylenic GABA	3·59 ± 0·31** (18)	4·40 ± 0·35** (6)	3·10 ± 0·23* (6)

GABA content was measured in different areas of the brain of mice, following i.p. injection of Picrotoxin (6 mg/kg), at the onset of seizure, or 60 min after the injection of phenobarbital (100 mg/kg) or γ-acetylenic GABA (l00mg/kg). The values are mean±SEM for the number of experiments indicated in parentheses. GABA content expressed as μmol/g tissue.
*Significance of difference: $P<0·01$; **$P<0·001$, compared to control values.

One min before the onset of convulsion GABA level was reduced significantly (by 24%, to 1·25±0·11 μmol/g, $P<0·025$). In 6 animals which recovered from convulsion GABA content of the sensory motor cortex increased to 1·45±0·08 μmol/g 20 min after the onset of seizure (table 2).

Table 2. Effect of Picrotoxin on GABA content of sensory motor cortex of mice pretreated with phenobarbital and γ-acetylenic GABA.

	Pretreatment		
	Saline	γ-Acetylenic GABA	Phenobarbital
Control	1·64 ± 0·06 (20)	3·59 ± 0·31 (18)	1·59 ± 0·08 (11)
	100%	100%	100%
Pre-convulsive stage	1·25 ± 0·11 (8)	2·19 ± 0·14 (9)	1·46 ± 0·05 (5)
	76%	61%	92%
	$P<0·025$	$P<0·005$	NS
Convulsive stage	1·09 ± 0·10 (5)	2·01 ± 0·11 (10)	1·54 ± 0·04 (8)
	66%	56%	97%
	$P<0·01$	$P<0·005$	NS
Post-convulsive stage	1·45 ± 0·08 (6)	2·85 ± 0·32 (6)	1·58 ± 0·06 (7)
	88%	79%	99%
	NS	NS	NS

The values are mean ± SEM for the number of experiments indicated in parentheses, GABA content expressed as μmol/g tissue.
NS, Not significant.

Anti-convulsive action of phenobarbital

Although phenobarbital (100 mg/kg) delayed the onset of Picrotoxin convulsion, decreased the mortality rate, and in 6 cases out of 17 prevented the convulsion, it

did not change GABA content of the whole brain or of any of the tested areas. Similar results were observed during pre-convulsive, convulsive and post-convulsive stages (table 2).

Anti-convulsive action of γ-acetylenic GABA

γ-Acetylenic GABA (100 mg/kg) increased GABA levels in the whole brain by 2-3-fold. The maximum effects was seen in the cortical area; the effect was slightly lower in the spinal cord/medulla oblongata (table 1).

Intraperitoneal injection of picrotoxin (6 mg/kg) to mice pretreated 45 min earlier with γ-acetylenic GABA produced a maximum reduction in GABA content of 44% ($P<0{\cdot}005$) at the onset of seizure (table 2). One min before the onset of convulsion GABA content was clearly decreased by 39% and in the post-convulsive stage the effect was partially reversed (~20%, not significant).

Discussion

Picrotoxin, a potent convulsant which acts by blocking the inhibitory synaptic action of GABA, has been reported to produce a strong clonic/tonic convulsion with a high mortality rate and a significant reduction in GABA content of brain at the onset of convulsion (Loscher and Frey, 1977; Abdul-Ghani *et al.,* 1987).

The fact that the reduction in brain GABA content was localized to the cortical area, with no significant decrease in GABA content of cerebellum or spinal cord/ medulla oblongata (table 2), is of special interest since picrotoxin acts as an antagonist of GABA inhibitory action in different areas of the nervous system including brain stem (Straughan, 1974), spinal cord (Engberg and Thaller, 1970), cerebral cortex (Hill *et al.,* 1972) and cerebellar cortex (Bisti *et al.,* 1971).

The present report shows that γ-acetylenic GABA produced an increase in GABA content of 2–3-fold; the amount of increase differing among various brain regions, with the greatest change in cortical areas. The picrotoxin convulsion occurred in mice pretreated with γ-acetylenic GABA, even with relatively high levels of GABA in these animals. However, the convulsions occurred after a 44% decrease in GABA content in the pretreated animals. The decrease in GABA content was seen before the onset of convulsion, and was partially reversed in the post-convulsive stage. These results would explain the slight anti-convulsant properties of γ-acetylenic GABA with regard to picrotoxin seizures. Similar anti-convulsive activity was found against strychnine- and thiosemicarbazide-induced seizures (Schechter *et al.,* 1977).

Although phenobarbital has shown the best anti-convulsive activity (Abdul-Ghani *et al.,* 1987) it had no effect on brain GABA content regardless of the area tested. This was true in pre-convulsive, convulsive and post-convulsive stages (table 2). Previous results have shown no correlation between the anti-convulsive activity of phenobarbital and changes in extracellular GABA accumulation (Iversen and Johnston, 1971; Abdul-Ghani *et al.,* 1981). It is more likely that the anti-convulsive action of phenobarbital is due to its effect on the GABA receptor complex.

From the present study it is very clear that there is no simple relationship between whole brain GABA levels and the excitable state of the brain, since picrotoxin convulsion can occur in normal, reduced or even elevated brain GABA

Concentrations. The only consistent factor is a one-third reduction in GABA content before the onset of seizure. This becomes important in the light of the fact that the neuronal pool of GABA is approximately one-third of the total brain GABA content (Patel *et al.,* 1974; Sellstrom *et al.,* 1975).

On the other hand the protective action of anti-convulsants is produced by those which raise seizure threshold, either by elevating brain GABA level or by reducing brain aspartate or glutamate levels. Other investigators have shown that correlation does exist between the increase in synaptosomal GABA levels and the delay in the onset of seizures (Wood and Geddes, 1983).

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