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CHANGES IN BRAIN γ-AMINOBUTYRIC ACID CONCENTRATIONS FOLLOWING ACUTE AND CHRONIC AMPHETAMINE ADMINISTRATION AND DURING POST AMPHETAMINE DEPRESSION

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Abstract—The acute administration of *d*-amphetamine caused an increase in the γ-aminobutyric acid (GABA) content of the brain stem and thalamus. Acute chloropromazine treatment lowered the GABA concentration in the thalamus, olfactory lobes and brain stem. Although this neuroleptic antagonized the stereotypy induced by amphetamine, drug combination markedly increased the GABA content of all brain regions studied. Following the chronic administration of amphetamine for 2 weeks, the GABA content of the striatum and thalamus decreased even though the stereotyped behaviour was more marked than that occurring after acute amphetamine administration. During the period of post amphetamine depression, the GABA content of the striatum and brain stem were also decreased whereas that of the amygdala was increased. These studies suggest that the changes in the GABA content do not reflect changes in gross behaviour following amphetamine administration. Nevertheless, they do suggest that the inhibitory amino acid may play an ancillary role in determining the neuropharmacological profile of amphetamine.

Although the physiological role of γ-aminobutyric acid (GABA) as a neurotransmitter is not entirely accepted, there is mounting evidence to support the view that it is an inhibitory transmitter in the mammalian brain [1]. The highest concentrations of this amino acid are found in the globus pallidus, substantia nigra and hypothalamus of several mammalian species [2–4], and its possible role as an inhibitory neurotransmitter suggests that the release of dopamine in the striatum is regulated by an inhibitory feedback mechanism which originates in the caudate nucleus and terminates at GABA coded synapses on dopaminergic neurons in the substantia nigra [5, 6]. Such a view is further substantiated by the observation that the injection of GABA into the substantia nigra induces an increase in the concentrations of striatal dopamine [7].

It is well established that *d*-amphetamine releases dopamine from central synapses [8]. The stereotypy which occurs in rats after a high acute dose, or after administration of lower doses for several days, may result from an increased stimulation of dopamine receptors in the striatum [9, 10]. The possibility therefore arises that some of the neuropharmacological effects of *d*-amphetamine are partly attributable to changes in GABA metabolism. The aim of this study therefore was to study the effects of acute and chronic amphetamine administration of GABA in those brain regions in which changes in brain monoamines are known to occur. The study was extended to follow the changes in the GABA concentration during the period of post amphetamine withdrawal and following the acute administration of chlorpromazine to rats which had received an acute dose of amphetamine. In this way it might be possible to see if a causal or a coincidental relationship existed between the gross behaviour of the animals following pharmacological manipulation and the changes in the GABA concentration.

MATERIALS AND METHODS

Male Wistar rats (130–150 g) were used. They were randomly divided into groups of six with free access to food and fluid for the duration of the experiment. In the first experiment, changes in GABA concentration were assessed following the acute administration of amphetamine given either alone or together with chlorpromazine. In the second experiment, the chronic effects of amphetamine were assessed.

Acute study

All animals were i.p. injected. One group received 10 mg/kg of chlorpromazine, a second 5 mg/kg of *d*-amphetamine sulphate and the control group were injected with an equal volume of physiological saline. The final group was injected with chlorpromazine followed after 15 min by amphetamine.

One hour after drug administration the animals were killed by subjection to microwave radiation (2.5 kW for 5 sec). The skulls were opened and the brains removed immediately and by freehand dissection separated into the brain-stem, striatum, thalamus, olfactory lobes and amygdala, according to the method of Popov *et al.* [11]. Following the dissection procedure the brain areas were weighed and homogenised in a mixture of 0.01 N HCl and 0.4 N $HClO_4$, 50:50 (v/v) containing 0.1% EDTA. The samples were centrifuged. 0.1 ml of the clear supernatant was removed for the fluorimetric determination of GABA [12]. The samples were stored for a maximum period of 3 days at −20° before GABA was estimated.

Chronic study

Five groups of rats were given *d*-amphetamine in their drinking water. An equal weight of ascorbic acid was added to serve as an antioxidant. The control groups were given only ascorbic acid in their drinking water. The experimental animals were treated with increasing doses of the drug for 14 days. The dose of *d*-amphetamine given was 50 mg/l. for the first 3 days increasing to 100 mg/l. until day 7 and finally 200 mg/l. for the second week. The period of withdrawal varied for each experimental group: 12 hr, 36 hr, 48 hr and 7 days. The last experimental group was not withdrawn from amphetamine. The animals were killed by microwave irradiation. Further details as given in Acute study. All data were analyzed statistically using Student's '*t*' test.

RESULTS

Acute study

Amphetamine alone caused marked excitability and stereotyped, dyskinetic movement. The concentration of GABA in the brain stem and thalamus were increased while the other regions were unaffected (Table 1).

Chlorpromazine, which caused a marked decrease in ambulation, caused a significant decrease in the concentration of GABA in the brain stem, thalamus and olfactory lobes (Table 1). A combination of chlorpromazine with amphetamine resulted in a marked reduction in the amphetamine induced stereotypy. However, the concentration of GABA was markedly increased in all five brain regions studied (Table 1). Thus a disparity arises between the behaviour changes induced by the drugs alone or in combination, and the changes in the brain GABA concentration.

Chronic study

After 2 weeks administration, amphetamine caused a significant decrease in the GABA content of the thalamus and striatum (Table 2). At this time, the animals showed pronounced stereotyped activity (grooming, chewing bars of cage, increased ambulation). Some 12 hr after amphetamine withdrawal however, the animals became behaviourally depressed, their ambulation and rearing activity being particularly affected.

The daily drug intake increased from approximately 0.75 mg/rat/day (when dose of drug in the drinking water was 50 mg/l.) to 1.5 mg/rat/day (100 mg/l.) reaching a final intake of 3.0 mg/rat/day (200 mg/l.) for the last week of drug administration.

During the period of post amphetamine depression the GABA content of the striatum was significantly reduced (Table 2), only returning to the control level 7 days after withdrawal. Conversely, the concentration of GABA in the amygdala rose during the period of post amphetamine depression and was still significantly elevated 7 days after withdrawal when the animals were still behaviourally depressed. The reduction in the brain stem GABA concentration only occurred for up to 36 hr after drug withdrawal. No changes were apparent in the thalamus and olfactory lobes during the period of post amphetamine depression.

DISCUSSION

Acute amphetamine treatment causes a rise in the GABA content of the brain stem and thalamus. We have shown previously that this drug increases the GABA content of the whole rat brain [13], an effect which appears from the present study to be

Table 1. Effect of acute *d*-amphetamine treatment on GABA concentration

	Brain stem	Striatum	Thalamus	Olfactory lobes	Amygdala
Control	3.37 ± 0.3	4.50 ± 0.20	4.24 ± 0.31	4.09 ± 0.53	2.58 ± 0.2
CPZ	2.45 ± 0.08*	4.30 ± 0.54	3.20 ± 0.18	2.36 ± 0.20*	2.64 ± 0.1
AMPH	3.92 ± 0.23*	4.92 ± 0.45	6.14 ± 0.37*	3.23 ± 0.42	2.86 ± 0.3
CPZ + AMPH	5.47 ± 0.37	15.16 ± 1.03*†	8.13 ± 0.40*†	5.89 ± 0.73*†	4.66 ± 0.4*

* Results are expressed as a mean, in μmoles/g of brain tissue, ±S.E.M. ($N = 6$).
CPZ—Chlorpromazine (10 mg/kg, i.p.), AMPH—*d*-amphetamine (5 mg/kg, i.p.).
*Animals treated with drugs significantly different from untreated rats ($P < 0.05$).
† Animals treated simultaneously with chlorpromazine and amphetamine are significantly different from those treated with amphetamine alone ($P < 0.05$).

Table 2. Effect of chronic amphetamine treatment and drug withdrawal on GABA concentration

Treatment	Brain stem	Striatum	Thalamus	Olfactory lobes	Amygdala
Control	3.34 ± 0.33	4.15 ± 0.30	4.36 ± 0.20	4.00 ± 0.35	2.67 ± 0.07
Chronic Amphetamine	2.95 ± 0.15	3.48 ± 0.31*	3.55 ± 0.20*	3.79 ± 0.19	3.23 ± 0.39
12 Hr withdrawal	2.45 ± 0.15*	3.50 ± 0.17*	3.85 ± 0.35	4.00 ± 0.21	3.34 ± 0.22*
36 Hr withdrawal	2.68 ± 0.11*	3.67 ± 0.21*	3.85 ± 0.20	4.00 ± 0.35	3.01 ± 0.33
48 Hr withdrawal	2.98 ± 0.12	3.50 ± 0.11	3.95 ± 0.39	4.21 ± 0.59	3.12 ± 0.18*
7 Days withdrawal	3.16 ± 0.20	4.42 ± 0.14	4.36 ± 0.11	4.52 ± 0.12	4.02 ± 0.26*

Each value represents the mean (μmoles/g of brain tissue) ±S.E.M. of six animals.
* Difference between experimental and control group significant at $P < 0.05$.

confined to two main anatomical areas as other studies in our laboratory indicate that no appreciable changes occur in the cortex, hippocampus, cerebellum or midbrain. Whereas chlorpromazine was found to cause a decrease in the GABA content of some brain regions, combination with amphetamine dramatically potentiated the rise in GABA in all regions investigated. Thus while chlorpromazine antagonized the acute behavioural effects of amphetamine, it potentiated the effect of the stimulant drug on brain GABA concentrations, thereby suggesting that the changes in the concentration of the inhibitory transmitter are probably not primarily involved in causing the acute effects of amphetamine.

Chronic amphetamine administration causes a decrease in the striatal and thalamic GABA content; the effect on the thalamus was qualitatively different to that found after acute drug administration despite the fact that the stereotypy was more marked after chronic amphetamine treatment. A detailed analysis of the acute and chronic behavioural effects has been the subject of another study in the laboratories (Lynch and Kenny, submitted for publication).

During the period of post amphetamine depression, the GABA content of the striatum, and to a lesser extent of the brain stem, was reduced by return to control levels within 7 days of drug withdrawal. It was of particular interest to find that the GABA concentration in the amygdala rose throughout the period of post amphetamine depression, reaching a maximum 7 days after withdrawal; at this time the animals were still behaviourally depressed. As the amygdala forms an integral part of the limbic system, and may play a functional role in emotion [14], it would appear that at least some of the neuropharmacological and behavioural effects of amphetamine may be due to a modification of GABA metabolism in brain areas other than those concerned with locomotor activity.

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