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γ-AMINOBUTYRIC ACID METABOLISM IN RAT BRAIN FOLLOWING CHRONIC ORAL ADMINISTRATION OF ETHANOLAMINE O-SULPHATE

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Abstract—Chronic, oral EOS* administration resulted in a marked inhibition of rat whole-brain GABA-T activity and a significant increase in brain GABA concentrations. The maximum degree of GABA-T inhibition attained was 83 per cent, when GABA levels were 200 per cent of control values. A fixed dose of EOS produced a steady fall in GABA-T activity over the first 7 days of administration, when enzyme activity appeared to stabilize at 20–25 per cent of control values. Concurrently, GABA levels rose to a steady maximum value of approximately 240 per cent of control values. These changes were accompanied by significant reductions in whole-brain GAD activity. Chronic EOS also produced small but significant increases in brain content of alanine and taurine. No behavioural changes were seen following chronic EOS administration.

An enhancement of GABA neurotransmission has been suggested as a promising strategy in the search for an effective therapy for several pathological states; for example, Huntington's chorea [1], epilepsy [2] and schizophrenia [3].

Brain GABA concentrations can be increased by inhibiting GABA-T, the enzyme primarily responsible for GABA catabolism. It is therefore reasonable to propose that a suitable GABA-T inhibitor could form the basis of a therapy for diseases in which a brain GABA deficiency is implicated. Indeed, an encouraging result has been achieved using isoniazid (a non-specific GABA-T inhibitor [4]) which was found to produce a limited clinical improvement in patients suffering from Huntington's chorea [5].

EOS is an enzyme-activated inhibitor of GABA-T, acting specifically and irreversibly [6]. It has been used widely to raise brain GABA levels in experimental animals (for example, see refs. 7–9); the route of administration being directly into the brain or cerebrospinal fluid on the assumption that it cannot penetrate the blood–brain barrier. However, Leach and Walker [10] have shown that a high subcutaneous dose of EOS in mice inhibits brain GABA-T and raises GABA concentrations. This finding encouraged us to investigate the primary neurochemical effects of chronic oral administration of EOS. The results of some of this work have been reported in preliminary form [11].

MATERIALS AND METHODS

Ethanolamine O-sulphate. EOS was obtained as 2-aminoethylsulphuric acid from Koch-Light Laboratories, Colnbrook, Bucks., U.K. An aqueous solution of this compound is acidic owing to the presence of free sulphate ions. Sulphate was precipitated by the addition of barium hydroxide until the solution became alkaline and the precipitate removed by centrifugation. CO_2 was bubbled through the supernatant (by adding solid CO_2) until the pH approached neutrality and the precipitated barium carbonate was removed by centrifugation. The supernatant was passed through a column of Dowex 50 ion-exchange resin (column dimensions 20 × 1 cm; H^+ form) in order to remove any barium ions. The column eluate was concentrated by rotary evaporation and absolute ethanol added to precipitate the EOS as tiny, pure-white crystals. The precipitate was washed twice with ether and dried.

The infra-red spectrum of this material was found to be identical to that of an authenticated sample of EOS, the purity of which had been established by elemental analysis and NMR spectroscopy.

Animals. Female Wistar rats weighing 150–300g and housed 4 or 5 to a cage were used in all neurochemical experiments. The rats were supplied with either normal drinking water (controls) or a dilute solution of EOS in distilled water. The liquid consumption of each cage of rats was measured daily. In an initial experiment the effect of various concentrations of EOS on brain GABA metabolism was assessed after 12 days of drug administration. A second experiment investigated the time-course of the neurochemical changes produced by a fixed dose (5 mg/ml) of EOS. Finally, in order to evaluate the specificity of chronic EOS administration, whole brain concentrations of 7 amino acids were measured in rats given 5 mg/ml EOS for 21 days.

Biochemical determinations. Amino acid levels and the activities of GABA-T and GAD were assayed in homogenates of whole brain. Rats were stunned by a blow to the head, decapitated, and the brain homogenized in 20 ml of ice-cold distilled water within 40 sec of death. GABA-T activity was measured by the method of Salvador and Albers [12], and

* Abbreviations used: GABA, γ-aminobutyric acid; GABA-T, 4-aminobutyrate: 2-oxoglutarate aminotransferase (EC 2.6.1.19); EOS, ethanolamine O-sulphate; GAD, L-glutamate decarboxylase (EC 4.1.1.15).

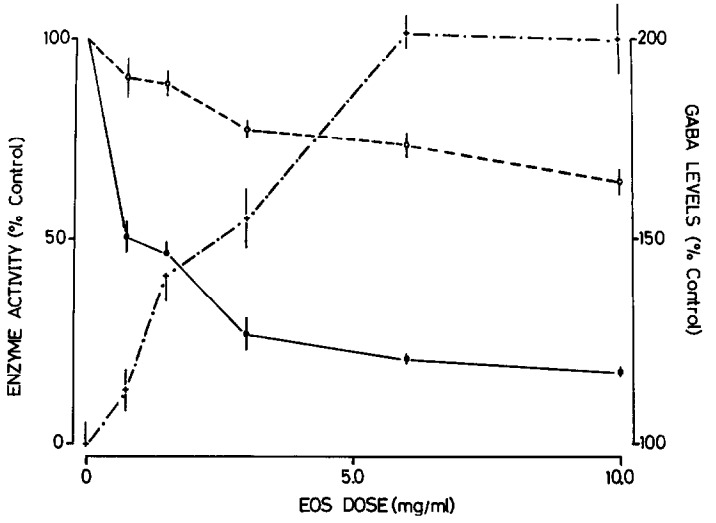

Fig. 1. Activities of GAD (○--○), GABA-T (●—●) and GABA levels (+ - · - +) in whole brain following 12 days administration of various concentrations of EOS dissolved in the drinking water. All values are means ± S.E.M. (N = 4) expressed as a percentage of control. All changes with respect to controls are significant ($P < 0.05$, Student's t-test), except GABA at the lowest EOS dose and GAD at the lower two doses. Control values are given in Table 1.

GAD activity by the fluorimetric method of Lowe *et al.* [13]. Amino acids were assayed by the micro-dansylation procedure of Briel and Neuhoff [14] using ^{14}C-amino acid internal standards as described by Snodgrass and Iversen [15].

Animal behaviour. In order to detect any general behavioural effects of chronic EOS administration, animals were tested on an automated holeboard [9]. Using a system of infra-red beams and detectors, this apparatus records the general locomotor activity and exploratory behaviour of individual rats over a 10 min test period. The number and duration of head dips into holes cut into the floor of the apparatus were used as an index of exploratory activity. Male hooded Lister rats weighing 450–500 g were used in this experiment. Nine rats given EOS 9 mg/ml for 21 days were tested against a group of 9 control rats.

RESULTS

Chronic oral administration of EOS to rats was found to inhibit brain GABA-T and raise GABA levels. The effects of various concentrations of EOS, administered in the drinking water for 12 days, are illustrated in Fig. 1. GABA-T was significantly inhibited by all doses of EOS; the highest dose reducing the enzyme activity to 17 per cent of control. Brain GABA concentration was increased by all doses of EOS; the increase produced by the lowest dose being non-significant. GABA levels were increased to 200 per cent of control at the two highest doses.

The activity of GAD was found to be lower in the brains of EOS-treated animals than in controls. The effect of the two lowest doses was non-significant.

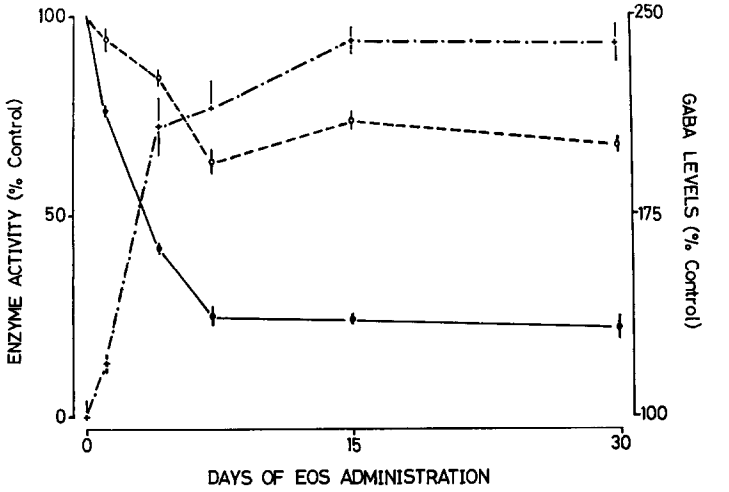

Fig. 2. Brain GAD (○--○), GABA-T (●—●) and GABA (+ - · - +) after various periods of EOS (5 mg/ml) administration. Values are means ±S.E.M. (N = 5) expressed as a percentage of control. All changes with respect to controls are significant ($P < 0.05$. Student's t-test), except GAD at the first time point.

Table 1. The effect of chronic oral EOS administration on whole-brain GAD, GABA-T and levels of amino acids*

	Controls (N = 9)	EOS-treated (N = 10)
Alanine	0.70 ± 0.02	0.80 ± 0.04†
Aspartate	3.55 ± 0.18	3.71 ± 0.17
GABA	2.20 ± 0.10	3.23 ± 0.20‡
Glutamate	7.14 ± 0.22	7.66 ± 0.22
Glutamine	4.09 ± 0.19	3.58 ± 0.28
Glycine	1.06 ± 0.05	1.09 ± 0.04
Taurine	4.20 ± 0.12	4.54 ± 0.10†
GAD	41.3 ± 1.4	32.1 ± 1.0‡
GABA-T	86.6 ± 2.5	21.4 ± 1.1‡

* Amino acid concentrations are expressed as μmoles/g wet wt and enzyme activity as μmoles/g wet wt/hr. All values are means ± S.E.M.
† $P < 0.05$, Student's t-test.
‡ $P < 0.005$, Student's t-test.

At the highest dose of EOS, GAD activity was 64 per cent of control.

The time course of the neurochemical changes produced by 5 mg/ml EOS is shown in Fig. 2. At all the time points investigated, the effects on GABA-T activity and GABA levels were significant. After 30 days, GABA-T activity was reduced to 22 per cent of control and GABA levels raised to 240 per cent of control values. As in the first experiment, GAD activity was found to be reduced in the brains of the EOS-tested animals. This effect was significant only after at least 7 days of EOS administration, with GAD activity being reduced to 67 per cent of control at 30 days.

The results presented in Table 1 show the effect of chronic oral EOS treatment on the brain concentrations of 7 amino acids and on GAD and GABA-T activities. As expected, GABA-T was inhibited and GABA levels correspondingly increased. However, EOS also produced increases in both alanine and taurine levels, although these increases (14 and 7 per cent, respectively) were small compared with the rise in GABA levels (47 per cent). GAD activity was significantly reduced to 78 per cent of control values.

Animal behaviour. The effects of the various doses of EOS on the mean daily water consumption of each cage of rats are shown in Table 2. The corresponding approximate doses per kg body wt are also shown. Only the highest dose of EOS appeared to seriously affect water intake.

Table 2. The effect of EOS on daily water consumption

EOS concentration in drinking water (mg/ml)	Daily water consumption per rat (ml)*	Mean daily dose of EOS per rat (mg/kg)
0	23.4	—
0.75	21.0	93
1.50	21.3	182
3.00	21.5	368
6.00	17.9	612
10.00	13.0	741

* These values are derived from the daily water consumption of each cage of 4 rats.

Table 3. Holeboard behaviour of hooded Lister rats after chronic EOS administration*

	Duration†	Dips‡	Activity§
Controls	125.5 ± 17.5	22.4 ± 3.7	313.3 ± 25.5
EOS-treated	136.6 ± 39.8	23.9 ± 5.6	257.4 ± 26.7

* Values are means ± S.E.M. (N = 9).
† Duration is the time spent (sec) head dipping over the 10 min test period.
‡ Dips are the total number of head dips in 10 min.
§ Activity is in arbitrary counts recorded automatically.

Observation of the rats in their cages and actual handling of the animals revealed no obvious behavioural differences between controls and any of the EOS-treated animals. These initial observations were confirmed by the results of the holeboard experiment, which are shown in Table 3. There was no significant difference in either exploratory behaviour or general locomotor activity between controls and drug-treated animals.

DISCUSSION

Chronic administration of EOS by the oral route was found to be an effective method of producing marked elevation of rat whole brain GABA content. Despite the polar nature of the EOS molecule, it appears to penetrate the blood–brain barrier sufficiently to inhibit brain GABA-T and raise GABA levels. Even at the relatively low doses of 0.75 and 1.50 mg/ml (equivalent to approximately 90 and 180 mg/kg/day, respectively), brain GABA-T was significantly inhibited and GABA levels raised after 12 days administration. The form of the curve of GABA-T inhibition against time shows that the activity of the enzyme decreases quite rapidly over the first few days of administration of a fixed dose of EOS, but thereafter stabilizes to a value approximately 20–25 per cent of control. This stable minimum value may reflect a balance between the rate of inhibition of GABA-T by EOS and the rate of synthesis of new enzyme. As one might expect, the increase in brain GABA content follows a similar time-course to the inhibition of GABA-T, reaching a maximum value of approximately 240 per cent of control. These changes in GABA levels were accompanied by significant reductions in whole brain GAD activity. This phenomenon has also been observed after chronic [16] and acute [17] treatment of rats with γ-vinyl GABA, another specific and irreversible inhibitor of GABA-T. High concentrations of both EOS [6] and γ-vinyl GABA [18] have been shown not to affect GAD activity *in vitro*, suggesting that the observed *in vivo* effects on GAD do not represent a direct inhibition of the enzyme by these drugs. An alternative explanation is that a high brain GABA concentration can feed back to inhibit either the activity of existing enzyme or to inhibit the synthesis of new enzyme (or both). This

feedback could be mediated by GABA itself or by a GABA metabolite. Some degree of GAD inhibition may therefore be an inevitable consequence of chronically elevating brain GABA concentrations.

In addition to its effect on GABA, chronic EOS administration was also found to produce small, but significant, rises in brain alanine and taurine content. These changes may represent direct, non-specific actions of EOS, although this possibility is made unlikely by the lack of evidence for direct effects of EOS on any brain enzyme other than GABA-T. Alternatively, the chronic blockade of GABA catabolism may invoke compensatory adaptations in brain amino acid metabolism. The observed changes in GAD activity and in alanine and taurine levels may represent such adaptations.

Chronic EOS administration was found not to produce any changes in general behaviour of the animals. High, acute doses of GABA-T inhibitors produce a characteristic syndrome [19], the major symptoms of which are behavioural depression, ataxia, hunched posture, piloerection and hypothermia. It is possible that these behavioural effects are not expressed until whole brain GABA levels are increased to values in excess of those attained by our chronic EOS treatment. However, we have found that although chronic EOS administration alone produces no obvious behavioural changes, it can markedly potentiate the CNS effects of drugs such as pentobarbitone and halothane (A. Fletcher and L. J. Fowler, unpublished observations).

In conclusion, we have shown chronic oral administration of EOS to be a convenient method of significantly increasing whole brain GABA levels in experimental animals without producing changes in general behaviour. It is therefore reasonable to propose that EOS could be tested in clinical situations where less specific GABA-T inhibitors have already been employed.

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