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CHRONIC ADMINISTRATION OF THE GABA-TRANSAMINASE INHIBITOR ETHANOLAMINE O-SULPHATE LEADS TO UP-REGULATION OF GABA BINDING SITES

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Abstract—In rats receiving the γ-aminobutyric acid (GABA)-transaminase inhibitor ethanolamine *O*-sulphate (EOS) in their drinking water for up to 28 days, the number of $GABA_A$ and $GABA_B$ binding sites was increased compared to controls. There was no change in binding affinity at $GABA_A$ or $GABA_B$ sites. One week after EOS withdrawal, the number of $GABA_A$ and $GABA_B$ sites in previously treated EOS rats did not differ from controls. There was no difference in the number or affinity of benzodiazepine binding sites between EOS-treated and control rats during EOS administration or withdrawal. There was no difference in the stimulation of benzodiazepine binding by GABA (alone or in the presence of NaCl) during EOS administration. Cortical and cerebellar GABA concentration was increased 3.2- to 4.6-fold and cortical glutamate decarboxylase (GAD) activity reduced 30–42%. The current required to induce electroshock convulsions did not differ between EOS-treated rats and control rats during EOS administration. We speculate that the stimulus for the increased number of $GABA_A$ and $GABA_B$ binding sites is a reduction in GABA release subsequent to a reduction in GAD activity.

γ-Aminobutyric acid (GABA)† is firmly established as a major inhibitory neurotransmitter in the mammalian CNS. Alterations in the transmitter function of GABA have been implicated in several CNS disorders such as epilepsy, anxiety and Huntington's disease [1–4]. Enhancing GABA-mediated neurotransmission has been advocated as a potential therapeutic strategy in their treatment and there is increasing evidence that some clinically effective drugs (e.g. benzodiazepines and barbiturates) enhance GABA neurotransmission by interacting with the GABA-receptor complex [5]. Other potential mechanisms for enhancing GABA neurotransmission include direct-acting GABA agonists, inhibitors of GABA re-uptake and inhibitors of the primary catabolic enzyme for GABA, GABA-transaminase (GABA-T, γ-aminobutyrate 2-oxoglutarate aminotransferase, EC 2.6.1.19). In the last decade a number of specific catalytic inhibitors (suicide substrates) of GABA-T have been developed including ethanolamine *O*-sulphate, γ-vinyl GABA, γ-acetylenic GABA, gabaculine and isogabaculine [6, 7]. All these drugs given acutely to animals increase cerebral GABA concentration and protect from a range of drug-induced seizures, electroshock seizures and reflex epilepsy [8, 9]. The long-term effects of GABA-T inhibitors have been less extensively studied.

GABA receptors exist as a complex consisting of GABA recognition sites, chloride ion channels and at least two associated modulatory sites, at which benzodiazepines (BZ), many barbiturates and certain convulsant drugs interact [5]. The GABA recognition site is termed the $GABA_A$ site. Recently a novel GABA receptor termed the $GABA_B$ site has been described which is pharmacologically distinct from the $GABA_A$ site and is not associated with benzodiazepine sites or chloride ion channels [10].

The present experiments were designed to examine the effects of prolonged elevation of cerebral GABA concentration on GABA and associated binding sites. We have determined [^{3}H]GABA binding (A and B sites), [^{3}H]FNM binding and GABA stimulation of [^{3}H]FNM binding to cerebral cortical membranes of rats treated orally with the GABA-T inhibitor EOS for up to 28 days and 7 days after drug withdrawal. We have also determined the effects of EOS administration on electroshock-induced seizures.

MATERIALS AND METHODS

Materials. EOS was purchased from Koch-Light Ltd. and purified by the method of Fletcher and Fowler [11]. Radiochemicals (4-amino-*n*[2,3-^{3}H]butyric acid, sp. radioact. 61–74 Ci/mmole; [*N*-methyl-^{3}H]flunitrazepam, 72–81 Ci/mmole; and L-[1-^{14}C]glutamic acid, 50–59 mCi/mmole) were purchased from Amersham International. Isoguvacine

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† Abbreviations: GABA, γ-aminobutyric acid; GABA-T, γ-aminobutyrate 2-oxoglutarate aminotransferase; GAD, glutamate decarboxylase; EOS, ethanolamine *O*-sulphate; FNM, flunitrazepam; CC_{50}, convulsive current 50; K_D, equilibrium dissociation constant; R_T, maximal number of binding sites.

was kindly given by Dr. N. G. Bowery and Dr. P. Krogsgaard-Larsen, and baclofen by Ciba-Geigy.

Animals. Male Wistar rats (mean starting wt 150 ± 10 g) were housed 8–10 to a cage, on a 12 hr light cycle beginning at 7 a.m. and water consumption was determined over 7 days. Rats then received either tap water (controls) or a 5 mg/ml solution of EOS *ad libitum*. Fluid consumption was measured daily and the rats were weighed every 2–3 days. At the stated times after initiation of drug administration, three groups of control and EOS rats were treated as follows: group 1 ($n = 6$) were decapitated, the brains rapidly removed and half the cerebral cortex and the cerebellum rapidly frozen (within 30 sec of decapitation) in liquid nitrogen for subsequent determination of GABA concentration. The remaining cerebral cortex was placed on ice for subsequent glutamic acid decarboxylase (L-glutamate 1-carboxylase, EC 4.1.1.15, GAD) activity determination. A second group of rats ($n = 10$–12) was killed by decapitation, the brains were removed and cerebral cortical membrane fractions prepared (for $GABA_A$ and $GABA_B$ binding [12], for benzodiazepine binding [13]) and stored at −20° for subsequent binding studies. In a third group of rats ($n = 12$) the threshold for electroshock-induced convulsions was determined by the 'up and down' method [14]. Stimulation (trains of square wave pulses at 50 Hz for 5 sec) was through ear-clip electrodes using a constant current stimulator. Tonic extension of the hindlimbs was taken as the end-point. Results are presented as convulsive current 50 (CC_{50}), the current inducing hindlimb extension in 50% of the group of animals. Electroshock testing was carried out between 10 and 11 a.m. and each rat was used for only one determination.

Assays. GABA concentration was determined by a radioreceptor assay and GAD activity by the release of $^{14}CO_2$ from L-[1-^{14}C]glutamate as previously described [15].

$GABA_A$ binding. For $GABA_A$ binding, membranes were thawed, treated with 0.02% Triton X-100 [12] (30 min at 37°), centrifuged and washed four times with 50 volumes (v/w) of 50 mM Tris–citrate buffer, pH 7.1. [^{3}H]GABA binding (16 concentrations, 0.5–300 nM) was performed at 0° as previously described [13]. Specifically bound [^{3}H]GABA was defined as that displaceable by 10^{-4} M GABA. Scatchard plots of $GABA_A$ binding were curvilinear and were fitted to a two-component binding system, and binding constants determined by a computerized iterative, non-linear fitting programme.

$GABA_B$ binding. For $GABA_B$ binding, membranes were thawed, washed 4 times with 100 volumes of 50 mM Tris–HCl, pH 7.4, containing 2.5 mM $CaCl_2$, and [^{3}H]GABA binding (8 concentrations, 10 nM–6 μM) was determined at room temperature (20–22°) in the same buffer containing 40 μM isoguvacine as previously described [10]. Specific $GABA_B$ binding was defined as that displaceable by 10^{-4} M (±)-baclofen.

BZ binding. Membranes were thawed, washed 3 times with 100 volumes of 50 mM Tris-citrate, pH 7.1, and [^{3}H]FNM binding (8 concentrations, 0.05–15 nM) was determined at 0° by a filtration assay [13]. Specific [^{3}H]FNM binding was defined as that displaceable by 2 μM clonazepam. [^{3}H]FNM binding was also determined at a single ligand concentration (0.25 nM) and a range of GABA concentrations (0.05–100 μM) in the presence and absence of 200 mM NaCl.

Scatchard analysis of [^{3}H]$GABA_B$ and [^{3}H]FNM from control and EOS-treated rats were well-fitted by single component plots and K_D (equilibrium dissociation constant) and R_T (maximal number of binding sites) were determined by computerized linear regression. All binding assays were repeated on at least 3 occasions. Binding to membranes from control and EOS-treated rats was always compared in the same experiment and the order of assay varied randomly.

Protein concentration. Protein concentration was determined by the method of Lowry *et al.* [16], using a bovine serum albumin standard.

RESULTS

The consumption of EOS solution was equivalent to a mean daily dose ranging from 642 ± 28 mg/kg at 7 days to 618 ± 8 mg/kg at 28 days (Fig. 1). Fluid consumption in the EOS-treated rats was significantly less than controls by 12–15% and returned to control levels on drug withdrawal (Fig. 1). The mean increase in body weight was also significantly less in EOS-treated rats (Fig. 1).

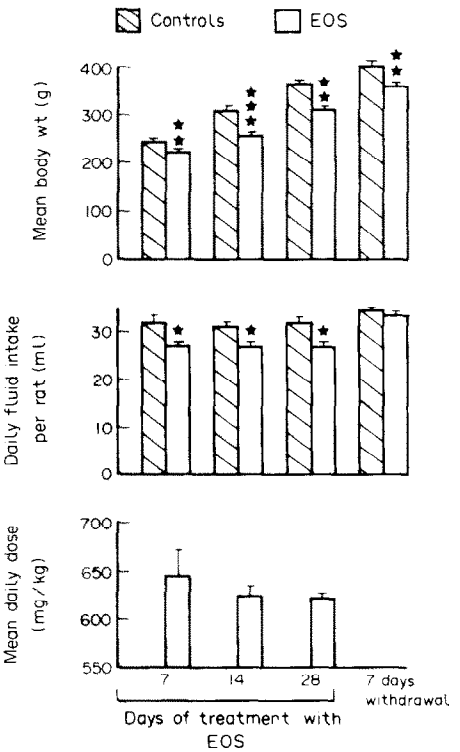

Fig. 1. Body weight (g), daily fluid intake (ml) and daily dose of EOS-treated and control rats ($n = 30$ for each group except after drug withdrawal where $n = 20$). The results are means ±S.E.M. (indicated by the error bars). Significant differences between EOS-treated and control rats are denoted by * $P < 0.01$, ** $P < 0.001$ and *** $P < 0.0001$ (Student's t-test).

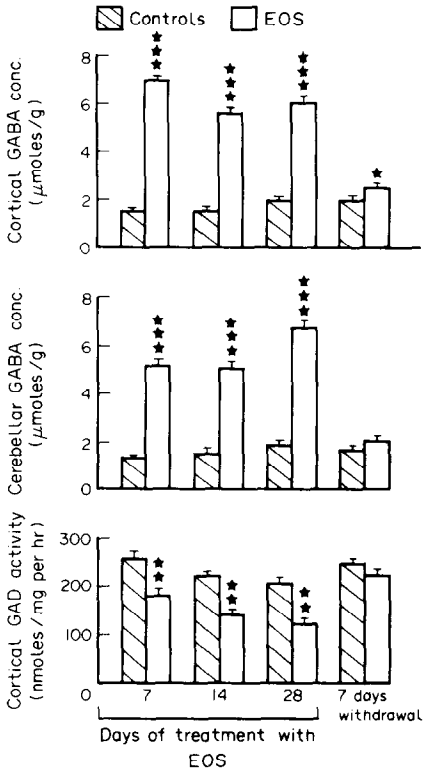

Fig. 2. Cortical and cerebellar GABA concentration (μmole/g wet wt) and cortical GAD activity (nmol glutamate converted/mg protein per hr) in EOS-treated and control rats ($n = 6$ for each group). The results are means ± S.E.M. (indicated by error bars). Significant differences between EOS-treated and control rats are denoted by $^{*}P < 0.01$, $^{**}P < 0.001$ and $^{***}P < 0.0001$ (Student's t-test).

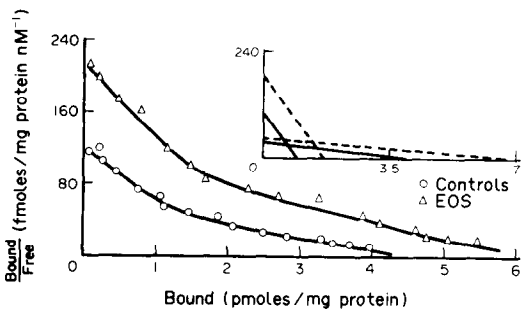

Fig. 3. A typical Scatchard plot of specific [^{3}H]GABA binding to $GABA_A$ sites in cerebral cortical membranes prepared from control rats and rats treated for 14 days with EOS. The inset shows the two binding sites derived from computer-fitting of the same data (solid line, controls; dashed line, EOS-treated).

Table 1. Electroshock susceptibility of EOS-treated and control rats

Duration of treatment (days)		Convulsive current (CC_{50}) mA
7	CONTROL	28.3 (26.9–29.9)
	EOS	23.7 (21.2–26.4)
14	CONTROL	30.6 (27.4–34.1)
	EOS	36.7 (29.6–45.7)
28	CONTROL	40.3 (36.4–44.6)
	EOS	39.1 (37.1–41.4)

Groups of 10–12 EOS-treated or control rats were subjected to electroshock, and the current required to produce tonic hindlimb extension in 50% of the group (CC_{50}) was determined [14]. Values are means with 95% confidence range shown in parentheses.

A marked and sustained increase in cerebral cortical (3.2- to 4.6-fold) and cerebellar (3.4- to 4-fold) GABA concentration was seen during EOS administration (Fig. 2). GAD activity in the cerebral cortex was reduced progressively by 30% at 7 days to 42% at 28 days (Fig. 2). One week after the withdrawal of EOS, cerebellar GABA concentration and cortical GAD activity had returned to control levels, while cortical GABA concentration remained slightly elevated (Fig. 2).

There was a progressive increase in the CC_{50} required to induce electroshock convulsions in control rats (Table 1), which is probably related to the increase in body weight. There was no significant difference in the CC_{50} value between EOS-treated rats and control rats at any time of EOS treatment (Table 1).

The binding of [^{3}H]GABA to cortical $GABA_A$ sites yielded curved Scatchard plots at all times in control and EOS-treated rats. A typical plot is shown in Fig. 3. Binding of [^{3}H]GABA was greater in EOS-treated rats than controls at all times during EOS administration. Fitting of the Scatchard plots to a two-site (low and high affinity) model indicated that the increased binding in EOS-treated rats was due to an increased number of low affinity (at 7, 14 and 28 days) and high affinity (at 14 and 28 days) binding sites without any change in the affinity of the sites (Table 2). One week after EOS withdrawal there was no significant difference in the parameters of $GABA_A$ binding sites between control and previously treated EOS rats.

There was also an increase in the number but not

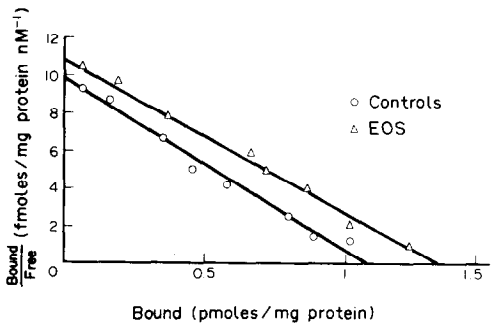

Fig. 4. A typical Scatchard plot of specific [^{3}H]GABA binding to $GABA_B$ sites in cerebral cortical membranes prepared from control rats and rats treated for 28 days with EOS.

Table 2. [^{3}H]GABA binding to $GABA_A$ sites in cerebral cortex from EOS-treated and control rats

Duration of treatment (days)		High affinity		Low affinity	
		K_D	R_T	K_D	R_T
7	CONTROL	8.9 ± 0.6	0.83 ± 0.13	79 ± 12	3.56 ± 0.13
	EOS	10.2 ± 0.9	1.36 ± 0.18	72 ± 6	4.64 ± 0.45*
14	CONTROL	8.9 ± 1.8	0.96 ± 0.09	119 ± 33	4.11 ± 0.44
	EOS	9.2 ± 0.2	1.65 ± 0.11*	177 ± 18	6.83 ± 0.48*
28	CONTROL	5.2 ± 1.2	1.02 ± 0.14	84 ± 9	3.63 ± 0.18
	EOS	5.5 ± 0.4	1.67 ± 0.19*	89 ± 3	5.49 ± 0.48*
7 days withdrawal	CONTROL	5.3 ± 0.7	1.11 ± 0.11	77 ± 7	3.07 ± 0.13
	EOS	6.1 ± 1.4	1.49 ± 0.25	83 ± 16	3.37 ± 0.07

Groups of EOS-treated and control rats were killed by decapitation and cerebral cortical membranes prepared and [^{3}H]GABA binding to $GABA_A$ sites was determined as in Materials and Methods. Specifically bound radioactivity was transformed to Scatchard plots which were curved and the kinetic parameters of binding to a two-component system were fitted with a computerized iterative non-linear fitting programme. K_D is the equilibrium dissociation constant (nM) and R_T the maximal number of binding sites (pmol/mg protein). Values are means ± S.E.M. for at least three determinations. Significant differences between EOS-treated rats and concurrent controls are denoted by *$P < 0.01$ (Student's t-test).

Table 3. [^{3}H]GABA binding to $GABA_B$ sites in cerebral cortex from EOS-treated and control rats

Duration of drug treatment (days)		K_D	R_T
7	CONTROL	126 ± 16	1.19 ± 0.09
	EOS	116 ± 9	1.58 ± 0.07**
14	CONTROL	165 ± 11	1.33 ± 0.06
	EOS	170 ± 12	1.78 ± 0.08**
28	CONTROL	134 ± 16	1.11 ± 0.08
	EOS	142 ± 5	1.39 ± 0.04**
7 days withdrawal	CONTROL	185 ± 11	1.44 ± 0.54
	EOS	194 ± 9	1.22 ± 0.46

Groups of EOS-treated and control rats were killed by decapitation and cerebral cortical membranes prepared and [^{3}H]GABA binding to $GABA_B$ sites was determined as in Materials and Methods. Specifically bound radioactivity was transformed to Scatchard plots and K_D and R_T values were determined by linear regression analysis. Values are means ± S.E.M. for at least three determinations. Significant differences between EOS-treated rats and concurrent controls are denoted by **$P < 0.001$ (Student's t-test).

the affinity of cortical $GABA_B$ binding sites during EOS administration (Fig. 4 and Table 3), which had returned to control values 1 week after drug withdrawal.

There was no difference in the K_D or R_T values of cortical [^{3}H]FNM binding between control or EOS-treated rats during drug administration or 1 week of drug withdrawal (Table 4). GABA alone and GABA in the presence of 200 mM NaCl produced a dose-dependent enhancement of [^{3}H]FNM binding in cortical membranes prepared from control and EOS-treated rats (Table 5). There was no significant difference in GABA or GABA and NaCl stimulation of [^{3}H]FNM binding between control and during EOS treatment (Table 5). NaCl alone produced a 30–43% and 34–46% enhancement of [^{3}H]FNM in control and EOS-treated rats, respec-

Table 4. [^{3}H]FNM binding to benzodiazepine sites in cerebral cortex from EOS-treated and control rats

Duration of treatment (days)		K_D	R_T
7	CONTROL	3.1 ± 0.2	1.64 ± 0.05
	EOS	3.2 ± 0.2	1.79 ± 0.12
14	CONTROL	3.0 ± 0.2	1.83 ± 0.19
	EOS	2.8 ± 0.2	1.92 ± 0.27
28	CONTROL	3.2 ± 0.1	2.03 ± 0.33
	EOS	2.7 ± 0.2	2.08 ± 0.24
7 days withdrawal	CONTROL	2.7 ± 0.2	2.15 ± 0.14
	EOS	2.8 ± 0.3	2.34 ± 0.11

Legend as Table 3 except that [^{3}H]FNM binding was determined to benzodiazepine binding sites.

Table 5. GABA stimulation of specific [^{3}H]FNM binding to cortical membranes of EOS-treated and control rats

Duration of drug treatment (days)	GABA conc. (M)	% Increase in [^{3}H]FNM No NaCl			200 mM NaCl		
		10^{-6}	10^{-5}	10^{-4}	10^{-6}	10^{-5}	10^{-4}
7	CONTROL	33 ± 6	54 ± 8	64 ± 8	32 ± 4	56 ± 11	74 ± 6
	EOS	39 ± 4	50 ± 6	64 ± 4	35 ± 5	61 ± 7	72 ± 6
14	CONTROL	25 ± 7	45 ± 3	62 ± 2	21 ± 1	56 ± 8	81 ± 5
	EOS	23 ± 1	36 ± 5	58 ± 3	17 ± 9	55 ± 1	75 ± 6
28	CONTROL	22 ± 6	39 ± 9	61 ± 8	39 ± 5	68 ± 8	83 ± 3
	EOS	26 ± 9	45 ± 9	66 ± 6	40 ± 7	69 ± 10	78 ± 9
7 days withdrawal	CONTROL	30 ± 1	58 ± 5	65 ± 4	63 ± 10	78 ± 10	84 ± 9
	EOS	26 ± 1	51 ± 1	52 ± 2	37 ± 2*	61 ± 13	81 ± 4

The specific [^{3}H]FNM binding (0.25 nM) was determined in the absence and presence of GABA and GABA and 200 mM NaCl. Results are the mean (±S.E.M.) % increase in baseline binding (no NaCl or 200 mM NaCl) induced by GABA. Experiments were repeated on at least three occasions. Significant differences are indicated by $^*P < 0.05$.

tively, with no difference between EOS-treated rats and concurrent controls.

DISCUSSION

Previous studies [11, 17] have demonstrated that prolonged oral administration of EOS to rats is an effective method of inhibiting GABA-T and producing marked and sustained increases in brain GABA concentration. The greater increase in GABA concentrations than previously reported [17] is probably due to higher drug intake (618 mg/kg compared to 344 mg/kg [11]). The reduced gain in body weight in the EOS-treated rats is compatible with this interpretation, since EOS produces dose-related decreases in food consumption and body weight [18, 19].

The main finding of this study is that prolonged treatment with EOS results in an increase in the number of both $GABA_A$ and $GABA_B$ binding sites. To our knowledge, this is the first report of such an increase during prolonged treatment with a GABA-T inhibitor. No difference in cortical [^{3}H]GABA binding was observed 1, 7 or 21 days after withdrawal of γ-vinyl GABA (250 mg/kg i.p. daily to rats for 21 days) [20]. Twice daily treatment of rats with γ-acetylenic GABA (10 mg/kg) for 14 days resulted in a decreased number of striatal [^{3}H]muscimol binding sites from day 7 to 1 day after drug withdrawal, followed by a 66% increase 3 days after withdrawal [21]. Decreased [^{3}H]muscimol binding 1 day after drug withdrawal was also seen after 14 days' treatment with the less specific GABA-T inhibitors amino-oxyacetic acid and isonicotinic acid hydrazide, and, as with γ-acetylenic GABA, was restricted to the striatum, binding being unchanged in the frontal cortex, hippocampus and cerebellum [22]. These apparent discrepancies may be due to detailed differences in the GABA-T inhibitors used or may be related to dosage and hence the degree of GABA-T inhibition and the extent of GABA accumulation.

Behavioural experiments with EOS are compatible with an enhancement of GABA neurotransmission resulting from the increased cerebral concentrations of GABA [23, 24]. However, enhanced receptor stimulation might be expected to cause down-regulation of receptors [25] rather than the up-regulation observed in this study.

The increased GABA binding is not a result of higher concentrations of GABA within the final membrane preparation since this would result in decreased binding. Indeed, any remaining GABA within the membrane preparations after the extensive washing employed would result in our underestimating the number of binding sites.

Although EOS is a selective inhibitor of GABA-T, with no direct effect on GAD or other cerebral transaminases or GABA uptake [6, 26], it is weakly active at the $GABA_A$ receptor. EOS causes displacement of [^{3}H]GABA binding from $GABA_A$ sites *in vitro* with IC_{50} values of 67 μM [26], 180 μM against 30 nM [^{3}H]GABA [27] and 54 μM against 10 nM [^{3}H]GABA (C. Sykes and R. Horton, unpublished results). Behavioural studies suggest EOS is a weak direct agonist at $GABA_A$ receptors [27]. This is in contrast to other GABA-T inhibitors (such as γ-vinyl GABA and γ-acetylenic GABA) which have weak antagonistic action at $GABA_A$ receptors [27, 28]. γ-Acetylenic GABA and γ-vinyl GABA mimic the dyskinetic effects of the GABA antagonists picrotoxin and bicuculline when injected focally into the rat striatum in high doses [27]. Focal injection of EOS not only failed to produce such dyskinetic effects but like GABA and muscimol rapidly reversed the effects induced by γ-acetylenic or γ-vinyl GABA [27]. This weak direct agonist action of EOS could result in high receptor occupancy and low ion channel activation, and in itself this may lead to a compensatory up-regulation of GABA receptors. Since we do not know the free EOS concentration in the brains of our rats, we can only speculate on this point. However, such an effect is unlikely to explain the increased number of $GABA_B$ sites since EOS (up to 500 μM) *in vitro* did not displace [^{3}H]GABA binding (10 nM) from $GABA_B$ sites (C. Sykes and R. Horton, unpublished results).

In agreement with previous reports [11, 17], we

found a progressive reduction in GAD activity. The reduced GAD activity after EOS is thought to reflect a feedback suppression of *de novo* synthesis of GAD apoenzyme by the increased cerebral concentration of GABA [11, 17]. Reduced GAD activity has been reported after chronic elevation of GABA with other GABA-T inhibitors [29–31]. The control of GAD activity *in vivo* is complex [32] and not well understood but it seems likely that a 40% reduction in GAD activity must result in a reduced rate of GABA synthesis. Seizures in experimental animals induced by GAD inhibitors are thought to result from reduced synthesis and release of GABA and occur when GAD activity (determined under similar conditions to those used in this study) has been reduced to a comparable or lesser extent to that seen after prolonged EOS treatment [33, 34]. The site of the increased GABA after prolonged EOS treatment is unknown but it is likely to be both neuronal and glial [35]. If the increase in neuronal GABA fails to compensate for the reduction in GABA synthesis due to reduced GAD activity, then this could result in reduced GABA release and decreased stimulation of GABA receptors. We suggest this as the most likely stimulus for the up-regulation of GABA binding sites.

Acute administration of EOS (i.p. or intracerebroventricularly) protects mice from electroshock, pentylenetetrazol and sound-induced seizures, and this has been attributed to an enhancement of GABA-mediated neurotransmission [8, 36, 37]. However, we found no protection against electroshock convulsions in rats treated with EOS for 7, 14 or 28 days despite large increases in brain GABA concentration. An elevated threshold for electroshock convulsions in mice has been reported after oral EOS treatment for 4 days, but not after 8 or 12 days [38]. Failure of the acute anticonvulsant effects of other GABA-T inhibitors (e.g. γ-vinyl GABA and γ-acetylenic GABA) to be maintained on more prolonged administration has also been reported [38]. A common event appears to be the reduction in GAD activity. Noteworthy is the appearance of spontaneous EEG spikes and 'grand mal' seizures from the third day in rats treated twice daily with γ-acetylenic GABA [30]. γ-Acetylenic GABA, unlike γ-vinyl GABA or EOS, has a direct inhibitory effect on GAD activity [39], and 50% reduction in whole brain GAD activity was reported after 2 days' treatment with γ-acetylenic GABA [30]. Such results are compatible with a decrease in GABA release as GAD activity is reduced.

The nature, location and functional significance of the increased GABA binding sites during EOS treatment are unknown. The ability of GABA to stimulate BZ binding is thought to reflect a coupling between low affinity $GABA_A$ sites and BZ sites [40]. The observed increase in low affinity $GABA_A$ sites without a corresponding change in BZ binding suggests that the additional $GABA_A$ sites which develop during EOS treatment are not functionally coupled to BZ sites. This may imply that the additional sites are not part of a GABA receptor–ionophore complex. While this does not exclude a post-synaptic location on neurones receiving a GABA input, other sites, at which there is less evidence for a functional coupling between GABA and BZ receptors, need to be considered. Autoreceptors capable of regulating the release of GABA from GABAergic neurones have been reported [41] and their density may be modulated by the synaptic concentration of GABA. It is unclear whether such autoreceptors are preserved and labelled by [^{3}H]GABA in our membrane preparation. Finally, we do not know if the additional GABA binding sites we observed are functional, but it is possible that their development is stimulated by, and in part compensates for, the reduction in GAD activity.

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