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Adaptation of the $GABA_A$-receptor complex in rat brain during chronic elevation of GABA by ethanolamine *O*-sulphate

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1 Slice preparations of rat cuneate nucleus were used for studies on the γ-aminobutyric acid $GABA_A$-receptor complex following chronic and acute pretreatment with GABA-α-ketoglutarate aminotransferase (GABA-T) inhibitors.

2 The whole brain GABA concentration was significantly increased 2.9 fold and 2.6 fold following treatment with ethanolamine *O*-sulphate (EOS, orally) for 15–30 days and 56–64 days, respectively. One hour after a single injection of γ-acetylenic GABA (GAG) i.p., there was a significant 2.1 fold increase in whole brain GABA.

3 Superfusion of a slice with muscimol or the GABA uptake inhibitor nipecotic acid depolarized the afferent nerve fibres. These effects were potentiated by flurazepam (1 μM) and pentobarbitone (10 μM) and antagonized by picrotoxin (3 μM, 30 μM).

4 Following 15–30 days of EOS-treatment, the depolarization response to muscimol was decreased and that to nipecotic acid increased. These changes were no longer significant by 56–64 days of pretreatment. The acute dose of GAG did not affect the depolarization response to muscimol but increased that to nipecotic acid.

5 The potentiations of muscimol by flurazepam (1 μM) and pentobarbitone (10 μM) were enhanced following chronic EOS treatment (15–64 days). The enhancement of flurazepam was less after 56–64 days than after 15–30 days pretreatment whereas the enhancement of pentobarbitone was similar at both times. Acute GAG treatment had no effect.

6 The potency of picrotoxin as an antagonist of muscimol was reduced following chronic EOS treatment; the enhancement was less after 56–64 days than after 15–30 days pretreatment. Acute GAG treatment caused only a very small reduction in picrotoxin potency.

7 Possible adaptations in the $GABA_A$-receptor complex and its modulation during chronic elevation of brain GABA are discussed.

Introduction

Malfunctions of the γ-aminobutyric acid (GABA) system have been implicated in a number of neurological disease states including epilepsy, Huntington's chorea, Parkinson's disease and tardive dyskinesia (Perry *et al.*, 1973; Lloyd & Hornykiewicz, 1973; Tower, 1976; Lloyd *et al.*, 1981; Fibiger & Lloyd, 1984).

Biochemical evidence for impaired GABAergic function is available for some of these conditions (for review see Fariello & Ticku, 1983). If efforts are to be made to activate the GABAergic system in patients with such disorders it is important to determine the long-term effects of GABAmimetic drugs. Brain GABA concentration can be increased by inhibiting GABA-α-ketoglutarate aminotransferase (GABA-T), the enzyme responsible for GABA catabolism. Various reports have shown that chronic administration of GABA-T inhibitors causes neurochemical effects and changes in GABA-receptor binding (Perry *et al.*, 1979; Fletcher & Fowler, 1980; Ferkany *et al.*, 1980; Rumigny *et al.*, 1981; Sykes *et al.*, 1984). The physiological relevance of those changes has been less extensively studied.

The cuneate nucleus slice preparation has been

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shown to be a suitable functional system for studies on the $GABA_A$-receptor complex. The $GABA_A$-receptor complex contains a bicuculline-sensitive receptor for GABA in association with sites for benzodiazepines, barbiturates and picrotoxin that regulate the responses to GABA and the GABA agonist muscimol. GABA and muscimol depolarize the dorsal funiculi fibres. Picrotoxin antagonizes GABA and its analogue muscimol, while benzodiazepines and barbiturates potentiate responses to GABA and muscimol (Simmonds, 1978; 1980; 1981).

In the present investigation we were interested in whether a prolonged elevation in whole brain GABA concentration may alter the responsiveness of the $GABA_A$-receptor and associated sites. The depolarization responses to muscimol and nipecotic acid as well as the potency of picrotoxin and the effects of the potentiating drugs were determined in cuneate nucleus slices from rats chronically treated with the irreversible GABA-T inhibitor, ethanolamine *O*-sulphate (EOS; Fowler & John, 1972). γ-Acetylenic GABA (GAG) was used as an acute GABA-T inhibitor and 4-deoxypyridoxine as an inhibitor of glutamate decarboxylase (GAD).

Methods

Male Wistar rats (180–200 g) were provided with EOS (5 mg kg^{-1}) in drinking water for 15 to 64 days. Fluid consumption and body weight were measured daily. Other rats were injected acutely with γ-acetylenic GABA (GAG; 200 mg kg^{-1}, i.p., 62.5 min before killing) or 4-deoxypyridoxine (400 mg kg^{-1}, i.p., 62.5 min before killing).

In order to prevent the post mortem increase in GABA all rats were injected with 3-mercaptopropionic acid 2.5 min before killing (Van der Heyden & Korf, 1978; Lindgren, 1983). The rats were stunned and killed by decapitation. One slice containing the cuneate nucleus was prepared from each side of the isolated medulla oblongata as previously described (Simmonds, 1978; 1980).

Muscimol was used routinely as the $GABA_A$-receptor agonist and applications were alternated with acute applications of nipecotic acid, a blocker of GABA uptake. The doses used under control conditions were usually 2.5 μM and 5 μM for muscimol and 0.5 mM and 1 mM for nipecotic acid. These doses were selected to give responses in the lower part of the dose-response curve. To check whether the condition of the slices was changed with time, the depolarization response following 6 mM KCl was measured regularly. Muscimol and nipecotic acid were superfused for periods of 2 min and for KCl a 5 min period was used. Recovery periods of about 20 min were allowed between the drug-perfusions.

In order to determine the effects of pretreatment with EOS, GAG or 4-deoxypyridoxine, experiments on slices from a control rat and a pretreated rat were performed on the same day or within 1–3 days of each other. This allowed paired comparisons between slices from control and pretreated rats. With the two slices from each rat, the effects of flurazepam and pentobarbitone were tested on one slice and the effects of picrotoxin on the other slice (e.g. Figure 1). An equilibration time of about 60 min was allowed with each change of drug or concentration superfusing the tissue and the superfusion of the drugs was maintained during the redetermination of muscimol and nipecotic acid dose-response lines. Potentiation or antagonism was measured as the leftward or rightward shift, respectively, of their log dose-response lines.

In a separate series of experiments the *in vitro* effects of GAG and GABA on the depolarization responses to muscimol and nipecotic acid on slices from untreated rats were investigated. Following the control reponses to muscimol and nipecotic acid, the dose-response lines were redetermined in the presence of GAG 1 mM (perfused for 60 min) or GABA 10 μM (perfused for 30 min).

GABA was assayed in frozen whole brain samples homogenized in 6% perchloric acid (1:10 w/v). The GABA content of the supernatant was kindly estimated by Dr A. Aitken on a LKB Biokrom amino acid analyzer.

The sources of drugs were as follows: ethanolamine *O*-sulphate (EOS), γ-aminobutyric acid (GABA), picrotoxin, nipecotic acid, 3-mercaptopropionic acid (MPA), pentobarbitone sodium salt and 4-deoxypyridoxine from Sigma; muscimol from Fluka AG; fluazepam (Dalmane) was a generous gift from Roche Products Ltd; γ-acetylenic GABA (GAG) was a generous gift from Merrell Dow Research Institute. EOS was kindly purified by Dr L.J. Fowler according to the method of Fletcher & Fowler (1980). All drugs used for perfusions were dissolved directly in the Krebs medium, which contained (mM): NaCl 118, KCl 2.1, KH_2PO_4 0.93, $CaCl_2$ 2.5, $MgSO_4$ 2.2, $NaHCO_3$ 25 and glucose 11. When intraperitoneally injected, 4-deoxypyridoxine and GAG were dissolved in 0.9% w/v NaCl solution (saline). MPA was dissolved in saline and the pH adjusted to about 6 with NaOH. Statistical comparisons were made by a paired *t* test.

Results

Ethanolamine O-sulphate dosage

The chronically EOS-treated rats gained weight at a rate not significantly different from that of the controls. Fluid consumption in the EOS-treated rats was

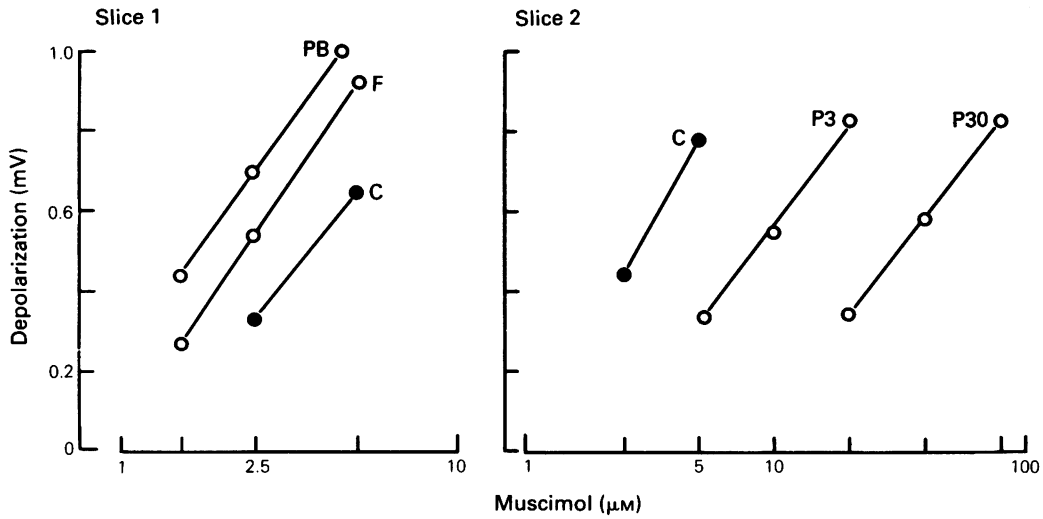

Figure 1 Effects of flurazepam (F), pentobarbitone (PB), and of picrotoxin (P) on log dose-response lines for muscimol. The results represent a single experiment from one animal and each point is a single response. Log dose-response lines were obtained under control (C) conditions in the two slices. In slice 1, flurazepam 1 μM and pentobarbitone 10 μM shifted the muscimol dose-response lines to the left in an approximately parallel fashion. Addition of picrotoxin, 3 μM and 30 μM, to slice 2 caused an antagonism of muscimol and shifted the dose-response lines to the right.

somewhat less than that in the controls after treatment for 56–64 days. The failure of fluid intake to increase with the weight of the rats resulted in a decrease in the mean daily dose of EOS, expressed as mg kg^{-1}, at 56–64 days as compared to 15–30 days of EOS-treatment (Table 1).

γ-Aminobutyric acid levels

A marked and sustained increase in whole brain GABA concentration was seen during chronic EOS administration. Acute treatment with GAG also increased the GABA concentration whereas the administration of 4-deoxypyridoxine did not significantly affect the whole brain GABA concentration (Table 2).

Responses to muscimol, nipecotic acid and K$^+$

Muscimol and nipecotic acid each produced concentration-related depolarizations of the dorsal funiculus fibres in the cuneate nucleus slice. A similar onset of action was seen with both drugs although a somewhat slower recovery was seen following muscimol. The

Table 1 Body weight, daily fluid intake and daily dose of ethanolamine *O*-sulphate (EOS) in treated and control rats.

	Days of treatment			
	15–30 days		56–64 days	
	Control	*EOS*	*Control*	*EOS*
Body weight (g)	306 ± 7.7	291 ± 10.6	428 ± 10.8†††	401 ± 13.6†††
Daily fluid (ml)	26 ± 1.1	24 ± 1.1	2.7 ± 0.5	24 ± 0.2***
Daily dose of EOS (mg kg^{-1})	0	413 ± 26.5	0	303 ± 10.7†

The values are means ± s.e.mean of 5–11 animals. Statistical significances of the differences between EOS-treated and control rats (***$P < 0.001$) and, of the differences between 15–30 days of treatment and 56–64 days of treatment (†$P < 0.05$; †††$P < 0.001$) were calculated using Student's *t* test.

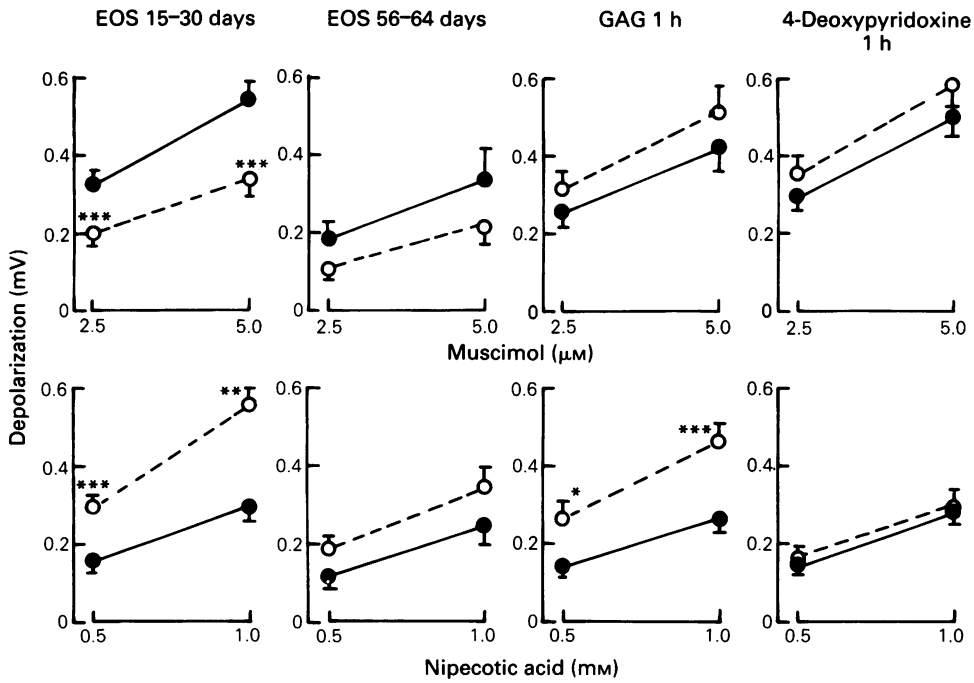

Figure 2 Effects of oral ethanolamine *O*-sulphate (EOS) administrations for 15–30 days and 56–64 days, γ-acetylenic GABA (GAG, 200 mg kg^{-1}, i.p., 62.5 min before killing) and 4-deoxypyridoxine (400 mg kg^{-1}, i.p., 62.5 min before killing) on responses to muscimol and nipecotic acid of dorsal funiculus fibres. Each point is the mean of $n = 17$ for EOS 15–30 days, $n = 6$ for EOS 56–64 days, $n = 8$ for GAG and $n = 15$ for the 4-deoxypyridoxine pretreated group; vertical lines indicate s.e.mean. Filled symbols represent values from control rats and open symbols represent values from pretreated rats. Statistical significances were calculated using paired *t*-test (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

Table 2 The concentration of γ-aminobutyric acid (GABA) in the whole rat brain following treatment with ethanolamine *O*-sulphate (EOS; 5 mg ml^{-1} in drinking water), γ-acetylenic GABA (GAG; 200 mg kg^{-1}, i.p.) or 4-deoxypyridoxine (400 mg kg^{-1}, i.p.)

Treatment (time of treatment)	*GABA* (μg g^{-1} brain tissue)	
Control	233 ± 9.4	(13)
EOS (15–30 days)	664 ± 26.4***	(9)
EOS (56–64 days)	604 ± 29.0***	(5)
GAG (1 h)	495 ± 23.0***	(10)
4-Deoxypyridoxine (1 h)	206 ± 14.0	(8)

The values are means ± s.e.mean with the number of experiment in parentheses. Statistical significances of the differences from the control group were calculated using Student's *t* test (***$P<0.001$).

responses to muscimol were markedly decreased following pretreatment with EOS for 15–30 days (Figure 2). This effect was no longer significant after 56–64 days of EOS-treatment. Pretreatment with GAG or 4-deoxypyridoxine did not influence the responses to muscimol.

In contrast; the responses to nipecotic acid were increased in slices from rats pretreated with EOS for 15–30 days but this was no longer significant after 56–64 days. Responses to nipecotic acid were also increased by pretreatment with GAG but were not affected by 4-deoxypyridoxine.

Elevation of the K^+ concentration to 6 mM from the 3 mM normally present in the Krebs medium was regularly used as a control depolarization test. The depolarization responses to K^+ were not changed in slices from rats pretreated with EOS, GAG or 4-deoxypyridoxine compared to the responses seen in slices from control rats (data not shown).

Perfusion of the control slices for 2 min with MPA (1 mM), GAG (1 mM) or 4-deoxypyridoxine (4 mM) did not produce any depolarization of the cuneate

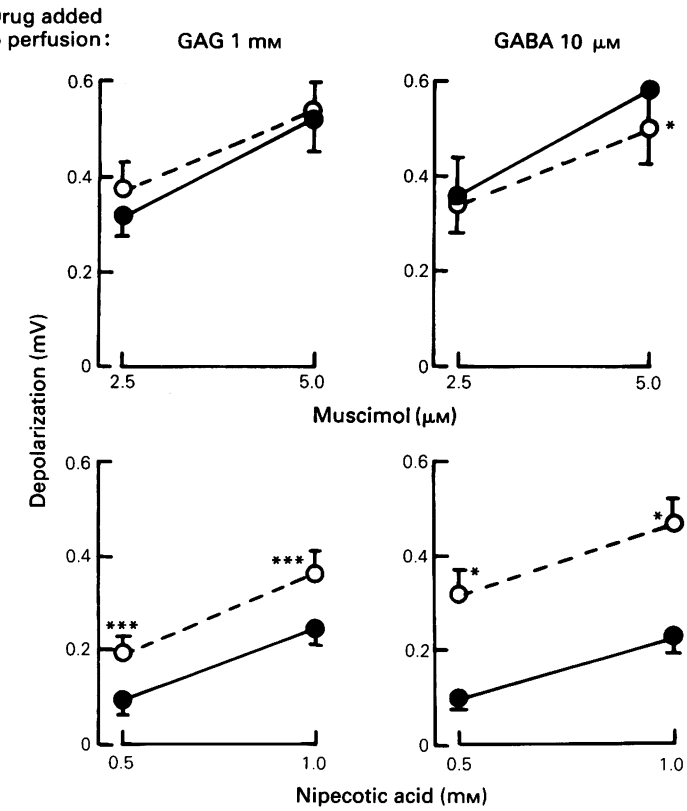

Figure 3 Effects of γ-acetylenic GABA (GAG, 1 mM) and γ-aminobutyric acid (GABA, 10 μM) on responses to muscimol and nipecotic acid. Following the control responses to muscimol and nipecotic acid, the dose-response lines were redetermined in the presence of GAG (perfused for 60 min) or GABA (perfused for 30 min). Filled symbols represent control responses to muscimol and nipecotic acid. Open symbols represent the responses to muscimol and nipecotic acid when GAG ($n = 6$) or GABA ($n = 3$) was added to the perfusion. The values are means with s.e.mean indicated by vertical lines. Statistical significances were calculated using paired t test (*$P < 0.05$; ***$P < 0.001$).

nucleus (data not shown). In a separate series of experiments, *in vitro* procedures expected to elevate GABA in the slices were investigated on responses to muscimol and nipecotic acid. The depolarization response to nipecotic acid was significantly increased when cuneate slices from untreated rats were superfused with Krebs medium containing GAG (1 mM) or GABA (10 μM) (Figure 3). Responses to muscimol were unaffected except for a very small decrease in response to the higher dose of muscimol during superfusion with GABA.

Flurazepam

The presence of flurazepam 1 μM induced a parallel shift of the muscimol dose-response line to the left by 0.19 ± 0.022 log unit (mean ± s.e.mean of 16 values, $P < 0.001$) in slices from control rats. Pretreatment with EOS enhanced the potentiation of muscimol by flurazepam (Table 3). This was statistically significant when both 15–30 days and 56–64 days pretreatment groups were combined. Some inconsistency of this effect prevented the results from the individual groups reaching significance. Pretreatments with GAG and 4-deoxypyridoxine were without effect.

The depolarization effect of nipecotic acid was also potentiated by flurazepam. The nipecotic acid dose-response lines were shifted to the left by 0.15 ± 0.030 log unit (mean ± s.e.mean, $n = 12$, $P < 0.001$) following superfusion with flurazepam. Pretreatment with EOS tended to diminish the potentiation of nipecotic acid by flurazepam, although this effect was only significant after 15–30 days of EOS-treatment (Table 4).

Table 3 Potentiation of muscimol by flurazepam on cuneate nucleus slices of control and pretreated rats

Treatment (time)	*Control*	*Pretreated*
EOS (15–64 days)	0.17 ± 0.023 (12)	0.31 ± 0.054* (12)
EOS (15–30 days)	0.15 ± 0.026 (9)	0.32 ± 0.073 (9)
EOS (56–64 days)	0.23 ± 0.030 (3)	0.29 ± 0.013 (3)
4-Deoxypyridoxine (1 h)	0.17 ± 0.029 (8)	0.15 ± 0.032 (8)
GAG (1 h)	0.14 ± 0.063 (4)	0.14 ± 0.074 (4)

Effects of oral chronic ethanolamine *O*-sulphate (EOS) administration, γ-acetylenic GABA (GAG, 200 mg kg^{-1}, i.p., 62.5 min before killing) and 4-deoxypyridoxine (400 mg kg^{-1}, i.p., 62.5 min before killing) on the potentiation of muscimol by flurazepam (1 μM) measured as lateral shifts (log unit) of the muscimol log dose-response line. All rats were treated with 3-mercaptopropionic acid (50 mg kg^{-1}, 2.5 min before killing). The values are means ± s.e.mean, (*n*). Statistical significances between control and pretreated groups were calculated using paired *t* test (*$P < 0.05$).

Table 4 Potentiation of nipecotic acid by flurazepam on cuneate nucleus slices of control and pretreated rats

Treatment (time)	*Control*	*Pretreated*
EOS (15–64 days)	0.11 ± 0.018 (9)	0.06 ± 0.033 (9)
EOS (15–30 days)	0.11 ± 0.020 (6)	0.01 ± 0.028* (6)
EOS (56 ± 64 days)	0.11 ± 0.045 (3)	0.17 ± 0.033 (3)
4-Deoxypyridoxine (1 h)	0.16 ± 0.038 (8)	0.17 ± 0.024 (8)
GAG (1 h)	0.16 ± 0.081 (4)	0.06 ± 0.053 (4)

Effects of oral chronic ethanolamine *O*-sulphate (EOS) administration, γ-acetylenic GABA (GAG, 200 mg kg^{-1}, i.p., 62.5 min before killing) and 4-deoxypyridoxine (400 mg kg^{-1}, i.p., 62.5 min before killing) on the potentiation of nipecotic acid by flurazepam (1 μM) measured as lateral shifts (log/unit) of the nipecotic acid log dose-response line. All rats were treated with 3-mercaptopropionic acid (50 mg kg^{-1}, i.p., 2.5 min before killing). The values are means ± s.e.mean, (*n*). Statistical significances between control and pretreated groups were calculated using paired *t* test (*$P < 0.05$).

Pentobarbitone

Pentobarbitone 10 μM caused a leftward shift of the muscimol log dose-response line by 0.30 ± 0.030 log unit ($n = 16$, $P < 0.001$) in control slices. This potentiation of muscimol was clearly increased following EOS-treatment for both 15–30 days and 56–64 days (Table 5). Pretreatment with GAG or 4-deoxypyridoxine had no effect.

The nipecotic acid dose-response line was shifted to the left by 0.20 ± 0.036 log unit ($n = 12$, $P < 0.001$) following pentobarbitone superfusion. Pretreatment with EOS, GAG or 4-deoxypyridoxine did not influence the potentiation of nipecotic acid by pentobarbitone (data not shown).

Picrotoxin

In all experiments, picrotoxin displaced the muscimol log dose-response curve to the right in an approximately parallel fashion. In 15 slices from control rats, the mean rightward shift of the muscimol dose-response line by 3 μM picrotoxin was 0.58 ± 0.027 log unit ($P < 0.001$) and by 30 μM picrotoxin 1.09 ± 0.038 log unit ($P < 0.001$). As seen in Table 6, the antagonism of muscimol by picrotoxin (3 μM and 30 μM) was

Table 5 Potentiation of muscimol by pentobarbitone on cuneate nucleus slices of control and pretreated rats

Treatment (time)	*Control*	*Pretreated*
EOS (15–64 days)	0.26 ± 0.032 (12)	0.39 ± 0.035*** (12)
EOS (15–30 days)	0.24 ± 0.040 (9)	0.37 ± 0.046** (9)
EOS (56–64 days)	0.31 ± 0.024 (3)	0.44 ± 0.020*** (3)
4-Deoxypyridoxine (1 h)	0.29 ± 0.038 (8)	0.31 ± 0.033 (8)
GAG (1 h)	0.23 ± 0.086 (4)	0.24 ± 0.088 (4)

Effects of oral chronic enthanolamine *O*-sulphate (EOS) administration, γ-acetylenic GABA (GAG, 200 mg kg^{-1}, i.p., 62.5 min before killing) and 4-deoxypyridoxine (400 mg kg^{-1}, i.p., 62.5 min before killing) on the potentiation of muscimol by pentobarbitone (10 μM) measured as lateral shifts (log unit) of the muscimol log dose-response line. All rats were treated with 3-mercaptopropionic acid (50 mg kg^{-1}, i.p., 2.5 min before killing). The values are means ± s.e.mean, (*n*). Statistical significances between control and pretreated groups were calculated using paired *t* test (**$P < 0.01$; ***$P < 0.001$).

Table 6 Antagonism of responses to muscimol by picrotoxin on cuneate nucleus slices of control and pretreated rats

Treatment (time)	*Picrotoxin* (3 μM) control	*Picrotoxin* (3 μM) pretreated	*Picrotoxin* (30 μM) control	*Picrotoxin* (30 μM) pretreated
EOS (15–64 days)	0.59 ± 0.031 (12)	0.35 ± 0.036*** (12)	1.12 ± 0.043 (12)	0.83 ± 0.047*** (12)
EOS (15–30 days)	0.61 ± 0.034 (9)	0.34 ± 0.039*** (9)	1.14 ± 0.054 (9)	0.80 ± 0.052*** (9)
EOS (56–64 days)	0.54 ± 0.070 (3)	0.39 ± 0.096 (3)	1.05 ± 0.059 (3)	0.91 ± 0.110 (3)
4-Deoxypyridoxine (1 h)	0.59 ± 0.043 (8)	0.53 ± 0.023 (8)	1.10 ± 0.065 (8)	1.01 ± 0.023 (8)
GAG (1 h)	0.63 ± 0.047 (5)	0.49 ± 0.059* (5)	1.15 ± 0.086 (5)	1.01 ± 0.085 (5)

Effects of oral chronic ethanolamine *O*-sulphate (EOS) administration, γ-acetylenic GABA (GAG, 200 mg kg^{-1}, i.p., 62.5 min before killing) and 4-deoxypyridoxine (400 mg kg^{-1}, i.p., 62.5 min before killing) on the antagonism of muscimol by picrotoxin 3 μM and 30 μM. All rats were treated with 3-mercaptopropionic acid (50 mg kg^{-1}, i.p., 2.5 min before killing). The values are means ± s.e.mean (*n*), measured as the rightward shifts (log unit) of the muscimol log dose-response line. The statistical significances beween control and pretreated groups were calculated using paired *t* test (*$P < 0.05$; ***$P < 0.001$).

clearly reduced following 15–30 days of EOS-treatment. However, this effect was no longer significant after 56–64 days of EOS-treatment. Pretreatment with GAG slightly decreased the antagonistic effect of picrotoxin 3 μM but not 30 μM. 4-Deoxypyridoxine had no effect.

The depolarization response to 6 mM KCl increased by 20–30% during superfusion with picrotoxin. This effect was seen in control slices as well as in slices from pretreated rats (data not shown).

Discussion

The aim of this study was to detect any adaptations in the $GABA_A$-receptor complex that might ensue from chronic elevation of GABA levels in the brain. An important aspect was to distinguish between effects resulting directly from the raised GABA concentration itself, which should be apparent both acutely and chronically, and true adaptations which require chronic elevation of GABA. To make this distinction, we used a 1 h pretreatment with GAG as the main acute condition, supplemented with a few experiments where GAG or GABA was added *in vitro* to tissues from untreated animals. Our interpretation of this study depends largely on a comparison of the results from these acute conditions with the results from chronic elevation of GABA by EOS pretreatment.

Acute elevations of GABA, whether by GAG pretreatment or addition directly to the cuneate nucleus slice of GAG or GABA itself, had little or no effect on the sensitivity of the $GABA_A$-receptor complex to the agonist muscimol. The GAG pretreatment was sufficient to sedate the rats but did not cause the receptor desensitization that can sometimes occur with elevated concentrations of GABA agonists (Desarmenien *et al.*, 1980; Brown & Scholfield, 1984). However the responses to nipecotic acid were significantly enhanced. Since nipecotic acid has only a very low affinity for GABA receptors (Krogsgaard-Larsen *et al.*, 1981) but is an inhibitor of both neuronal and glial uptake processes for GABA (Krogsgaard-Larsen & Johnston, 1975), it seems likely that the responses of the cuneate nucleus to nipecotic acid were due to blockade of the uptake of GABA. This implies that there was a process of release and uptake of endogenous GABA going on within the cuneate nucleus slice and that the rate of this process was increased when raised GABA levels resulted in increased responses to nipecotic acid.

Acute elevation of GABA by pretreatment with GAG had no effect on the potency of flurazepam or pentobarbitone as potentiators of muscimol. There was a minor reduction in the potency of picrotoxin as an antagonist of muscimol but this effect was seen only at the lower concentration of picrotoxin. Thus, acute pretreatment with GABA-T inhibitor had little effect on the properties of the $GABA_A$-receptor complex.

In contrast, chronic treatment with EOS caused some clear quantitative changes in the pharmacology of the $GABA_A$-receptor complex. The elevation of GABA achieved in the present experiments was very similar to that reported by Fletcher & Fowler (1980). Like them, we also found no obvious behavioural effects of the EOS pretreatment, in contrast to the acute sedation observed following injection of GAG, even though the brain levels of GABA were slightly

higher in the EOS-pretreated rats. This in itself might suggest some adaptation to the acute effects of elevated GABA.

In slices of cuneate nucleus from rats pretreated for 15–30 days with EOS, there was a significant reduction in the responses to muscimol. This could have resulted from receptor desensitization due to the rise in endogenous GABA or from an adaptation of the $GABA_A$-receptor complex. The interpretation must be cautious, however, because of the unusually high values of the control responses to muscimol. In the same experiments, an opposite change in the responses to nipecotic acid was found. This can be explained in the same way as the similar enhancements in nipecotic acid responses following acute GAG, assuming that such an effect obscured the reduced responsiveness of the GABA receptor. For both muscimol and nipecotic acid, these changes were no longer statistically significant by 56–64 days of pretreatment. One possible explanation for this is that the elevated GABA redistributed into compartments that contributed less to the extracellular concentration of GABA. Such a development might be expected to reduce any adaptations induced by the increased GABA tone.

Chronic pretreatment with EOS also caused some changes in the potentiation of muscimol by flurazepam and pentobarbitone and in the antagonism of muscimol by picrotoxin. The potentiating effects of flurazepam and pentobarbitone were significantly enhanced; for flurazepam, the enhancement was greater after 15–30 days than after 56–64 days pretreatment while, for pentobarbitone, the enhancements were similar at both times. The antagonistic effect of picrotoxin, on the other hand, was reduced and the reduction was greater after 15–30 days than after 56–64 days pretreatment. Although, inexplicably, the potentiations of nipecotic acid by flurazepam and pentobarbitone were not enhanced, the results with muscimol provide clear evidence of adaptation.

To investigate the consequences of reduced GABA concentrations, the acute effects of a GAD inhibitor were determined. Pretreatment of rats with 4-deoxypyridoxine in a dose that caused increased irritability and convulsive episodes failed to reduce brain GABA and had no effect on the responses to muscimol, nipecotic acid, flurazepam, pentobarbitone or picrotoxin. Although this dose of 4-deoxypyridoxine has previously been reported to reduce rat brain GABA concentration (Bayoumi *et al.*, 1972; Lindgren & Andén, 1984), it may not be possible to achieve a reduction in GABA concentration and at the same time avoid the complication of convulsions.

Overall, our results with chronic elevation of brain GABA indicate a changing pattern of adaptation between 15–30 days and 56–64 days of EOS pretreatment. The changes in sensitivity of the $GABA_A$-receptor complex to flurazepam, pentobarbitone and picrotoxin suggest that the receptors for these drugs may have physiological functions, presumably involving endogenous mediators. A peptide candidate for the benzodiazepine site has been proposed (Costa & Guidotti, 1985) and the possibility of an endogenous steroid acting in the same way as pentobarbitone has also been suggested (Harrison & Simmonds, 1984; Majewska *et al.*, 1986). If the postulated endogenous mediators continued to exert their regulatory activity on the $GABA_A$-receptor complex *in vitro*, two types of adaptation would be possible: a change in the synthesis and release of endogenous mediators and a change in the synthesis of new $GABA_A$-receptor complexes with respect to rate of synthesis and/or the inclusion of regulatory sites. There is a report (Sykes *et al.*, 1984) that the numbers of high and low affinity $GABA_A$-binding sites increase in the brains of rats treated chronically with EOS while the number of benzodiazepine binding sites does not change. These data do not provide a simple explanation of the results that we present here and more extensive binding studies need to be made. Evaluation of the possible role of endogenous mediators must await their identification.

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