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Some neurochemical effects of chronic oral administration of ethanolamine *O*-sulphate to rats

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Ethanolamine *O*-sulphate (EOS), a specific irreversible inhibitor of GABA-transaminase (GABA-T) (Fowler & John, 1972) has been widely used to raise brain GABA levels in experimental animals. In most instances EOS has been administered directly into the CSF on the assumption that peripheral administration is ineffective. However, when administered in a high dose subcutaneously (Leach & Walker, 1977) EOS was found to effectively inhibit GABA-T and elevate brain GABA concentrations. In this report we investigate oral administration.

Wistar rats (120–200 g) of either sex were given either normal drinking water (controls) or a dilute solution of EOS in distilled water. Daily liquid consumption was measured. The effect of various concentrations of EOS on brain GABA-T (Salvador & Albers, 1959) and GABA levels (Sutton & Simmonds, 1974) were measured after 12 days. Brain homogenates were prepared in ice-cold distilled water within 40 s of decapitation. The time-course of the effects of a fixed dose (5 mg/ml) were measured in a separate experiment.

The results in Table 1 show that oral EOS inhibited GABA-T in a dose-dependent manner. However, the increase in GABA was not as clearly dose-dependent. Similarly, the fixed dose (5 mg/ml) of EOS caused progressively greater inhibition of GABA-T up to 14 days (the maximum time period studied). After 1 day on 5 mg/ml EOS, GABA-T activity was 86.2 ± 3.9% of controls ($P < 0.02$, $n = 5$) and at 14 days was 22.6 ± 1.88% of controls ($P < 0.001$, $n = 3$). At these times GABA levels were 171.4 ± 16.5% ($P < 0.01$, $n = 4$) and 178.9 ± 19.5% ($P < 0.001$, $n = 3$) of controls, respectively.

It is possible that when GABA-T is chronically inhibited a compensatory mechanism operates to prevent GABA levels from becoming excessively high. Preliminary experiments suggest that glutamate

Table 1 The effect of various concentrations of EOS on brain GABA and GABA-T after 12 days

EOS (mg/ml)	*n*	*GABA-T*	*GABA*	*Mean daily water consumption per rat (ml)*
Controls	5	100	100	23.4
0.75	5	73.5 ± 3.6*	126.5 ± 8.0****	21.0
1.50	5	48.3 ± 2.7*	143.3 ± 11.0***	21.3
3.00	5	38.7 ± 2.8*	188.3 ± 2.8*	21.5
6.00	3	23.1 ± 3.0*	149.8 ± 7.5**	17.9
10.0	3	16.5 ± 0.2*	171.0 ± 9.3**	13.0

Values are percentage control ± s.e. Control GABA-T activity 115.3 ± 1.8 μmol g^{-1} h^{-1}. Control GABA levels 3.95 ± 0.23 μmol/g. **** $P < 0.05$, *** $P < 0.02$, ** $P < 0.01$, * $P < 0.001$, compared with controls (Student's *t*-test).

decarboxylase activity may be depressed in rats chronically dosed with EOS.

These initial experiments show that EOS is orally active and could form the basis of a therapy for pathological states in which a GABA deficiency is implicated. An example is Huntington's Chorea, which has been treated with partial success using isoniazid (Perry, Macleod & Hanson, 1977), a non-specific inhibitor of GABA-T.

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The effect of some anaesthetic agents on [^{3}H]-GABA release from rat brain slices

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Barbiturates prolong pre- and postsynaptic inhibitions in the mammalian central nervous system which are thought to be mediated by the neurotransmitter γ-aminobutyric acid (GABA), (Eccles, Schmidt & Wallis, 1963; Nicoll, Eccles, Oshima & Rubia, 1975). It is of interest therefore to determine the mechanism by which these phenomena occur, and several studies have suggested that barbiturates inhibit GABA transport (Cutler, Markowitz & Dudzinski, 1974) and have synergistic agonist actions at GABA receptors (Lodge & Curtis, 1977). The present investigation is concerned with the effect of barbiturate and, for comparison, some non-barbiturate anaesthetics on depolarization-induced [^{3}H]-GABA release from rat brain slices.

The cerebral cortices were removed from adult Wistar rats and chopped into small slices (0.1 × 0.1 × 1.0 mm approx.) which were then incubated with 0.1 μM [^{3}H]-GABA for 15 min at 37°C. The slices were then collected by filtration and superfused with warm oxygenated Krebs-phosphate solution containing 10 μM amino-oxyacetic acid to prevent metabolism of [^{3}H]-GABA. Exposing the slices to 40 mM K^+ for 9 min resulted in a 5.2 ± 0.2 (s.e. mean, $n = 64$) fold increase in the fractional efflux rate constant. Barbiturates added to the superfusing medium failed to influence the spontaneous efflux of [^{3}H]-GABA, however they inhibited the increase in release due to elevated K^+ concentration. The concentration for 50% inhibition of K^+-evoked release was 100 μM for thiopentone, 200 μM for pentobarbitone and 600 μM for methohexitone. By contrast the non-barbiturate anaesthetics ketamine (5–100 μM) and urethane (5–30 mM) did not alter either spontaneous or K^+-elicited [^{3}H]-GABA efflux.

Since the concentration of these agents for anaesthesia in rats falls within the ranges tested in this study (assuming even distribution throughout the body water) it is tempting to infer that the barbiturate-induced inhibition of GABA release may be related to anaesthesia. However, the non-barbiturate anaesthetics did not influence GABA release and so it would appear more likely that these effects seen in cortical tissue are not causal events in anaesthesia but rather represent interesting side effects possibly related to agonist action by the barbiturates at presynaptic GABA receptors.

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