retinal subcellular particles on density gradients, following the incubation of rabbit retinae with labelled GABA 0.1 μM or 5 mM (uptake mainly by the high or low affinity process respectively). Each retina of a pair was incubated at 37°C with [^{14}C] or [^{3}H]-GABA and then homogenized separately in sucrose (0.32 M). The resulting homogenates were mixed and subjected to subcellular fractionation using 0.4-1.8 M linear sucrose gradients (Atterwill & Neal, 1973). Gradient fractions were assayed for radioactivity by double-label liquid scintillation spectrometry.

A partial separation occurred between the particulate populations which had accumulated labelled GABA mainly by the high or the low affinity uptake processes (Figure 1). The separation was also obtained when reverse labelling conditions were used. In control experiments when both retinae were incubated with labelled GABA (0.1 μM), no separation occurred.

The morphology of the particles responsible for the accumulation of GABA at different concentrations has not yet been determined. A preliminary electron microscopic study of the P2 fraction has revealed the presence of not only nerve-ending particles similar to those found in brain subcellular preparations, but also other particles which may be of glial origin. It is hoped to determine whether these different types of particle accumulate GABA by the high or the low affinity uptake mechanism by the use of electron microscope autoradiography.

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Effects of some clinically used anaesthetics on the enzymes of γ-aminobutyric acid metabolism

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Recent work has emphasized the involvement of brain γ-aminobutyric acid (GABA) metabolism during audiogenic seizures and convulsions (Wood, Pesker & Urton, 1972; Matin & Kar, 1973; Simler, Cielielski, Maitre, Randrianarisova & Mandel, 1973) although no direct correlation between GABA levels and convulsive threshold appears to exist. The main problems in examining compounds that might have specific effects on glutamic acid or GABA metabolism are, first, that glutamate decarboxylase (GAD) and GABA aminotransferase (GABA-T) are both pyridoxal dependent enzymes which are susceptible to inhibition by carbonyl agents such as hydroxylamines and hydrazines (Baxter, 1969) and, secondly, that GABA-T has to be strongly inhibited before any change in GABA concentration can be detected (Fowler, 1973). Drugs which increase the GABA level usually have anticonvulsant activity, e.g. di-*n*-propylacetic acid (Godin, Heiner, Mark & Mandel, 1969) whereas drugs which lower GABA concentration usually lower the convulsive threshold, e.g. paroxan (Matin & Kar, 1973).

The present work has examined the effects of several newer anaesthetic agents, particularly those which have been shown to possess excitatory side-effects (Barron & Dundee, 1967; Winters, Ferrar-Allado, Gueman-Flores & Alcara) on mouse brain GABA and glutamate levels *in vivo* and GAD from GABA-T activity *in vitro*. GAD activity was determined by trapping $^{14}CO_2$ released from L-[^{14}C] glutamate (25 mM) in the presence of pyridoxal phosphate. The activity was unaffected by pentobarbitone (5 mM), thiopentone (5 mM) or althesin (1.82 mM), the latter being the maximum concentration obtainable due to the low solubility of althesin. GAD was uncompetitively inhibited by methohexitone (K_I = 11.5-17.0 mM) and competitively inhibited with respect to glutamate by ketamine (K_I = 14-15 mM) and gammahydroxybutyrate (GHB; K_I = 8.7-10.5 mM). Also, it was confirmed that succinic semialdehyde non-competitively inhibited GAD (K_I = 4.0-6.25 mM). GABA-T was assayed using [2,3-^{14}C]-GABA and subsequent separation of the acid metabolites on Dowex resin. Both thiopentone and methohexitone produced inhibition which was not competitive with respect to GABA whereas ketamine and GHB had no effect on the enzyme activity at concentrations which inhibited GAD.

None of the drugs used produced any significant change in mouse brain levels of GABA, glutamate or glutamine. It is interesting that the dissociative anaesthetics tested, namely ketamine and GHB, competitively inhibited GAD, but did not affect GABA-T. Since the concentrations of drug used were chosen to be of the same order as the expected *in vivo* concentration following i.v.

administration of the drug, it might be expected that these drugs could produce a net decrease in cerebral GABA synthesis. The possibility that ketamine and GHB might interfere with glutamate binding at other sites in the brain is being examined.

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The release of [^{14}C]-taurine from slices of rat cerebral cortex and spinal cord evoked by electrical stimulation and high potassium ion concentrations

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In an attempt to elucidate the possibility that taurine (2-aminoethanesulphonic acid) is a neurotransmitter in the mammalian central nervous system, a study has been made of the release of [^{14}C]-taurine from slices of rat cerebral cortex and spinal cord. Slices of cerebral cortex and spinal cord obtained from freshly killed rats were incubated for 20 min with 1.0 μCi of radioactive taurine and thence transferred to a perspex perfusion chamber (Srinivasan, Neal & Mitchell, 1969).

Table 1 Factors affecting the release of [^{14}C]-taurine from slices of rat cerebral cortex and spinal cord

Experimental procedure	*Cerebral cortex*		*Spinal cord*	
	Fractional rate constant, f (min^{-1})		*Fractional rate constant, f (min^{-1})*	
	Control	*Test*	*Control*	*Test*
50 mM K^+	0.0021 ± 0.0002	0.005 ± 0.0007†	0.006 ± 0.002	0.01 ± 0.0008
100 mM K^{++}	0.002 ± 0.00006	0.007 ± 0.0006*	0.008 ± 0.001	0.01 ± 0.002
100 mM K^+ Ca^{++} omitted	0.002 ± 0.0002	0.003 ± 0.0002	–	–
100 mM K^+, Ca^{++} replaced by Mg^{++}	0.0016 ± 0.0004	0.002 ± 0.0002	–	–
Electrical stimulation	0.002 ± 0.0003	0.005 ± 0.0006†	0.005 ± 0.0005	0.006 ± 0.0007
Electrical stimulation, Ca^{++} replaced by Mg^{++}	0.0017 ± 0.0002	0.003 ± 0.0002†	0.004 ± 0.0006	0.005 ± 0.0005

Tissue slices were incubated with 1.0 μCi of [^{14}C]-taurine for 20 min, transferred to the perfusion chamber and perfused with Krebs bicarbonate Ringer solution. After 20 min, the slices were either stimulated electrically (5 ms, 100 Hz, 20 mA for 30 s) or the potassium concentration was increased. The values are the mean fractional rate constants (min^{-1}) of between 3 and 8 experiments ±s.e. mean. Significantly different from controls (Students *t*-test).

* $P < 0.001$. † $P < 0.01$.