6.4-7.0 illustrates that amphibian and mammalian GAD are not identical.

4-Aminobutyrate-2-ketoglutorate-aminotransferase (GABA-T) activity was determined by the method of Hall & Kravitz (1967). Its activity showed less regional variation than that of GAD but again the activity in the tectum (5.24 ± 0.5 (μmol/h)/g wet weight) and mid brain (4.5 ± 0.1 (μmol/h)/g) was higher than that of other regions.

It therefore appears that, in the frog brain, GABA is produced by the action of GAD on glutamic acid and is then transaminated by a GABA-T to succinic semialdehyde which is subsequently oxidized to succinic acid. This metabolic pathway closely resembles that which, in the mammalian system, has been designated the GABA shunt. The relatively high activity of GAD and GABA-T in the tectum and mid brain suggest that GABA shunt is particularly important in these regions.

The demonstration that GABA, together with its synthesizing and inactivating enzymes is relatively concentrated in the tectum and mid brain of the frog suggests a transmitter role for GABA in these tissues.

R.A. Yates is an M.R.C. Junior Research Fellow.

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Partial separation of retinal subcellular particles accumulating labelled γ-aminobutyric acid (GABA) at high and low concentrations

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The rabbit retina possesses both a high and a low affinity uptake process for GABA, the apparent Km values of the two processes being 10 μM and 250 μM respectively. The possibility that different cell types might possess only the high affinity, or only the low affinity uptake mechanism, was investigated by examining the distribution of

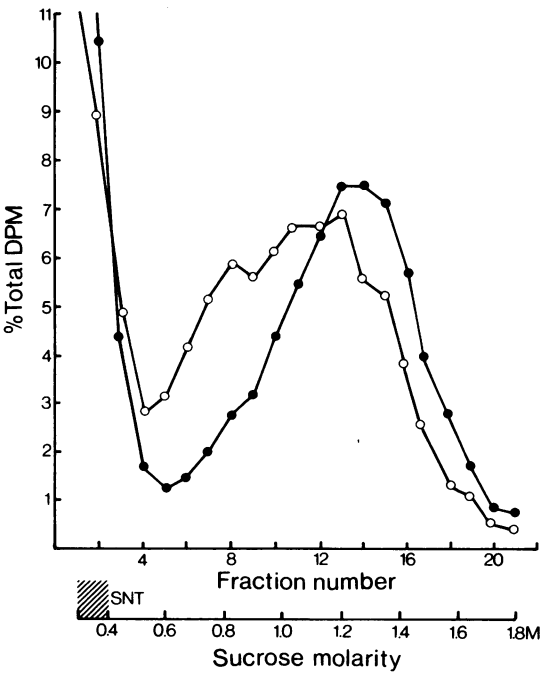

Fig. 1 Distribution of [^{14}C]-GABA (○), 5 mM, and [^{3}H]-GABA (●), 0.1 μM, on a linear continuous sucrose gradient (0.4-1.8 M). In both cases the results are expressed as a percentage of the total radioactivity (DPM) recovered in all fractions from the gradient.

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.