GABA antagonists then they might be expected to mimic picrotoxin in GP. We have investigated this possibility.

Female Sprague-Dawley rats (170–190 g) were fitted with a bipolar stimulating electrode in one neostriatum and a cannula in the ipsilateral GP as previously described (Crossman, Lee & Slater, 1977b). Biphasic pulses (0.1 – 0.3 mA; 25 Hz) caused contralateral head-turning. The duration of threshold stimulation needed for a 90° head-turn was recorded 10 times at 2 min intervals. Drugs were dissolved in 1 μl of saline and injected into GP following which the testing procedure was repeated.

When picrotoxin was injected into GP every animal produced an immediate decrease in the latency of the head-turn response (e.g. 2 μg: latency decrease −55%; $P < 0.001$; $n = 45$). This was a dose-dependent effect within the range 0.25 – 2.0 μg. No statistically significant effects were recorded following saline (1 μl) in GP. In contrast, the potent GABA agonist muscimol slowed the head-turn (e.g. 10 ng: +106%; $P < 0.001$; $n = 12$). A dose-related effect was obtained (1–25 ng). Slowing of the head-turn was also recorded following injection into GP of either 50 μg of GABA (+38%; $P < 0.05$; $n = 5$) or 10 μg of the GABA uptake inhibitor (−)-2,4-diaminobutyric acid (+31%; $P < 0.01$; $n = 19$).

Of the convulsants tested only (+)-tubocurarine (1 – 2 μg) appeared to mimic picrotoxin in facilitating the head-turn (e.g. 1 μg: −24%; $P < 0.001$; $n = 7$). Bicuculline slowed the head-turn response (e.g. 4 μg: +63%; $P < 0.001$; $n = 7$). A similar effect was observed with penicillin G (e.g. 4 μg: +54%; $P < 0.01$; $n = 5$). Leptazol (10 – 50 μg) had no effect on head-turning.

This animal model reveals a clear difference in the effects of convulsants in GP. (+)-Tubocurarine mimics the action of the GABA antagonist picrotoxin while bicuculline and penicillin G behave more like GABA agonists. Some analogous findings with these compounds have been reported by others using iontophoresis (Hill, Simmonds & Straughan, 1971, 1973; Krnjević, Puil & Werman, 1977). This disparity in the actions in GP of supposed GABA antagonists might be resolved by further studies on the morphology of inhibitory synapses and the nature of the GABA receptors in GP.

L.A.L. is an SRC student.

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Glial cell function and the GABA-feedback hypothesis

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An impressive body of evidence now suggests that glial cells of the CNS have far more important functions than a simply 'supportive' role.

Kuffler & Nicholls (1966) suggested that glia may play a significant role in neurotransmission by taking up potassium ions released during prolonged depolarisation of neurones. Increased glial cell potassium stimulates production of GABA which may be translocated back to nerve endings to modulate neurotransmitter release by interaction with presynaptic receptors (Tower & Young, 1973).

We have attempted to separate neurones and glial cells from mammalian CNS with a view to determining differences in specificity of drug action. In the course of the study we have detected differences in the two general cell types which may support the GABA hypothesis mentioned above.

Slices of rat cerebral cortex were incubated for 30 min at 37°C in an imidazole/HCl buffer (50 mM, pH 7.4) containing sucrose (0.5 M) and glucose (10 mM) and then forced through nylon bolting cloth (130 μm pore size) to dissociate the cells. The resulting suspension was layered on to a discontinuous sucrose gradient of 0.9, 1.45 and 2.0 M sucrose and centrifuged at

100,000 *g* for 30 minutes. The fraction lying at the 0.9/1.45 M interface contained glial cells while the fraction at the 1.45/2.0 M interface contained neurones.

Microscopy revealed some possible contamination of the neuronal fraction by glia or debris but no neurones could be seen in the glial fraction. The respiratory rates of the two fractions were similar (500 nmol O_2.mg protein $^{-1}h^{-1}$) but the carbonic anhydrase (4.4 ± 0.2 μmol CO_2.mg protein $^{-1}h^{-1}$) and Na^+,K^+-ATPase (7.7 ± 2.3 μmol.Pi.mg protein $^{-1}h^{-1}$) activities of the glial fraction were two fold and seven fold higher respectively than those of the neuronal fraction. A manometric method (modification of Meldrum & Roughton 1933) was used for carbonic anhydrase and the method described previously (Gilbert & Wyllie, 1976) for ATPase. Sodium-activated magnesium-dependent ATPase activity (Gilbert & Wyllie, 1975) was evident in both fractions but only the activity of the neuronal fraction was sensitive to inhibition by the anticonvulsant sodium valproate (1mM).

The Na^+,K^+-ATPase activity of the glial fraction was markedly dependent on the medium potassium concentration in the range 0–20 mM whereas the activity of the neuronal fraction was not, and, in addition, the glial fraction exhibited potassium-activated ATP-hydrolysing activity which was not significant in the neuronal fraction.

These results suggest there are differences in enzyme activities in glial cells and neurones which may be linked to ion pumps; that the sensitivities of the cells to drugs differ, and in particular, that activities are detectable in the glial fraction which are compatible with the hypothesis that these cells are specialised for removing potassium ions from the interstitial fluid.

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Actions of γ-acetylenic GABA on single central neurones in rat

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γ-Acetylenic GABA (GAG; RMI 71645) is a potent enzyme-activated irreversible inhibitor of GABA-transaminase. It increases brain GABA concentrations (Jung, Lippert, Metcalf, Schechter, Böhlen & Sjoerdsma, 1977) and has a significant anticonvulsant effect (Schechter, Tranier, Jung & Sjoerdsma, 1977). However, at high dose levels (~250 mg/kg i.p. or 50 μg and above when administered by injection into the cerebral ventricles), GAG produces marked excitation, myoclonic jerks and occasionally convulsions (Palfreyman, Huot, Lippert & Schechter, 1978). We have investigated the effects of GAG on the activity of single neurones to see if an explanation for this stimulant effect can be found.

Extracellular recordings were made from GABA-sensitive cells in the medullary reticular formation and the nucleus accumbens of urethane-anaesthetised rats. Drugs were applied iontophoretically from multibarreled micropipettes. The majority of cells were firing spontaneously but some were driven by the continuous application of (±)-homocysteic acid.

GAG reduced the firing rate of approximately 25% of the cells studied. This effect was rapid in onset and its time-course was similar to that of the ejection current.

In a slightly larger proportion of neurones GAG clearly and reversibly antagonised the actions of GABA, an effect which sometimes considerably outlasted the period of GAG application. This antagonism could be overcome if the GABA ejection current was increased, indicating that the antagonism was competitive.

GAG's depression of neuronal firing rate would be expected to contribute to the anticonvulsant effects previously attributed solely to the established action of GABA-transaminase inhibition. The behavioural excitation seen at high dose levels, on the other hand, might reflect GABA antagonism at the single-cell level as described here.