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# Effect of centrally acting drugs on the uptake of γ-aminobutyric acid (GABA) by slices of rat cerebral cortex

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**Summary**

1. The effects of centrally acting drugs on the uptake of $^3$H-γ-aminobutyric acid (GABA) by slices of rat cerebral cortex have been studied.

2. Many centrally acting drugs at concentrations of 0·1–1·0 mM significantly inhibited the uptake of $^3$H-GABA by cortical slices, but the only classes of drugs in which all members consistently produced inhibition of uptake were the phenothiazines, tricyclic antidepressants, and butyrophenones.

3. The receptor blocking drugs; phentolamine, propranolol, thymoxamine, mepyramine, and diphenhydramine at concentrations of 0·5–1 mM also significantly reduced the uptake of $^3$H-GABA. However, atropine, hexamethonium and (+)-tubocurarine had little effect on the uptake of $^3$H-GABA by cortical slices.

4. Centrally acting drugs, which did not significantly inhibit $^3$H-GABA uptake, included barbiturates, local anaesthetics, hallucinogens, monoamine oxidase inhibitors, anticonvulsants, and convulsants (except picrotoxin).

5. Chlorpromazine, prochlorperazine, L-2,4,diaminobutyric acid, desmethylimipramine, and iprindole inhibited the uptake of $^3$H-GABA by 50% (IC50) at concentrations of 30–100 μM. The most potent inhibitor of $^3$H-GABA uptake was *p*-chloromercuriphenylsulphonate (IC50=18 μM).

6. With the exception of L-2,4,diaminobutyric acid, an outstanding characteristic of these drugs was their complete lack of specificity. Thus at the IC50 for GABA, *p*-chloromercuriphenylsulphonate, chlorpromazine, prochlorperazine, iprindole, desmethylimipramine, apomorphine and diphenylhydramine also inhibited the uptake of radioactive glycine, alanine, noradrenaline, and 5-hydroxytryptamine. The uptake of the latter two compounds was often inhibited to a greater extent than GABA, glycine and alanine.

7. Kinetic analysis indicated that the inhibition of $^3$H-GABA by *p*-chloromercuriphenylsulphonate, chlorpromazine, and desmethylimipramine was non-competitive. L-2,4,Diaminobutyric acid reduced the uptake of $^3$H-GABA by a 'mixed' type of inhibition.

8. The present results do not support the suggestion that some centrally acting drugs may produce their effects by reducing the uptake of GABA in the brain after its release from inhibitory nerve terminals. Conceivably, the design of compounds which interfere effectively with the mechanisms of GABA operated synapses may lead to the introduction of whole new groups of centrally acting drugs.

## Introduction

γ-Aminobutyric acid (GABA) is probably an important inhibitory synaptic transmitter substance in the central nervous system (Krnjević & Schwartz, 1967; Obata, Ito, Ochi & Sato, 1967; Krnjević, 1970; Hebb, 1970). However, the mechanism by which GABA is inactivated after its release from inhibitory nerve endings is unknown. It seems unlikely that enzymic degradation is of major importance, since inhibitors of GABA-oxoglutarate aminotransferase do not markedly potentiate or prolong neurally evoked inhibition or the inhibitory effects of iontophoretically applied GABA (Obata *et al.*, 1967; Krnjević & Schwartz, 1968).

The brain possesses a specific uptake process for GABA (Iversen & Neal, 1968) and this may provide an effective mechanism for terminating its physiological effects after its release from presynaptic nerve terminals (Iversen & Neal, 1968; Curtis, Duggan & Johnston, 1970; Goodchild & Neal, 1972). If such an inactivating system for GABA is important in the brain, then it is conceivable that some centrally acting drugs might produce their effects by reducing the uptake of GABA after its release from nerve endings. Presumably, such an action would tend to potentiate inhibitory processes in those areas of the brain where GABA is a transmitter substance.

In the present study, the effect of some centrally acting drugs on the GABA uptake system has been investigated by determination of their effects on the uptake of $^3$H-GABA by slices of rat cerebral cortex. A preliminary report of these experiments has been published previously (Harris, Hopkin & Neal, 1972).

## Methods

The uptake of $^3$H-GABA by slices of rat cerebral cortex was estimated as described previously (Iversen & Neal, 1968). Briefly, slabs of rat cortex were sliced with a mechanical tissue chopper to produce small pieces of tissue ($0{\cdot}1 \times 0{\cdot}1 \times$ approx. $2{\cdot}0$ mm) and slices equivalent to 10 mg wet weight were placed in 10 ml of oxygenated Krebs-bicarbonate medium. After preincubation with the drug for 15 min at 25° C, $^3$H-GABA was added to the medium to give a final concentration of $5 \times 10^{-8}$M ($0{\cdot}1$ μCi/ml). The incubation was then continued for a further 10 minutes. The slices were recovered by rapid filtration and washed twice with 5 ml of ice-cold medium. The tissue was then dissolved in Soluene Tm 100 (Packard) and the radioactivity was measured by liquid scintillation counting after the addition of 10 ml of phosphor (1% butyl PBD, Ciba, in toluene). The accumulation of radioactivity in the tissue was taken as a measure of GABA uptake, since in previous experiments in which the same incubation conditions were used, it was found that more than 97% of the radioactivity accumulated in cortical slices was unchanged $^3$H-GABA (Iversen & Neal, 1968; Iversen & Johnston, 1971). The temperature of 25° C was used as previous studies had shown this to be optimal for cortical accumulation of $^3$H-GABA (Iversen & Neal, 1968).

The effect of some of the drugs on the uptake of $^{14}$C-glycine ($6 \times 10^{-7}$M), $^3$H-L-alanine ($6 \times 10^{-7}$M), $^3$H-(±)-noradrenaline ($1{\cdot}3 \times 10^{-7}$M) and $^3$H-5-hydroxytryptamine ($5{\cdot}8 \times 10^{-8}$M) by cortical slices was also studied. The procedure was identical to that described for GABA, except that the medium used for experiments with noradrenaline and 5-hydroxytryptamine contained ascorbic acid ($1{\cdot}1$ mM) and the disodium salt of ethylene diamine tetra-acetic acid ($0{\cdot}13$ mM).

The tissue was preincubated with the drugs, as preliminary experiments showed that this procedure ensured maximum inhibition of $^3$H-GABA uptake in the incubation conditions described.

*Materials*

The incubation medium was oxygenated Krebs-bicarbonate Ringer of the following composition (g/l.):

NaCl 6·92, KCl 0·354, $CaCl_2$ 0·28, $MgSO_4$ 0·144, $KH_2PO_4$ 0·162, $NaHCO_3$ 2·1, D-glucose 2·0. The medium was continuously gassed with a mixture of oxygen (95%) and carbon dioxide (5%).

GABA-2,3-[$^3$H], specific activity 2 Ci/mmole, was obtained from New England Chemical GmvH, 5072 Dreieichenenhain, West Germany. Glycine-1-[$^{14}$C], specific activity 41·5 mCi/mmole; L-alanine-[$^3$H] (G), specific activity 100 mCi/mmole; 5-hydroxytryptamine-[$^3$H] (G) creatinine sulphate, specific activity 8·5 Ci/mmole; (±)-noradrenaline-7-[$^3$H], specific activity 12 Ci/mmole; were obtained from the Radiochemical Centre, Amersham, England.

## Results

TABLE 1. *Centrally acting drugs which significantly reduced the uptake of $^3$H-GABA by slices of rat cerebral cortex* ($P<0·05$). *Each result is the mean of 4–8 experiments. The* S.E. *of the means was in the range 5–10%. Control cortical slices accumulated $^3$H-GABA to produce tissue : medium ratios of* 41±2·1 (*mean*±S.E. *of mean of 6 determinations*)

| Drug | Concentration (mM) | Uptake (% of control) |
|---|---|---|
| Tricyclic antidepressants | | |
| Desipramine | 0·2 | 2 |
| Imipramine | 0·2 | 9 |
| Dichlorimipramine | 0·2 | 45 |
| Opipranol | 0·2 | 27 |
| Amytryptyline | 0·6 | 8 |
| 3,Dimethylaminoimipramine | 0·2 | 12 |
| Nortryptyline | 0·2 | 9 |
| Iprindole | 0·5 | 3 |
| Phenothiazines | | |
| Promethazine | 0·5 | 0 |
| Chlorpromazine | 0·5 | 1 |
| Methotrimeprazine | 0·5 | 0·5 |
| Trifluoperazine | 0·5 | 0·5 |
| Fluphenazine | 0·5 | 0·8 |
| Thioridazine | 0·5 | 0·8 |
| Thioproperazine | 0·5 | 0·9 |
| Perphenazine | 0·5 | 3 |
| Prothipendyl | 0·5 | 9 |
| Prochlorperazine | 0·5 | 27 |
| Butyrophenones | | |
| Haloperidol | 1·0 | 0 |
| Fluoropipamide | 1·0 | 66 |
| Droperidol | 1·0 | 62 |
| Spiperone | 1·0 | 14 |
| Fluanisone | 1·0 | 1 |
| Moperone | 1·0 | 44 |
| Miscellaneous | | |
| Chlordiazepoxide | 0·5 | 82 |
| Diazapam | 0·5 | 14 |
| Tetrabenazine | 0·5 | 60 |
| Amiphenazol | 1·0 | 64 |
| Picrotoxin | 0·5 | 71 |
| Methadone | 1·0 | 12 |
| Pethidine | 1·0 | 69 |
| Levorphanol | 0·5 | 62 |
| Amantadine | 0·5 | 72 |
| Apomorphine | 0·5 | 4 |

### *Centrally acting drugs*

Many centrally acting drugs significantly reduced the uptake of $^3$H-GABA by cortical slices. However, the only classes of drugs in which all members consistently produced inhibition of uptake were the phenothiazines, tricyclic antidepressants, and butyrophenones (Table 1).

The centrally acting drugs that had no significant effect on $^3$H-GABA uptake by cortical slices are shown in Table 2. These included barbiturates, local anaesthetics, hallucinogens, monoamine oxidase inhibitors, anticonvulsants and convulsants (except picrotoxin).

TABLE 2. *Centrally acting drugs which did not significantly reduce the uptake of $^3$H-GABA by slices of rat cerebral cortex. Drugs were tested at a concentration of* 1·0 mM *except tetrahydrocannabinol and bulbocapnine which were tested at* 16 μM *and* 0·2 mM *respectively*

| | |
|---|---|
| Morphine | Phenytoin |
| Acetylsalicylic acid | Phenobarbitone |
| Mescaline | Amylobarbitone |
| Tetrahydrocannabinol | Chloral hydrate |
| Bulbocapnine | Reserpine |
| Phenelzine | α-Methyl-*m*-tyrosine |
| Leptazol | |
| Bicuculline | |
| Strychnine | |

Some of the drugs which were relatively effective inhibitors of $^3$H-GABA uptake are known to block peripheral synapses or to be local anaesthetics. Therefore the effect of some local anaesthetics and receptor blocking drugs on the uptake of $^3$H-GABA by the cortex was investigated. The results are summarized in Table 3. The uptake of $^3$H-GABA by cortical slices was greatly reduced by phentolamine, propranolol, thymoxamine, mepyramine, and diphenhydramine at concentrations of 0·5–1·0 mM. Atropine had a small but significant inhibitory effect, whilst hexamethonium, (+)-tubocurarine and methysergide were without action. Cocaine and procaine did not affect $^3$H-GABA uptake when used at a concentration of 1·0 mM.

TABLE 3. *Effect of receptor blocking drugs and local anaesthetics on uptake of $^3$H-GABA by slices of rat cerebral cortex. Each result is the mean of* 6 *to* 10 *experiments.* *=($P<0.05$). *The* S.E. *of the means was in the range* 4–10%

| Drug | Concentration (mM) | $^3$H-GABA uptake (% control) |
|---|---|---|
| Thymoxamine | 0·5 | 25* |
| Phentolamine | 1·0 | 15* |
| Propranolol | 0·5 | 51* |
| Diphenhydramine | 1·0 | 37* |
| Mepyramine | 1·0 | 17* |
| Hexamethonium | 1·0 | 97 |
| Atropine | 0·5 | 76* |
| (+)-Tubocurarine | 2·0 | 86 |
| Procaine | 1·0 | 95 |
| Cocaine | 1·0 | 94 |

### *Structural analogues and enzyme inhibitors*

The most active analogue was L-2,4,diaminobutyric acid, which at a concentration of 0·1 mM, reduced the uptake of $^3$H-GABA to 43% of the control values. β-Guanidinopropionic acid was also a relatively potent inhibitor of GABA uptake, inhibiting uptake to 44% of the controls, but the 2,3 isomer of diaminobutyric

acid and allylglycine had only small inhibitory effects at a concentration of 0·1 mM (Table 4) reducing the uptake of GABA to 71% and 61% of the controls respectively. The enzyme inhibitor, *p*-chloromercuriphenylsulphonate was the most potent inhibitor tested in the present experiments and at a concentration of 0·1 mM this compound reduced the uptake of $^3$H-GABA to 4·9% of the controls. These results are very similar to those obtained by Iversen & Johnston (1971) in their more extensive study of GABA analogues.

TABLE 4. *Effect of GABA analogues and enzyme inhibitors on uptake of $^3$H-GABA by slices of rat cerebral cortex. Each result is the mean of 6 to 8 experiments. The* S.E. *of the means was in the range 3–11%. All these compounds significantly inhibited the uptake of $^3$H-GABA* ($P<0.05$)

| Compound | Concentration (mM) | $^3$H-GABA uptake (% control) |
|---|---|---|
| *p*-Chloromercuriphenylsulphonate | 0·1 | 5 |
| L-2,4,Diaminobutyrate | 0·1 | 43 |
| L-2,3,Diaminobutyrate | 0·1 | 71 |
| β-Guanidinopropionate | 0·1 | 44 |
| Allyl-glycine | 1·0 | 61 |
| β-Hydroxy-γ-aminobutyrate | 0·1 | 41 |

## *Comparison of potencies of inhibitors*

Eight compounds representative of the different classes of drugs found to be relatively effective inhibitors of $^3$H-GABA uptake were tested over a range of concentrations to determine an IC50 value (Fig. 1) (i.e. the concentration of drug

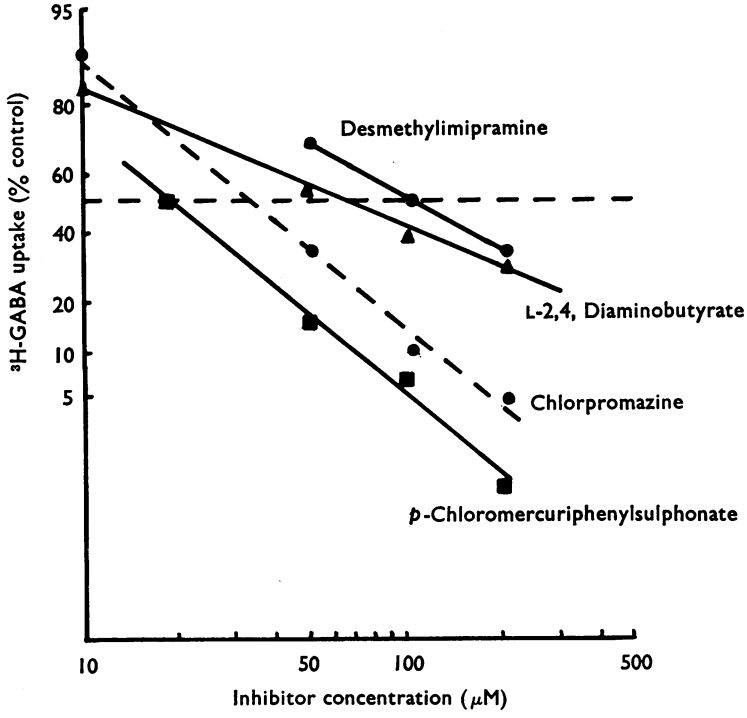



FIG. 1. Determination of IC50 values for inhibitors of $^3$H-GABA uptake in rat cerebral cortex. Each point is the mean of at least four determinations in which the tissue was preincubated for 15 min with various concentrations of the drug and then for 10 min after the addition of $^3$H-GABA ($5\times10^{-8}$M). The uptake of $^3$H-GABA is plotted on a probability scale against the inhibitor concentration (log scale) to obtain the IC50 value=inhibitor concentration producing 50% inhibition of $^3$H-GABA uptake.

which inhibited the uptake of $^3$H-GABA by 50%). These results allowed the compounds tested to be placed in order of potency and showed that the most potent inhibitors of uptake were *p*-chloromercuriphenylsulphonate and chlorpromazine (Table 5). Prochlorperazine, L-2,4,diaminobutyric acid, iprindole, desmethylimipramine and apomorphine were also relatively potent inhibitors of uptake. The only receptor blocking drug tested, diphenhydramine, was at least an order of magnitude less potent than chlorpromazine in its effectiveness in inhibiting $^3$H-GABA uptake.

### *Comparison of specificity of inhibitors*

The specificity of the inhibitors was tested by measuring the effect of each drug, at its IC50 for GABA, on the uptake of $^{14}$C-glycine, $^3$H-L-alanine, $^3$H-5-hydroxytryptamine and $^3$H-(±)-noradrenaline. The results, which are summarized in Table 5, show that only L-2,4,diaminobutyric acid specifically inhibited the uptake of $^3$H-GABA by cortical slices. The other drugs tested, all significantly inhibited the

TABLE 5. *Effect of drugs on $^3$H-GABA uptake by slices of rat cerebral cortex expressed as the concentration of drug which reduced the uptake by 50% (IC50), and the effect of these drugs on the uptake of $^{14}$C-glycine (gly), $^3$H-alanine (ala), $^3$H-5-hydroxytryptamine (5-HT) and $^3$H-noradrenaline (NA) when applied to the tissue at the IC50 for GABA*

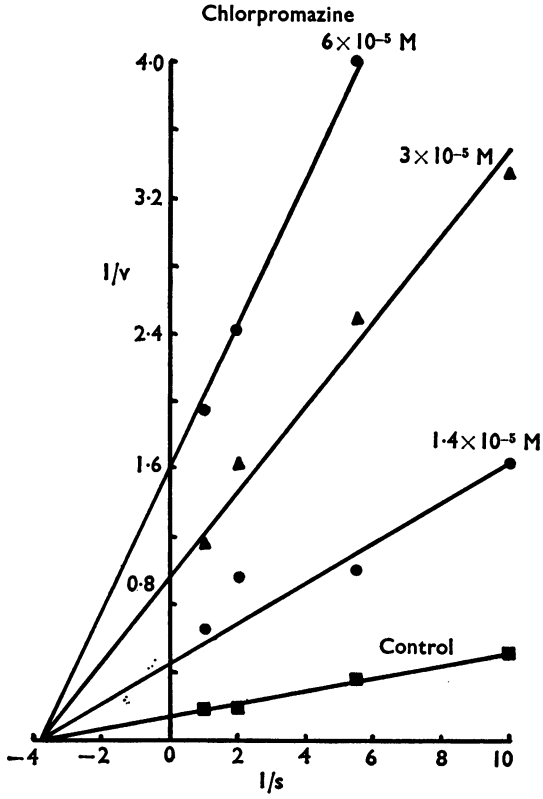
| Compound | IC50 (μM) | Uptake (% of control) at IC50 for GABA | | | |
|---|---|---|---|---|---|
| | $^3$H-GABA | $^{14}$C-Gly | $^3$H-Ala | $^3$H-5-HT | $^3$H-NA |
| *p*-Chloromercuriphenylsulphonate | 18 | 29·6 | 45·7 | 33·1 | 24·9 |
| Chlorpromazine | 32 | 52·4 | 59·5 | 7·8 | 10·9 |
| Prochlorperazine | 50 | 27·1 | 53·2 | 7·8 | 45·5 |
| L-2,4,Diaminobutyric acid | 66 | 95·1* | 98·0* | 86·4* | 92·9* |
| Iprindole | 78 | 25·0 | 42·4 | 6·1 | 12·6 |
| Desmethylimipramine | 100 | 27·5 | 48·2 | 0 | 7·0 |
| Apomorphine | 130 | 42·2 | 57·6 | 84·5 | 0 |
| Diphenhydramine | 370 | 38·2 | 40·8 | 6·0 | 40·0 |

The results are the means of four to six experiments and are significantly different from the controls ($P<0.05$) except those marked * which are not significantly different from controls. The S.E. of the means were less than 10%.

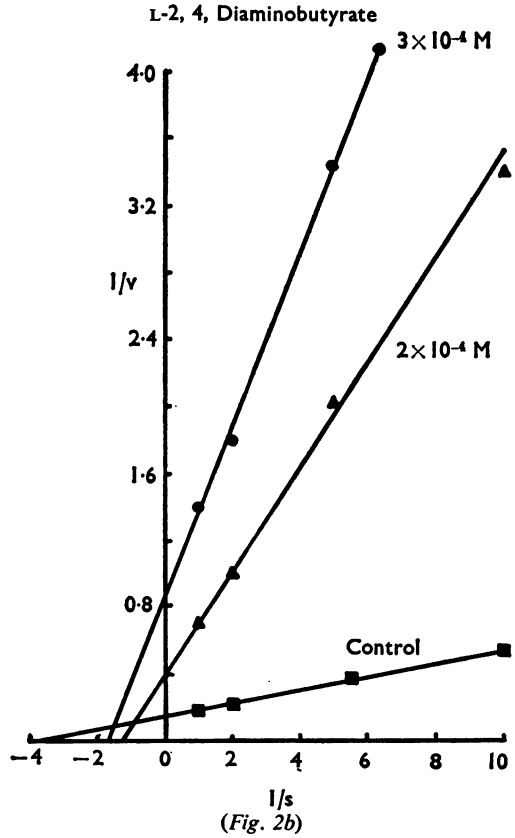
uptake of radioactive glycine, alanine, 5-hydroxytryptamine and noradrenaline. These drugs at the IC50 for GABA appeared to reduce the uptake of $^3$H-alanine to about the same extent as GABA but usually had a rather greater inhibitory effect on the uptake of $^{14}$C-glycine. The effect of the inhibitors on the uptake of biogenic amines was more variable; *p*-chloromercuriphenylsulphonate inhibited the uptake of 5-hydroxytryptamine and noradrenaline to about the same degree as the amino acids but, as might be expected, chlorpromazine and desmethylimipramine virtually abolished the uptake of 5-hydroxytryptamine and noradrenaline. Iprindole also inhibited the uptake of both noradrenaline and 5-hydroxytryptamine by cortical slices.

### *Kinetic analysis of inhibition*

The mode of action of the drugs inhibiting the uptake of $^3$H-GABA was not investigated, but a few of the drugs were examined in more detail to determine the type of inhibition involved. In these experiments, the rate of $^3$H-GABA uptake was estimated in the presence of several fixed concentrations of the inhibitor, over a range of $^3$H-GABA concentrations. Double reciprocal plots were then used in an attempt to determine whether the inhibition was competitive or non-competitive. The inhibition produced by *p*-chloromercuriphenylsulphonate, chlorpromazine and


Chlorpromazine
6×10⁻⁵ M
3×10⁻⁵ M
1·4×10⁻⁵ M
Control
4·0
3·2
2·4
1·6
0·8
1/v
−4
−2
0
2
4
6
8
10
1/s

(*Fig. 2a*)


L-2, 4, Diaminobutyrate
3×10⁻⁴ M
2×10⁻⁴ M
Control
4·0
3·2
2·4
1·6
0·8
1/v
−4
−2
0
2
4
6
8
10
1/s

(*Fig. 2b*)

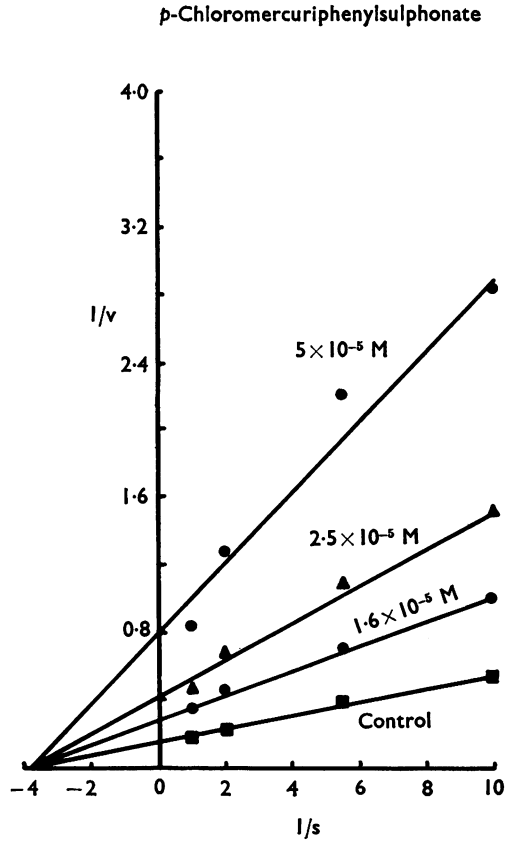



FIG. 2. Kinetic analysis of GABA uptake inhibition on slices of rat cerebral cortex. Uptake of $^3$H-GABA (v) was determined after incubation of tissue slices for 10 min in media containing various concentrations (s) of GABA in the presence or absence of several fixed concentrations of inhibitors. Each point is the mean value of at least four experiments. Units of v are $10^{-8}$ (mol/min)/g, and s=$10^{-4}$M.

desmethylimipramine was non-competitive (Fig. 2). Although L-2,4,diaminobutyric acid closely resembles GABA structurally, the inhibition of $^3$H-GABA uptake produced by this compound was of the mixed type (Fig. 2).

## Discussion

The present studies show that at relatively high concentrations many centrally acting drugs reduce the accumulation of $^3$H-GABA by slices of rat cerebral cortex. The mechanisms involved have not been studied, but the effects seem unlikely to be due to general metabolic depression of the tissue, since Gottesfeld & Elliott (1971) have shown that chlorpromazine and imipramine at concentrations similar to those used in the present experiments do not inhibit the oxygen uptake of brain slices. Probably the major effect of drugs on the accumulation of radioactive GABA is inhibition of the active uptake mechanism for GABA which is present in the mammalian central nervous system (Elliott & van Gelder, 1958; Iversen & Neal, 1968). However, as considerable exchange of $^3$H-GABA with the endogenous GABA stores may occur (Iversen & Neal, 1968; Neal & Iversen, 1969; Goodchild & Neal, 1972), it is possible that drugs might influence the net uptake of $^3$H-GABA

by affecting the efflux of the amino acid from the tissue. For this reason, the tissue was preincubated for 15 min to allow at least partial equilibration between the endogenous amino acid pools and the external medium, and the incubations with $^3$H-GABA were restricted to 10 min to minimize the influence of $^3$H-GABA efflux on the total amount of radioactivity accumulated by the tissue. It seems unlikely that effects on GABA metabolism affected the uptake of radioactivity, since previous experiments have shown that under the present conditions of incubation (i.e. small amount of tissue, 10 mg, large volume of medium, 10 ml) virtually all the radioactivity in the tissue is present as $^3$H-GABA (Iversen & Neal, 1968; Iversen & Johnston, 1971). In the present study, no attempt was made to determine whether drugs produced changes in the endogenous GABA concentration of the tissue.

The inhibitory actions of GABA on neurones may be terminated by re-uptake into nerve endings, neurones or neuroglia, since autoradiographic studies have shown that all three structures may accumulate $^3$H-GABA (Hökfelt & Ljungdahl, 1970; Ehinger & Falck, 1971; Bloom & Iversen, 1971; Lam & Steinman, 1971; Neal & Iversen, 1972). Thus, drugs could conceivably produce effects on the central nervous system by inhibiting the uptake processes for GABA present in these neural elements. However, such a mechanism of action seems unlikely for any of the centrally acting drugs tested in the present study because even the phenothiazines and tricyclic antidepressants, which were some of the most potent inhibitors of GABA uptake, required concentrations of 0·03 mM to 0·1 mM to inhibit the uptake of $^3$H-GABA by 50%. In contrast, the uptake of noradrenaline and 5-hydroxytryptamine by brain slices is similarly inhibited by much lower concentrations ($10^{-8}$ to $10^{-6}$M) of these drugs (Shaskan & Snyder, 1970; Ross, Renyi & Ogren, 1971).

The results with chlorpromazine and imipramine confirm other reports of the inhibitory action of these drugs on GABA uptake both by brain slices (Iversen & Johnston, 1971; Gottesfeld & Elliott, 1971) and crustacean nerve-muscle preparations (Iversen & Kravitz, 1968) and also on GABA binding to particles in mouse brain homogenates (Sano & Roberts, 1963).

The most potent inhibitor of GABA uptake was the enzyme inhibitor *p*-chloromercuriphenylsulphonate, which inhibited the uptake of $^3$H-GABA by 50% at a concentration of 18 $\mu$M. This compound when applied to neurones iontophoretically, potentiates the inhibitory actions of applied GABA and glycine (Curtis, *et al.*, 1970). These results are consistent with the suggestion that the uptake processes for glycine (Neal & Pickles, 1969; Neal, 1971; Johnston & Iversen, 1971) and GABA in the central nervous system may be concerned with inactivating the inhibitory amino acids following their release from nerve endings.

An outstanding characteristic of the drugs studied was their complete lack of specificity. Thus, in addition to inhibiting GABA uptake, *p*-chloromercuriphenylsulphonate, chlorpromazine, prochlorperazine, iprindole, desmethylimipramine, apomorphine and diphenhydramine, also inhibited the uptake of radioactive glycine, alanine, 5-hydroxytryptamine and noradrenaline. In general, these drugs inhibited the uptake of different amino acids to about the same extent. However, the uptake of 5-hydroxytryptamine and noradrenaline was often inhibited to a much greater extent. For example, desmethylimipramine at the IC50 for GABA virtually abolished the uptake of 5-hydroxytryptamine and noradrenaline. Not surprisingly

in view of their lack of specificity, kinetic analysis indicated that *p*-chloromercuriphenylsulphonate, chlorpromazine and desmethylimipramine inhibited the uptake of GABA non-competitively. It seems, therefore, that these drugs are capable of interfering with a large number of uptake mechanisms in the brain, although *in vivo*, the phenothiazines and the tricyclic antidepressants would presumably affect noradrenaline and 5-hydroxytryptamine uptake preferentially, as these amines are inhibited at much lower concentrations than would be required to interfere with amino acid uptake. Apart from any direct actions these compounds may have on neuronal activity, their lack of specificity suggests that they are unlikely to assist in identifying amino acid transmitters at synapses.

The only specific inhibitor of GABA uptake by cortical slices was L-2,4,diaminobutyric acid. This compound was a relatively potent inhibitor of GABA uptake (IC50=66 $\mu$M) and had no significant effect on the uptake of radioactive alanine, glycine, 5-hydroxytryptamine or noradrenaline. L-2,4,Diaminobutyric acid resembles GABA structurally, but the inhibition of GABA uptake by this compound was of the mixed type and not competitive. Although L-2,4,diaminobutyric acid inhibits the uptake of GABA specifically, it would not appear to be suitable for use in experiments designed to demonstrate the release of GABA from the brain, since it is reported to have its own inhibitory actions, when applied to neurones (Curtis & Watkins, 1960). However, this amino acid may be worth reinvestigation, as its inhibitory effects when applied to neurones iontophoretically, may be due to an indirect action, such as displacement of endogenous GABA, or inhibition of the uptake of GABA released from nerve endings.

The results of the present study suggest that it is unlikely that any of the centrally acting drugs at present available act primarily by inhibiting the uptake of GABA by the brain. If GABA indeed proves to be an important inhibitory transmitter in the central nervous system, then conceivably the design of compounds which interfere with the mechanisms of GABA operated synapses may lead to the introduction of whole new groups of centrally acting drugs.

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