Br. J. Pharmac. (1973), **49**, 37–51.

A comparative study of some convulsant substances as γ-aminobutyric acid antagonists in the feline cerebral cortex

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Summary

1. By the use of microiontophoretic techniques, quantitative estimates were obtained of the depressant effects of γ-aminobutyric acid (GABA) on single feline cortical neurones.

2. Picrotoxin, bicuculline, strychnine, (+)-tubocurarine, penicillin and leptazol were also applied microiontophoretically to single neurones. Sequential GABA applications were made before, during and after the microiontophoresis of these substances and any effects on the time course of the GABA depression were measured as an estimate of antagonism or potentiation of GABA.

3. (+)-Tubocurarine was found to be a potent GABA antagonist. Picrotoxin and bicuculline were rather less potent and strychnine and penicillin only weakly active as GABA antagonists. Leptazol appeared to be inactive against GABA depressions.

4. In addition, bicuculline and strychnine were found to be capable of potentiating the depressant action of GABA. This property was not shared by the other substances studied.

5. All the substances studied produced changes in neuronal firing rate that did not correlate with GABA antagonism.

6. In conclusion, several potent convulsants have been shown to be capable of GABA antagonism. It is not yet clear that this effect, rather than a direct effect on neuronal excitability, is the prime mechanism behind their convulsant properties.

Introduction

γ-Aminobutyric acid (GABA) is now widely accepted to be a major inhibitory transmitter in the cerebral cortex (Krnjević, 1970). As part of the search for a greater understanding of cortical inhibitory processes, the present investigation was undertaken into substances which might be capable of specifically antagonizing GABA. The substances so far reported to be GABA antagonists include bicuculline, picrotoxin, (+)-tubocurarine and penicillin (Curtis, Duggan, Felix & Johnston, 1970; Engberg & Thaller, 1970; Hill, Simmonds & Straughan, 1971; 1972a,b; Curtis, Game, Johnston, McCulloch & MacLachlan, 1972). All of these drugs are convulsant when applied topically to the cerebral cortex (Ajmone-Marsan, 1969; Banerjee, Feldberg & Georgiev, 1970; R. G. Hill,

unpublished observations) and it, therefore, seemed reasonable to compare these drugs with other known convulsants.

Since previous studies of GABA antagonism have been largely qualitative, a major objective of the present investigation was to make quantitative assessments of the relative potencies of different GABA antagonists when applied by the microiontophoretic technique. A limited number of the experiments included here were also the basis of a previous report on the quantitative method (Hill & Simmonds, 1973). In addition, certain other aspects of the findings have been communicated in preliminary form (Hill *et al.*, 1971 ; 1972a, b).

Methods

Investigations were performed on 50 adult cats (2·2 to 3·5 kg) of both sexes, anaesthetized with nitrous oxide and halothane in oxygen, and prepared as described previously (Hill & Simmonds, 1973). The continuity of blood flow in the pial blood vessels was examined from time to time with a stereoscopic dissecting microscope. Where respiration was judged to be inadequate, intermittent positive pressure ventilation was employed and arterial blood gas measurements showed that these animals stayed within the limits of pH, pCO_2, and pO_2 observed in spontaneously respiring animals.

The glass micropipettes, containing aqueous solutions of the drugs under test, were prepared and used as described by Hill & Simmonds (1973). Extracellular action potentials were recorded from neurones at all depths from 200 to 2,000 μm in the middle suprasylvian gyrus. As in previous studies (Straughan, Neal, Simmonds, Collins & Hill, 1971 ; Hill *et al.*, 1972a, b) the majority of neurones did not fire spontaneously and were, therefore, driven with a continuous minimal iontophoretic application of L-glutamate or DL-homocysteate. GABA depressions were obtained with rates of iontophoresis judged to produce complete inhibition of neuronal firing within approximately 60 seconds. Repeated applications of the same currents of GABA were made with an interval of one minute between the end of one application and the beginning of the next, as this was found to be an adequate period allowing a full return of the unit to control firing levels. In all experiments described, both GABA and the antagonist under test were applied from adjacent barrels of the same micropipette. The antagonist was applied continuously whilst the repeated applications of GABA were continued as before and, where possible, each GABA application was maintained until firing was completely inhibited. Following termination of the application of an antagonist, the applications of GABA were repeated until the responses had returned to control values.

Drugs

Amino-acids were all used at a concentration of 0·2 M and adjusted to a suitable pH for iontophoresis ; sodium L-glutamate (BDH) and sodium DL-homocysteate (Calbiochem) pH 8 with NaOH ; GABA (Sigma) and glycine (BDH) pH 3·5 with HCl.

The convulsants were expelled by iontophoresis or, when they were of limited dissociation, by a combination of iontophoresis and electro-osmosis (Curtis, 1964). The solutions were prepared as follows: bicuculline (K and K or Pierce Chemical)

5 mM, pH 3·5 in 150 mM NaCl; strychnine sulphate (Hopkin and Williams) 5 mM, pH 3·5 or neutral in 150 mM NaCl; picrotoxin (Sigma) 5 mM in 150 mM NaCl, no adjustment of pH; (+)-tubocurarine chloride (Burroughs Wellcome) 5 mM in 75 mM NaCl or saturated in distilled water, no adjustment of pH; leptazol (pentamethylenetetrazole) (Emanuel) 100 mg/ml i.e. approx. 1 M, no adjustment of pH; sodium benzyl penicillin (Crystapen-Glaxo) 150 mg/ml i.e. approx. 0·5 M, no adjustment of pH. Bicuculline and picrotoxin were characterized as pure samples by N.M.R. and mass spectrometry.

A barrel containing 1 M NaCl was used for current balancing and the recording barrel contained 3 M NaCl. All drug solutions were rendered particle free by centrifugation prior to filling the micropipettes.

Results

A total of 535 neurones were examined and all were found sensitive to the depressant effects of GABA. Of these, a sample of 272 were selected for antagonism studies, the selection criteria being a stable firing pattern and rate and a consistent response to GABA during several control applications. All neurones in this sample received applications of at least one postulated antagonist and, in addition, on thirty neurones dose-response relationships were established for two or sometimes three antagonists. A useful GABA antagonist should be readily expelled from a micropipette and the substances studied appeared to show a wide variation in their behaviour in this respect. In an attempt to give an estimate of the relative utility of the drugs studied, a comparison was made of the total number of neurones to which the postulated antagonist was applied and the number of neurones from which meaningful dose-response relationships for that substance were obtained. The percentage of neurones giving dose-response relationships was expressed as the percentage utility of the substance in question (see Table 1). This value takes into account blocking of the micro-

TABLE 1. *Comparison of success rate in microiontophoretic use of various drugs*

Drug	Total no. of neurones	Full study completed on	% Utility
Bicuculline	87	57	66
Strychnine	30	17	57
Picrotoxin	55	42	77
(+)-Tubocurarine	44	35	80
Leptazol	29	20	69
Penicillin	27	23	85

pipette, the depressant or destructive effects of drugs and loss of neurones due to relative movement of the pipette tip and recorded unit. Laboratory conditions and electrochemical properties of individual micropipettes obviously influenced these values but, although we would not necessarily expect the values themselves to be reproducible in another laboratory, we might expect that the relative utility of one substance compared with another would be reproducible.

Antagonism and potentiation

The ability of bicuculline to both antagonize (Curtis *et al.*, 1970) and potentiate (Godfraind, Krnjević & Pumain, 1970; Straughan *et al.*, 1971) GABA on cerebral cortical neurones has been well described in the literature and will not be considered in detail, other than in the final analysis of data.

Picrotoxin is less well documented and, although previously reported as inactive on cerebral cortical neurones (see Krnjević, 1970), it has now been demonstrated to possess GABA antagonist properties at this site (Hill *et al.*, 1972a). The method employed in the present study is illustrated in Fig. 1(A), which also shows antagonism of the depressant effects of GABA by picrotoxin. The action

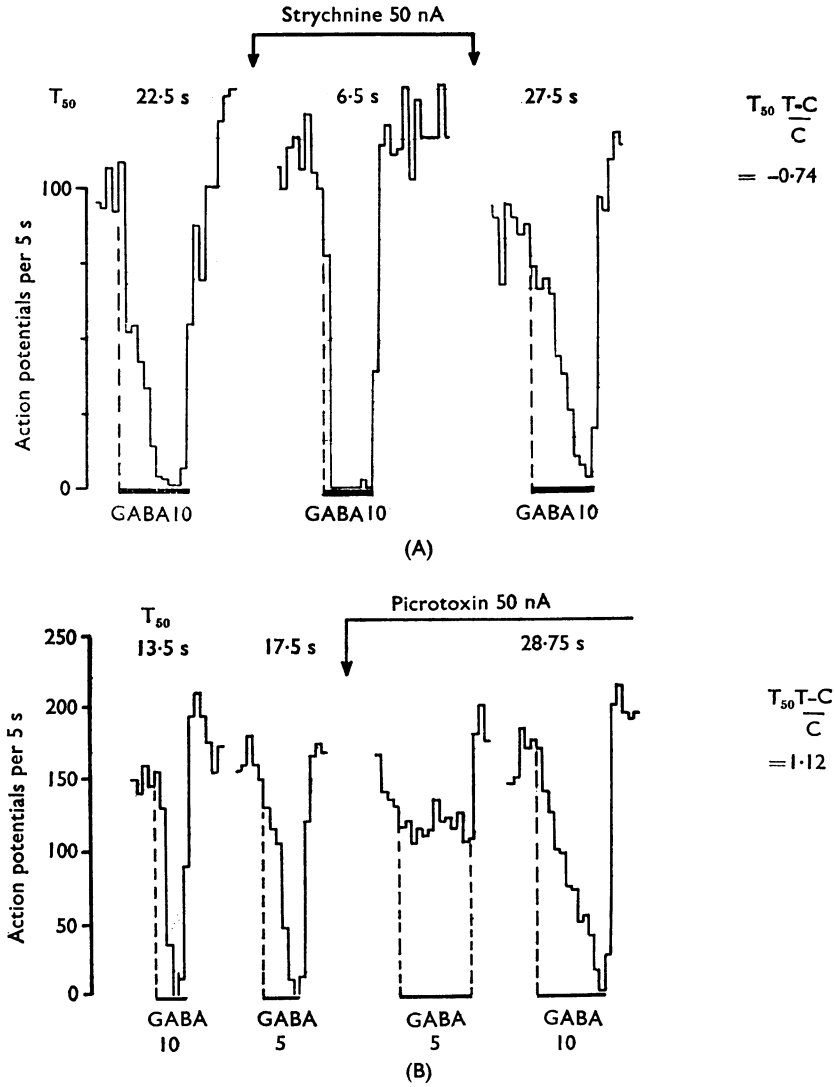

FIG. 1. Action potential counter traces showing depression of neuronal firing produced by microiontophoretic application of γ-aminobutyric acid (GABA). (A) Neurone in the left suprasylvian gyrus at a depth of 1,761 μm, driven by a continuous application of 10 nA of glutamate. Note that the time to reach 50% depression of firing (T_{50}) was shorter for the higher current of GABA (10 nA). Continuous application of picrotoxin, indicated by the arrow and bar, attenuated the depression produced by both high and low currents of GABA. In the case of the higher current of GABA this was seen as a prolongation of the time to achieve 50% depression of firing. (B) Trace for a second cortical neurone, found at a depth of 1,231 μm and firing spontaneously. The time course of the depression due to GABA 10 nA was clearly accelerated during the application of strychnine 50 nA, indicated by the arrows and bar. Clear recovery indicates the reversibility of this effect.

potential counter trace shows clearly that both 5 nA and 10 nA of GABA produced 100% depression of the firing of this neurone under control conditions. During the concurrent application of picrotoxin 50 nA, it was impossible to produce more than 30–40% depression of firing with GABA 5 nA, whereas 10 nA of GABA produced a 100% depression after a prolonged period of application. Where a plateau response such as that shown for 5 nA of GABA is taken as a quantitative measure of the effectiveness of the applied dose of picrotoxin, there are certain problems of interpretation, elegantly pointed out by Curtis, Duggan & Johnston (1971). For this reason we have chosen to use high currents of GABA which produce 100% depression throughout and to use the time-course of the depression as representing a cumulative dose-response relationship, since we believe this to have certain advantages (see Hill & Simmonds, 1973). For each application of GABA, therefore, we have measured the time taken to reach 50% depression of firing (T_{50}). In contrast to picrotoxin, strychnine often potentiated the depressant effects of GABA. In Fig. 1(B), the action potential counter trace shows clearly that the time-course of the GABA depression was accelerated during the application of strychnine.

Displacements of GABA response-time curves along the time axis have been expressed in the form: T_{50}(test)$-T_{50}$(control)/T_{50} (control), i.e. $T_{50}(T-C)/C$. T_{50}(test)$-T_{50}$ (control) was divided by T_{50} (control) to allow for the rate of rise

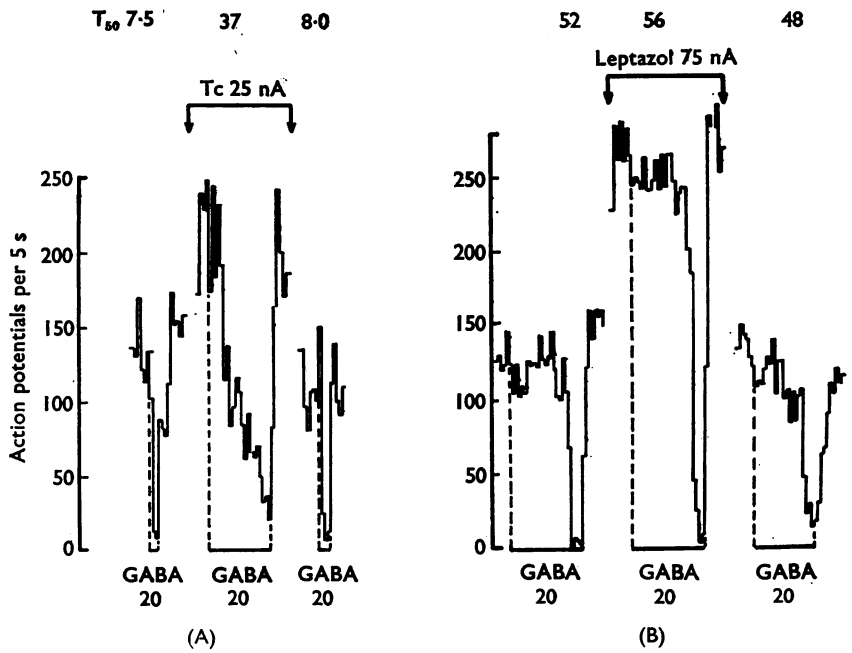

FIG. 2. (A). Neurone located at 1,115 μm in cerebral cortex, driven with a continuous application of glutamate 25 nA. The time course of the GABA depression of this unit was clearly prolonged during the iontophoresis of (+)-tubocurarine (Tc) 25 nA, and the firing rate of the neurone was considerably increased. Both these effects were seen to be completely reversible. $T_{50}(T-C)/C$ for this experiment was 3·80 (see text). (B). A second cortical neurone was used for this experiment. It was found at a depth of 900 μm and was firing spontaneously. Iontophoresis of leptazol (75 nA) produced a large increase in the firing rate, which completely reversed when the leptazol application was terminated. The time-course of GABA depression of this unit was little affected by the applications of leptazol, i.e. $T_{50}(T-C)/C=0{\cdot}14$ (see text).

of GABA concentration at the neurone (Hill & Simmonds, 1973). Experience with a large number of neurones and many replicate GABA applications has shown that $T_{50}(T-C)/C$ values in excess of 0·25 are likely to represent 'genuine' antagonisms and those negative values greater than −0·25 'genuine' potentiations. The larger the value the greater the effect. Both the displacements shown in Fig. 1 would therefore be well within the range of genuine effects.

Amongst the other substances that we have studied, (+)-tubocurarine (Tc) seemed likely to antagonize GABA (Hill *et al.*, 1972b) and also showed a marked excitant effect on cortical neurones. In Fig. 2, action potential counter records for both (A) Tc and (B) leptazol showed that both can have excitant effects. Inspection of the effects on GABA depression showed that only Tc was antagonizing GABA, even though the increase in firing rate produced by leptazol was more pronounced than that produced by Tc. These traces were obtained from two different cortical neurones and the marked differences in the time-course of control GABA responses on the two neurones probably reflect differences in neurone-pipette distances. It is clear that stable GABA responses can be obtained under both near and relatively distant recording situations when the similarity between control and recovery T_{50} values is compared in each example. In Fig. 3, a similar example of Tc antagonizing GABA is shown, in this case illustrated by dose-response curves rather than the action potential counter trace. Penicillin was also applied to the same neurone and did not produce any antagonism of GABA. Inspection of the trace of control firing rate *vs* time showed that both penicillin and Tc produced equivalent increases in firing rate.

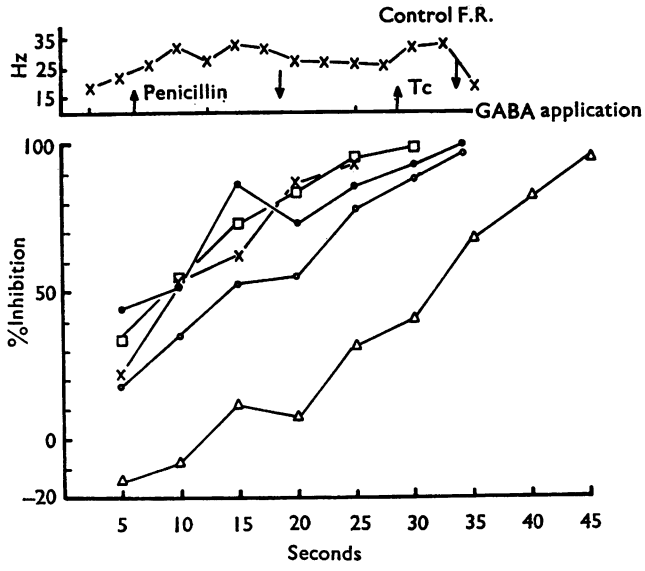

FIG. 3. Dose-response curves demonstrating the effects of penicillin and (+)-tubocurarine (Tc) on the depression of a cortical neurone by γ-aminobutyric acid (GABA) (10 nA). The upper part of the figure is a plot of the control firing rate in the 30 s period immediately preceding each GABA application. Penicillin and Tc were applied where indicated by the arrows. In the lower graph, mean dose-response curves are plotted at various times during the experiment: ×——× control, □——□ penicillin 50 nA, ○——○ recovery, △——△ Tc 25 nA, ●——● recovery. Although firing rate increases produced by the two drugs are clearly comparable, inspection of the dose-response curves reveals that only Tc shifted the GABA response line to the right, indicating antagonism. ($T_{50}(T-C)/C$ for Tc=1·70. The penicillin $T_{50}(T-C)/C$ was −0·16, well within the ±0·25 no effect range.)

Results obtained from all neurones to which antagonists were applied are shown in Fig. 4 as frequency distributions of antagonist potency in the form: $T_{50}(T–C)/C$ *vs* % of total number of neurones studied for each drug. From these results it appeared that leptazol was unlikely to antagonize GABA and that penicillin had only weak GABA antagonist properties (see also Curtis *et al.*,

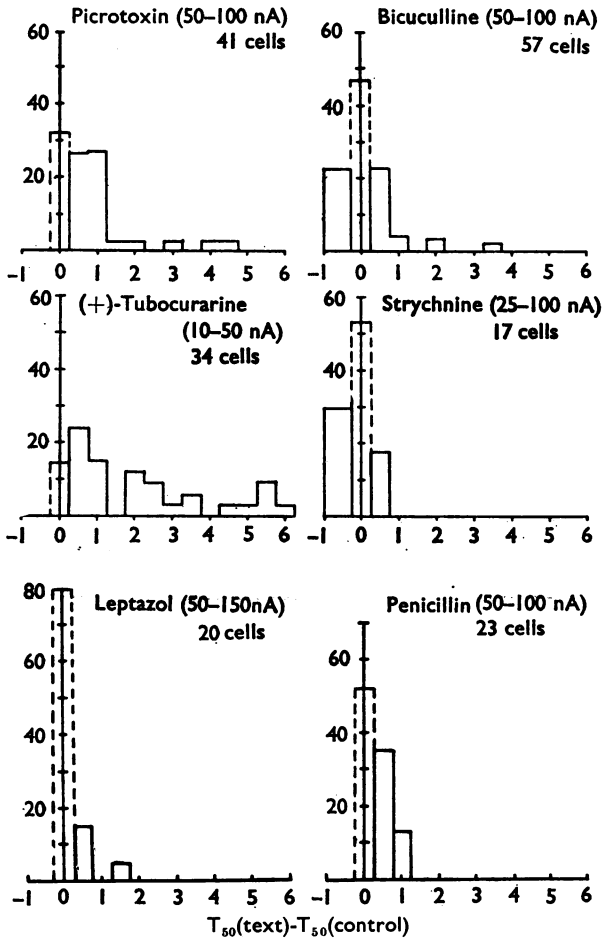

FIG. 4. Frequency distribution histogram for the $T_{50}(T–C)/C$ values obtained with all six substances studied. The vertical axis indicates, in each case, the percentage of the total neurones studied lying within each histogram block. The horizontal axis indicates $T_{50}(T–C)/C$ values, those lying to the left of the vertical axis showing potentiation and those to the right showing antagonism. The dotted column at the centre of each histogram represents the ±0·25 no effect range (see text).

1972). Strychnine, on the other hand, appeared more likely to potentiate the actions of GABA than to antagonize them under our experimental conditions. Clearly, the three most potent GABA antagonists were bicuculline, picrotoxin and Tc, although there was also a high incidence of GABA potentiations by bicuculline.

TABLE 2. *Comparison of the iontophoretic potencies of γ-aminobutyric acid (GABA) antagonists*

Drug	*n*	Mean iontophoretic current (nA)	Antagonism of GABA $T_{50}(T-C)/C$
Bicuculline	18	69±7	0·89±0·19
Picrotoxin	28	56±3	1·23±0·22
(+)−Tubocurarine	29	31±3*	2·34±0·37*

Each value is the mean±S.E.M. of individual values obtained only in those experiments where GABA was antagonized i.e. $T_{50}(T-C)/C>0·25$. *Significantly different ($P<0·05$) from corresponding values for bicuculline or picrotoxin.

Statistical comparisons of the iontophoretic potencies of bicuculline, picrotoxin and Tc as GABA antagonists are shown in Table 2. For each drug, the potency was determined as the mean $T_{50}(T-C)/C$ obtained only in those experiments where GABA was antagonized, i.e. $T_{50}(T-C)/C>0·25$. On this basis, the iontophoretic potency of Tc was significantly greater than that of either bicuculline or picrotoxin and this was achieved with significantly lower currents of application than were used for the latter two drugs. The precise applying currents used for each drug were determined empirically. If the first current used had no clear effect, then the current was increased until an antagonism or potentiation of GABA was produced or the neurone was depressed or excited by the test drug to a level at which we could no longer work. In the case of picrotoxin and bicuculline there was a limit to the current that could be passed through barrels containing these drugs, and this also functioned as a controlling factor. (+)-Tubocurarine, on the other hand, usually antagonized GABA depression with lower currents than those which produced problems with passage of current. It must be remembered, however, that these comparisons of iontophoretic potency do not necessarily indicate the relative molar potencies as there are variations in the rate at which the respective antagonists are expelled from a micropipette for a given current (see **Discussion**). However, in terms of practical microiontophoresis, Tc was the most potent and useful antagonist that we tested.

Firing rate effects

To investigate the relationship between GABA antagonism and change in firing rate caused by the antagonists, a representative sample of neurones from each group in Fig. 4 has been plotted in Fig. 5 as $T_{50}(T-C)/C$ *vs* % change in control firing rate. This figure demonstrates quite clearly that bicuculline had a mixture of depressant and excitatory effects, as did leptazol. Penicillin and Tc had predominantly excitatory effects, whereas picrotoxin and strychnine appeared more likely to depress neuronal firing rates. Overall there was no significant correlation between change in firing rate and effects on GABA depressions.

Firing rate effects were always dose-related, with the higher currents of Tc, penicillin and bicuculline tending to produce more excitation. The higher currents of picrotoxin and strychnine tended to be depressant, as noted by other workers (Galindo, 1969; Johnson, Roberts & Straughan, 1970), and lower currents of strychnine than those needed to influence the GABA response were frequently excitatory. Leptazol tended to have mixed effects and high currents could produce both excitation and depression, although the predominant effect in the dose range we have used was excitation.

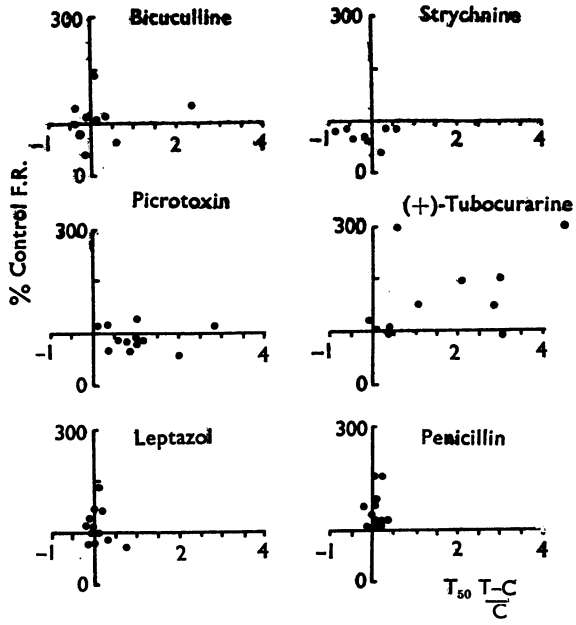

FIG. 5. Representative values of $T_{50}(T-C)/C$ for each substance, abstracted from Fig. 4, are plotted against their respective effects on neuronal firing rate (F.R.), in order to examine any possible correlation between GABA antagonism and change in firing rate. Correlation coefficients for each group were calculated, but there were no statistically significant correlations. (+)-Tubocurarine was the substance giving the highest correlation coefficient. ($R=0{\cdot}5167$, $P<0{\cdot}1>0{\cdot}05$.)

pH of drug solutions

Although six convulsant substances have been studied, only two appeared to produce potentiation of GABA depressions. These two substances, strychnine and bicuculline, were also the only two used at an acid pH (3·5). Accordingly we investigated the action of H^+ ions applied by iontophoresis from a solution of HCl/NaCl at pH 2–3 on the responses to GABA of seven cortical neurones. In none of these experiments was antagonism or potentiation of the GABA response observed, even though the rate and duration of applications of H^+ ions was frequently sufficient to produce destructive effects on the neurone being studied. In addition, strychnine also produced potentiation of GABA depression when used at neutral pH. We therefore concluded that potentiation of GABA responses was unlikely to be related to the pH of antagonist solutions in the micropipette.

Control agonists

An important point that has not been considered so far is the specificity of these agents as GABA antagonists. Glycine and glycine-like amino acids can be used as control agonists on cat cortical neurones with moderate success (Curtis *et al.*, 1970; Curtis, Duggan, Felix, Johnston & McLennan, 1971), although recent reports on rat cortical neurones would suggest that it is not always easy to separate antagonism of GABA and glycine (Biscoe, Duggan & Lodge, 1972). In an attempt to use glycine as a control agonist in our present study, it was

applied to 34 cortical neurones. It was considerably less potent than GABA, confirming the observations of other workers (Kelly & Krnjević, 1969; Johnson *et al.*, 1970), and the responses were not reproducible. A typical glycine response is illustrated in Figure 6. This clearly shows the common finding that recovery from the depressant effects of glycine had started before the iontophoretic current was terminated. For these reasons we considered it an unsuitable control substance for quantitative evaluation of GABA antagonists in the cerebral cortex. Glycine was also applied continuously to four neurones whilst GABA applications were made and no effect was seen on the GABA depression.

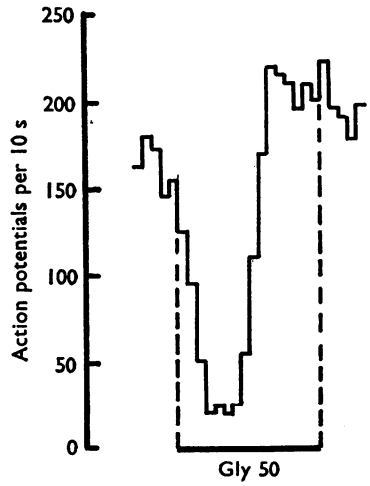

FIG. 6. Response of a cortical neurone, depth 2,127 μm, to microiontophoretic application of glycine 50 nA. Firing of this neurone was sustained by a continuous application of glutamate 25 nA. Note that recovery from the depressant effects of glycine had started before the application of glycine was terminated and that 100% depression was not achieved (see text).

Discussion

Six convulsant substances have been examined as potential antagonists of the depressant effects of GABA on feline cortical neurones. Four of these, bicuculline, picrotoxin, (+)-tubocurarine and penicillin, were clearly capable of antagonizing GABA, although bicuculline could also potentiate GABA. Strychnine more frequently potentiated GABA than antagonized it whilst leptazol was predominantly without effect.

Potency comparisons for these substances, in terms of the iontophoretic currents with which they were applied, must be circumspect as the actual mode of release (Curtis, 1964) determines the amount of drug expelled for a given current. Bicuculline, picrotoxin and strychnine were expelled largely by electro-osmosis and thus equivalent amounts of each should be expelled for a given current. Penicillin and leptazol, on the other hand, were expelled by direct iontophoresis and no true comparison of the amount released is possible, as transport numbers (Curtis, 1964) for these substances are at present unknown.

(+)-Tubocurarine was applied by iontophoresis from an aqueous solution in preliminary investigations (Hill *et al.*, 1972b), but subsequently it has been expelled by electro-osmosis from a saline solution with no apparent alteration in the

magnitude of its effect. In practical terms, release characteristics may sometimes be the main determinant of iontophoretic potency as a drug of moderate intrinsic potency which is readily released by iontophoresis is likely to show greater activity than a drug of higher intrinsic potency which is expelled from a micropipette only with extreme difficulty.

Picrotoxin has been shown to be a potent GABA antagonist at invertebrate synapses (Robbins & van der Kloot, 1958; Takeuchi & Takeuchi, 1969; Earl & Large, 1972) and some workers have found it active on mammalian central neurones (Galindo, 1969; Engberg & Thaller, 1970; ten Bruggencate & Engberg, 1971; Hill *et al.*, 1972a), whereas others have found it inactive (Krnjević, Randić & Straughan, 1966; Curtis, Duggan & Johnston, 1969). The reported inconsistencies of pictrotoxin as a GABA antagonist may be due to differences in technique and practical difficulties resulting from the low stability of picrotoxin solutions and the low solubility of the active picrotoxinin moiety (Bryan & Marshall, 1948; Ramwell & Shaw, 1963). In agreement with previous observations (Engberg & Thaller, 1970; ten Bruggencate & Engberg, 1971), a wide variation was seen in the effectiveness of picrotoxin-containing pipettes, even though they were filled with fresh picrotoxin solution and used on the day of preparation. When a pipette was found to contain pharmacologically active picrotoxin, however, consistent GABA antagonism could easily be demonstrated. Inactive pipettes were discarded after one or two trials and this may explain the rather higher percentage of antagonisms in our results as compared with those of Engberg & Thaller (1970), although the fact that we were studying different populations of neurones must not be discounted. Failure of earlier studies with picrotoxin may have been due to insufficient pipettes being filled to ensure an active sample, coupled with a low number of neurones studied (e.g. Krnjević *et al.*, 1966), but even more exhaustive studies have given negative results (Curtis *et al.*, 1969). In our hands, picrotoxin appeared to be an effective GABA antagonist provided that problems of expulsion of sufficient concentration of active drug from the micropipette could be surmounted. There was no correlation between change in neuronal firing rate and GABA antagonism and, additionally, no evidence of the interaction between iontophoretic glutamate and picrotoxin described by Galindo (1969) for cuneate neurones.

Bicuculline was first described as a GABA antagonist by Curtis *et al.* (1970) and this has subsequently been confirmed by other workers. More recently it has been demonstrated that bicuculline can also have potentiating effects on GABA depressions (Godfraind *et al.*, 1970; Straughan *et al.*, 1971) in addition to its antagonistic properties. Although picrotoxin showed no tendency to potentiate GABA, it is of similar potency to bicuculline as a GABA antagonist. An initial assessment (Hill *et al.*, 1972a) suggested that picrotoxin was the more potent but statistical analysis of the present results from a larger group of neurones shows that the relative iontophoretic potencies are indistinguishable ($P>0{\cdot}1$). Firing rate changes were more common with bicuculline than with picrotoxin, although there was no correlation between either potentiation or antagonism and firing rate. Sometimes bicuculline would produce destructive changes in the firing pattern resulting in the loss of the neurone under study. This effect was the principal reason for the rather lower percentage utility of bicuculline as compared with some of the other substances examined (Table 1).

Strychnine has been shown to antagonize glycine depression of spinal and cortical neurones (Curtis, Hösli & Johnston, 1968; Johnson *et al.*, 1970) and, in addition, large currents of strychnine can have some antagonistic effects on GABA responses in the cortex (Johnson *et al.*, 1970; Biscoe *et al.*, 1972). The present study confirms that strychnine can exert a weak GABA antagonist action and also indicates that it may sometimes potentiate GABA depressions. It is possible that the potentiating action of bicuculline may be due to a resemblance to strychnine which is not shared by the other substances studied and may be accounted for by direct interference with ion movement through the neuronal membrane, a property already suggested for strychnine by Araki (1965). This may explain the fact that strychnine had even more marked effects on neurones than bicuculline, producing changes in firing pattern and spike shape which are reflected in its low percentage utility.

(+)-Tubocurarine has only recently been shown to possess GABA antagonist properties (Hill *et al.*, 1972b) but has previously been observed to antagonize the depressant effects of acetylcholine, 5-hydroxytryptamine and noradrenaline (see Phillis, 1970, for review). The value of (+)-tubocurarine as a GABA antagonist would obviously be limited by such a lack of specificity but it should be noted that picrotoxin has also been shown to antagonize noradrenaline-induced depression of thalamic neurones (Phillis & Tebēcis, 1967) and that both picrotoxin and bicuculline will potently antagonize depression of cortical neurones by 5-hydroxytryptamine (D. W. Straughan, unpublished observations). The effects of (+)-tubocurarine on firing rate were generally excitatory and, to some extent, were related to the applying current. Although visual examination of the results suggested a positive correlation between increase in firing rate and GABA antagonism (Fig. 5) this was not statistically significant ($P<0{\cdot}1>0{\cdot}05$) and it was possible to find examples of large increases in firing rate without GABA antagonism and *vice versa*. Iontophoretically, (+)-tubocurarine was the most potent GABA antagonist that we studied, being significantly more potent than picrotoxin ($P<0{\cdot}05$) and bicuculline ($P<0{\cdot}01$). It was relatively easy to use and had a high percentage utility.

Penicillin has long been known to produce convulsions when applied topically to the cerebral cortex (Ajmone-Marsan, 1969) and it has now been shown to reduce the sensitivity of cortical neurones to microiontophoretic GABA (Clarke & Hill, 1972). More specific interference with the action of GABA was demonstrated in the amphibian spinal cord by Davidoff (1972) and recently Curtis *et al.* (1972) have shown that microiontophoretically applied penicillin is capable of weak GABA antagonism. In our hands, preliminary experiments with low expelling currents of penicillin (50 nA) showed no antagonism of GABA, whereas subsequent work with higher currents (100 nA) provided examples of weak GABA antagonism. After some twelve hours of use, it was possible to withdraw aliquots of solution from the micropipettes and demonstrate convulsive activity in the penicillin, when applied topically to the cerebral cortex. As the intact penicillin molecule is necessary for its convulsant action (Gutnick & Prince, 1971), it must be concluded that an estimate of four hours (Curtis *et al.*, 1972) for the viability of penicillin-filled pipettes is rather too short under our experimental conditions. Clear GABA antagonism was obtained up to one week after filling the pipettes and we conclude that the controlling factor in the effectiveness of

penicillin as a GABA antagonist is the magnitude of the expelling current. Overall, the potency of penicillin was low and not correlated with firing rate. It is interesting to compare the results with those for (+)-tubocurarine which had similar actions on firing rate.

Leptazol, although well known to be convulsant, has at present no known mode of action (see Esplin & Zablocka-Esplin, 1969). Other workers have found it without activity as a GABA antagonist (Krnjević *et al.*, 1966) and our results would agree with this finding, any apparent antagonisms coming into a population small enough to be accounted for by chance. Our pipettes obviously released a pharmacologically active drug as evidenced by both increases and decreases in firing rate. Occasional destructive effects on the neurone under study occurred and this is illustrated by the rather low percentage utility. A note of caution must be sounded, however, as leptazol is far less potent a convulsant than, for instance, picrotoxin (Banerjee *et al.*, 1970). Indeed, a sufficient concentration of leptazol to produce GABA antagonism may not be achieved in the area of the neurone when the drug is applied by iontophoresis.

Although five of the six substances studied may be described as GABA antagonists, that is not to say that any of the six substances were active solely by combination with the cortical GABA receptor. It would be easy to suggest alternative mechanisms by which the effectiveness of GABA could be reduced (see Clarke & Hill, 1972). The weaker antagonists we have studied have all been suggested to have non-specific effects. It has been suggested that strychnine has direct effects which can result in membrane depolarization (Ajmone-Marsan, 1969); similar properties have been attributed to penicillin (Ayala, Lin & Vasconetto, 1970) and recent work on leptazol (Gross & Woodbury, 1972) suggests that this substance may act by direct depolarization, linked to an increase in extracellular K^+ concentration. These properties may account for the firing rate changes seen with these drugs.

Biochemical effects of the convulsants examined may be important as alterations in removal or metabolism of GABA may influence its effective concentration at the receptor. However, the substances studied are all without marked effect on GABA uptake (G. G. S. Collins & Judy Hopkin, unpublished observations; Harris, Hopkin & Neal, 1973) and GABA transaminase (L. J. Fowler, M. S. Starr & Irene Sutton, unpublished observations). Such biochemical effects would, therefore, seem to be of little importance in the action of these drugs.

In conclusion we have found that several potent convulsants can function as GABA antagonists. However, all the convulsants studied had marked effects upon neuronal firing rate that were not correlated with GABA antagonism. Therefore, it is by no means certain that GABA antagonism is the sole mechanism by which these substances cause electrographic seizures when applied topically to the cerebral cortex.

We thank Mr. C. J. Courtice and Mr. T. Gonye for skilled technical assistance. This work was supported through a grant from the M.R.C. to D.W.S. and by a grant for equipment from the Wellcome Trust. R. G. H. is a Wellcome Research Student.

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(*Received November* 13, 1972)