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ANTAGONISM OF γ-AMINOBUTYRIC ACID AND GLYCINE BY CONVULSANTS IN THE CUNEATE NUCLEUS OF CAT

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1 Some convulsant substances have been applied to single neurones in the cat cuneate nucleus by microiontophoresis. Numerical values were derived for the effectiveness and selectivity of the substances as antagonists of γ-aminobutyric acid (GABA) and glycine.

2 (+)-Bicuculline methochloride was the most effective GABA antagonist and it also excited many neurones. It antagonized GABA in 93% of experiments but also antagonized glycine in 41% of experiments. In most experiments the antagonism of GABA was greater than the antagonism of glycine resulting in an overall selective antagonism of GABA that was statistically significant. Nevertheless, in only about one quarter of the individual experiments was the GABA antagonism substantial and the selectivity clearcut.

3 (+)-Bicuculline and picrotoxin were less easily applied to neurones by microiontophoresis and were found to antagonize GABA in 30% and 35% of experiments, respectively. They also antagonized glycine in 25% and 30% of experiments, respectively. Overall, neither substance could be shown to be selective, statistically, although in the few individual experiments where the GABA antagonism was substantial the antagonism was clearly selective.

4 (+)-Tubocurarine antagonized GABA in 59% and glycine in 32% of experiments and it also excited many neurones. Penicillin antagonized GABA in 33% of experiments without antagonizing glycine. Neither antagonist caused any substantial antagonisms of GABA and neither showed significant selectivity overall. (−)-Bicuculline methochloride, leptazol and bemegride antagonized GABA or glycine in less than 10% of experiments, although (−)-bicuculline methochloride excited most neurones.

5 Strychnine antagonized glycine in every experiment while antagonizing GABA in only 5% of experiments. In each individual experiment the antagonism of glycine was substantial and clearly selective, resulting in a statistically significant selectivity overall.

6 It is concluded that the selective glycine antagonist strychnine is considerably better than the presently available GABA antagonists for distinguishing between responses to GABA and glycine, when the antagonists are applied by microiontophoresis.

Introduction

In an earlier paper (Hill, Simmonds & Straughan, 1973a), we compared a number of convulsant substances as antagonists of γ-aminobutyric acid (GABA) in the cerebral cortex of cats. We were unable to examine the selectivity of these antagonists in distinguishing between GABA and glycine-evoked depressions of neuronal firing because glycine had so little effect on cortical neurones. We have, therefore, made further experiments in the cuneate nucleus, an easily accessible area where the neurones can be readily depressed by both GABA and glycine.

Of the substances we have examined, strychnine is well known as an antagonist of glycine and it has frequently been reported that picrotoxin and (+)-bicuculline will antagonize GABA (e.g. Galindo, 1969; Bruggencate & Engberg, 1971; Curtis, Duggan, Felix, Johnston & McLennan, 1971a; Curtis, Duggan & Johnston, 1971b). (+)-Bicuculline methochloride and (+)-tubocurarine are two more recently introduced antagonists of GABA (Johnston, Beart, Curtis, Game, McCulloch & MacLachlan, 1972; Hill *et al.*, 1973a; Collins & Hill, 1974) and we have also used three other convulsants, penicillin, leptazol and bemegride, whose mechanisms of action are less well elucidated.

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We have attempted to measure the effectiveness of these substances as antagonists of GABA and glycine and also to assign numerical values to their selectivity in distinguishing between the two agonists. To do this, we have used an analytical procedure (Hill & Simmonds, 1973; Simmonds, 1974) which is different from those used by previous investigators. It was hoped that such a study would allow comparison of the GABA receptors in the cuneate nucleus with those in the cerebral cortex and might also provide some clues to the mechanism of action of the convulsant substances. Furthermore, it has become clear that some quantitative comparisons of antagonist potency and selectivity *in vivo* are required to assist in the task of identifying GABA and glycine receptors *in vitro* (e.g. Young & Snyder, 1973; Zukin, Young & Snyder, 1974).

Methods

Surgical procedure

Experiments were made on a total of 26 cats of either sex weighing 2.0 to 3.5 kg and anaesthetized with 0.8 to 1.2% halothane in 70% N_2O and 30% O_2. Initially, the animals breathed spontaneously but, later, positive pressure artificial ventilation was sometimes applied, especially if neuronal firing became irregular. Rectal temperature was maintained at 37.5 ± 1°C.

The cat's head was mounted in a stereotaxic frame and flexed ventrally by 30°. A midline incision was made through the skin from the centre of the head down the nape of the neck. The cervical musculature was teased apart and the atlanto-occipital membrane exposed and removed. Since the head was flexed, a pressor foot (diameter 6 mm) could be placed centrally on the exposed area of medulla just caudal to the obex without removing the cerebellum. This substantially reduced movement of the underlying cuneate nucleus without any visible impairment of blood flow in the surface vessels. The pressor foot contained holes to allow the passage of microelectrodes. There was a continuous flow of c.s.f. over the surface of the tissue.

Experimental procedure

Seven-barrel glass micropipettes with tip diameters of 5–7 μm were prepared as described previously (Hill & Simmonds, 1973). Extracellular recordings of firing rate were made from neurones in the cuneate nucleus at depths of 0.6 to 2.6 mm from the surface of the medulla. No attempt was made to select neurones on the basis of their anatomical projections or firing patterns but all were depressed by GABA and glycine. Drugs were expelled from the micropipettes by iontophoresis and the experimental technique adopted was similar to that described before (Hill *et al.*, 1973a). Briefly, GABA and glycine were applied alternately with a period of 60 s elapsing between the end of one application and the beginning of the next. Each application was maintained until complete depression of neuronal firing was achieved, the expelling current being selected to give 50% reduction in firing rate in not less than 10 s and usually not more than 60 seconds. Once reproducible control responses were obtained, as characterized by the duration of amino acid application required to give 50% inhibition of firing (T_{50}), a 10–12 min continuous application of one of the convulsant drugs was made while the sequence of amino acid applications continued. Recovery from any effects of the drug was then followed.

Drugs

The drug solutions used in the pipettes were as follows: sodium DL-homocysteate (Calbiochem) or sodium L-glutamate (BDH), pH 8, 0.2 M; GABA (Sigma), pH 3.5, 0.2 M; glycine (BDH), pH 3.5, 0.2 M; (+)-bicuculline (K & K or Pierce Chemical), pH 3.5, 5 mM in 150 mM NaCl; strychnine sulphate (Hopkin & Williams), 5 mM in 150 mM NaCl; picrotoxin (Sigma), 5 mM in 150 mM NaCl; (+)-tubocurarine chloride (Burroughs Wellcome), 5 mM in 75 mM NaCl; (+)-bicuculline methochloride, 5 mM in 150 mM NaCl, and (−)-bicuculline methochloride 5 mM in 150 mM NaCl (both kindly prepared by Dr J.F. Collins, see Collins & Hill (1974)); leptazol (Emanuel), 1 M; bemegride (Nicholas), 30 mM in 150 mM NaCl; sodium benzyl penicillin (Crystapen-Glaxo), 0.5 M. Where no pH value is given, the pH of the drug solution was not adjusted.

All drugs were retained in the pipettes between periods of expulsion by appropriate retaining currents. For ionized drugs, 25 nA was usually sufficient although 40 nA was required for glycine. For drugs in NaCl solution, 10 or 15 nA was used.

Results

Recordings were obtained from 173 neurones. All the neurones were firing spontaneously and all were depressed by both GABA and glycine. Satisfactory studies with antagonists were completed on 89 neurones and more than one antagonist was studied on 42 of these. With nearly all neurones, the spontaneous firing rate was sufficiently high and steady for the study of depressant substances; on only eight neurones were continuous low currents of L-glutamate or DL-homocysteate used to increase the firing rates.

The currents of GABA and glycine generally found adequate were 20, 30 or 40 nA and, in most experiments, equal currents of the two amino acids

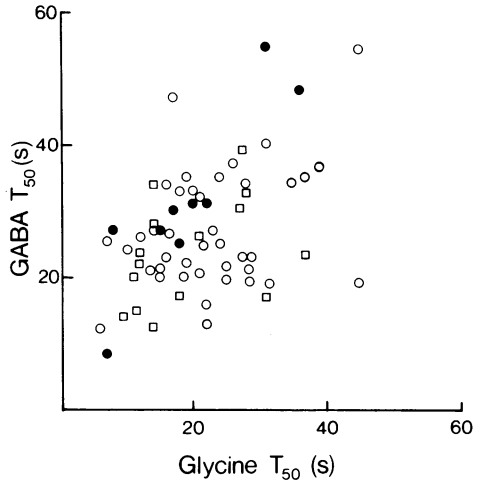

Figure 1 Comparison of the durations (s) of iontophoretic application of γ-aminobutyric acid (GABA) and glycine required to achieve 50% inhibition of neuronal firing (T_{50}). Each of the 64 points was obtained from a separate neurone. The same currents were used to apply both GABA and glycine in individual experiments, the values being 20 nA (□), 30 nA (●) or 40 nA (○).

were used. For a representative sample of 64 neurones, the times taken to achieve 50% inhibition of firing (T_{50}) with GABA and glycine are correlated in Figure 1. There was no clear difference in the effectiveness of GABA and glycine in depressing these neurones. In eight experiments, the control periods of repeated alternate applications of GABA and glycine extended to 20 min or more and during this time the responses showed little variation in T_{50}. All the values for GABA fell within ±20% of the mean in each experiment and 95% of the values for glycine fell within ±25% of the mean. Thus, in the studies of antagonists, we have regarded changes in T_{50} values of less than 25% as 'no effect'.

A typical record of the effects of GABA and glycine on the firing rate of a neurone is shown in Figure 2(a). When the retaining current was removed from the strychnine barrel to allow strychnine to diffuse out, the depressant effect of glycine was progressively antagonized. This was apparent as a slowing of the time course of the glycine response indicating that a higher concentration of glycine was needed to achieve a given level of response (see Hill & Simmonds, 1973; Simmonds, 1974). Eventually, sufficient antagonism had occurred that a glycine expelling current of 30 nA was inadequate to suppress completely the firing of the neurone. At the same time, the responses to GABA were unaffected. The T_{50} value for each response was determined and the values are plotted against elapsed time for the entire experiment in Figure 2(b). The progressive antagonism of glycine by strychnine is clearly seen to be selective and, in other experiments where the strychnine application was maintained for longer, the glycine response was completely suppressed without the response to GABA being affected.

Only occasionally did the antagonists of GABA show such clearcut selectivity. One of our best examples is illustrated in Figure 3 where (+)-bicuculline methochloride reversibly antagonized GABA. Compared with the antagonism of glycine by strychnine, the degree of GABA antagonism by (+)-bicuculline methochloride always reached a plateau much more rapidly and the recovery from antagonism was generally much quicker. The greater the degree of GABA antagonism achieved, the more selective the antagonism appeared to be. The marked excitation caused by (+)-bicuculline methochloride was frequently seen but was not a necessary concomitant of GABA antagonism.

In order to compare the effectiveness of all the substances tested as antagonists of GABA and glycine, the degree of antagonism achieved in each experiment was expressed as T_{50} (test-control)/T_{50} (control) (see Hill & Simmonds, 1973). The T_{50} (test) values were obtained from the responses to GABA and glycine recorded 6–10 min after the start of a continuous application of the test substance. The T_{50} (control) values were means of values obtained just before the application of test substance and values obtained after at least 6 min recovery from the test substance. The value of T_{50} (test-control)/T_{50} (control) obtained for the displacement of the GABA response was plotted against the value obtained for displacement of the glycine response in the same experiment. The accumulated results obtained with (+)-bicuculline methochloride, (+)-bicuculline, picrotoxin, strychnine, (+)-tubocurarine, (−)-bicuculline methochloride, leptazol, bemegride and penicillin are shown in Figure 4. Values in the range −0.25 to +0.25 on the ordinate scale represent no effect on the GABA response and the same range of values on the abscissa scale represents no effect on the glycine response. Larger positive values indicate antagonism and negative values indicate potentiation.

It is apparent from these results that (+)-bicuculline methochloride (25–50 nA) was the most consistently effective GABA antagonist amongst the substances tested, antagonizing GABA in 93% of experiments. In nearly half of those experiments the antagonism was sufficient to give a value of T_{50} (test-control)/T_{50} (control) in excess of 1.0. The second most consistent GABA antagonist was (+)-tubocurarine (25–50 nA) (59% of experiments), but in no experiment was the antagonism sufficiently great to give a value in excess of 1.0. Next came picrotoxin (50–100 nA), penicillin (75–100 nA) and (+)-bicuculline (50–100 nA) which

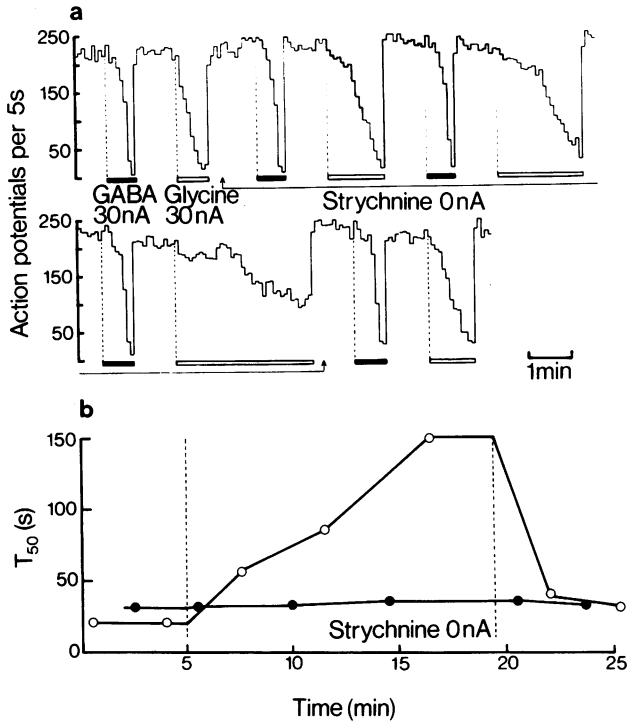

Figure 2 The selective antagonism of glycine by strychnine. (a) Histogram representation (5 s epochs) of the spontaneous firing rate of a neurone 1370 μm deep in the cuneate nucleus. Alternate micro-iontophoretic applications of γ-aminobutyric acid (GABA) 30 nA and glycine 30 nA were made throughout. Strychnine 0 nA was applied by removing the retaining current of −15 nA, thus allowing it to diffuse out of the pipette for the period indicated. (b) Time to 50% inhibition of neuronal firing (T_{50}) was measured for each response to GABA (●) and glycine (○) and plotted against elapsed time for the entire experiment.

antagonized GABA in 35%, 33% and 30% of experiments, respectively. In spite of these rather low figures, about one quarter of the picrotoxin antagonisms and two thirds of the (+)-bicuculline antagonisms had values in excess of 1.0. Strychnine (0–2 nA), (−)-bicuculline methochloride (25–50 nA), leptazol and bemegride (75–150 nA) were inactive with respect to antagonism of GABA.

As regards antagonism of glycine, it is clear that strychnine was the most consistently effective of the substances tested, antagonizing glycine in every experiment. In 95% of these experiments, the antagonism gave a value in excess of 1.0, in spite of the very low currents used. The second most consistent glycine antagonist was (+)-bicuculline methochloride (41% of experiments) but in only one sixth of those experiments did the antagonism give a value in excess of 1.0. Next came (+)-tubocurarine, picrotoxin and (+)-bicuculline which antagonized glycine in 32%, 30% and 25% of experiments, respectively. Nearly half of the (+)-tubocurarine antagonisms had values in excess of 1.0 but none of the picrotoxin or (+)-bicuculline antagonisms were over 1.0. Leptazol, bemegride, penicillin and (−)-bicuculline methochloride were inactive with respect to antagonism of glycine.

It is also interesting to note that (+)-bicuculline caused a considerable number of potentiations of GABA and glycine, as it did of GABA in the cerebral cortex (Hill *et al.*, 1973a).

The difference between the value of T_{50} (test-control)/T_{50} (control) obtained for GABA and that obtained for glycine in each experiment was taken as a measure of the selectivity of the test substance in antagonizing GABA or glycine. The greater the difference between the values, the greater was the selectivity demonstrated to be. In Table 1, these measures of selectivity are divided into seven consecutive ranges and the numbers of experiments falling into each range are shown. To determine whether any of the test substances demonstrated statistically significant selectivity overall in

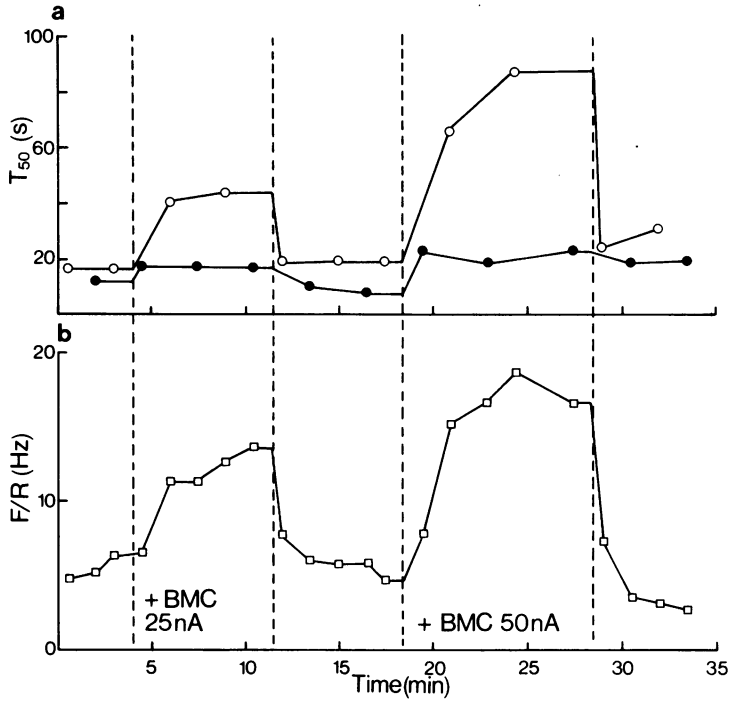

Figure 3 The selective antagonism of γ-aminobutyric acid (GABA) by (+)-bicuculline methochloride (BMC). (a) Shows values of T_{50} obtained from alternate microiontophoretic applications of GABA 40 nA (O) and glycine 40 nA (●). These results were obtained from a spontaneously firing neurone, 951 μm deep in the cuneate nucleus. (b) Shows the baseline firing rate of the neurone during the 30 s before each application of GABA or glycine. The values are plotted against elapsed time throughout the experiment.

antagonizing either GABA or glycine, the results were examined by the Wilcoxon matched-pairs signed-rank test. As indicated in Table 1, (+)-bicuculline methochloride was shown to antagonize GABA selectively rather than glycine, and strychnine to antagonize glycine selectively rather than GABA. None of the other test substances showed significant selectivity overall in their antagonism of GABA and glycine. However, it should be pointed out that in the individual experiments where picrotoxin and (+)-bicuculline antagonized GABA with values of T_{50} (test-control)/T_{50} (control) in excess of 1.0, the antagonisms could all be described as selective, since the value for GABA antagonism was always substantially greater than that for glycine antagonism. Similarly, in the individual experiments where (+)-tubocurarine antagonized glycine with values of T_{50} (test-control)/T_{50} (control) in excess of 1.0, the antagonisms could all be described as selective.

In some experiments, the test substances themselves changed the firing rates of the neurones in a reversible manner. To examine whether these effects were related to the ability of the test substance to antagonize GABA or glycine, values of T_{50} (test-control)/T_{50} (control) were plotted against the simultaneous effects on the overall background firing rates of the neurones (Figure 5). The most marked excitant effects were caused by (+)-bicuculline methochloride and (+)-tubocurarine; (−)-bicuculline methochloride was moderately excitant but no consistent excitant or depressant effects were seen with the remaining substances. With none of the test substances, however, was there any significant correlation between their displacement of background firing rate and their effects on responses to GABA or glycine.

The currents used to expel picrotoxin, (+)-bicuculline, leptazol, bemegride and penicillin were as high as could be applied without an excessive incidence of unacceptably noisy recordings and failure of the drug barrel to pass a steady current. Further prolongation of the drug applications would have had little effect since, after 10 min continuous application, the concentration of drug around the neurone must have come close to the asymptotic concentration for the current being passed. The lower currents used to expel (+)-bicuculline methochloride, (−)-bicuculline

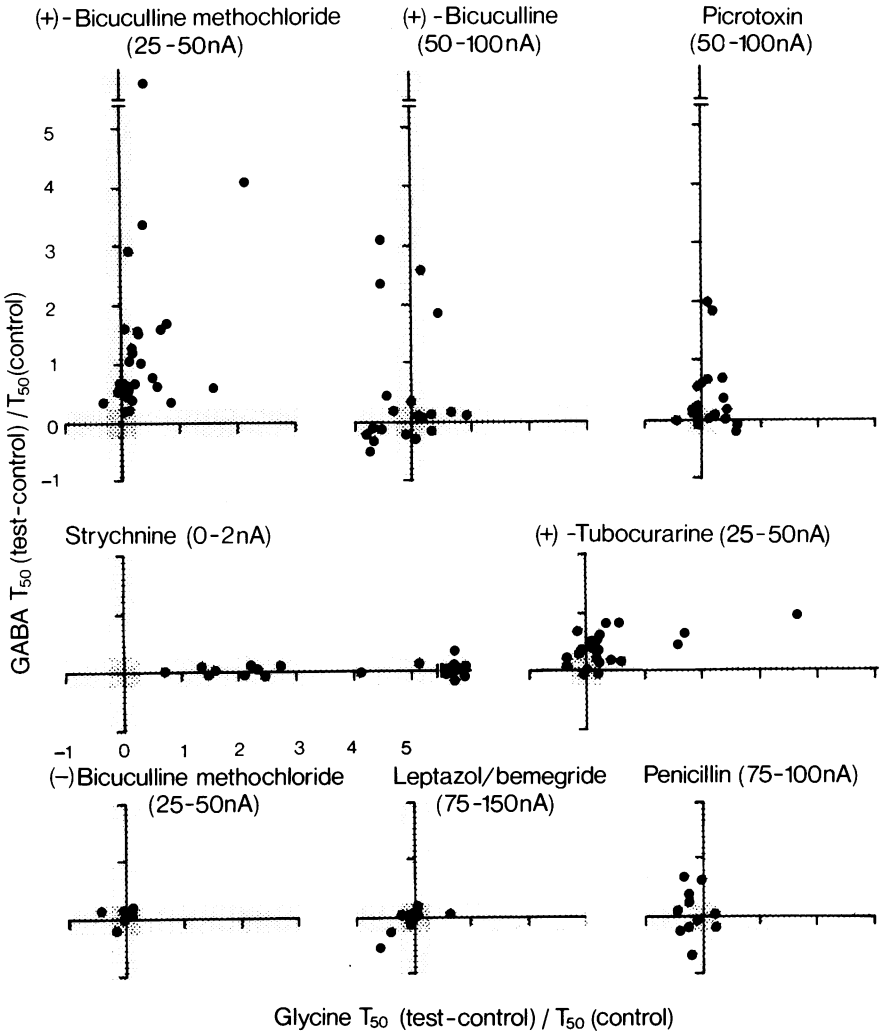

Figure 4 Effects of some convulsant substances on responses of neurones in the cuneate nucleus to γ-aminobutyric acid (GABA) and glycine. The sizes of the effects are expressed as T_{50} (test-control)/T_{50} (control) for GABA (ordinate scale) and glycine (abscissa scale). Each point represents a separate experiment. Negative values indicate potentiation and positive values antagonism. The stippled areas indicate the ±0.25 no effect zones. In some experiments, the antagonisms were sufficiently large that GABA or glycine, in the currents used, became unable to depress neuronal firing to 50%; in these cases, the point is plotted at the far end of the scale.

methochloride and (+)-tubocurarine reflect the limitation which the marked excitatory actions of these substances placed on the amounts that could be applied to neurones. On the other hand, the currents used to expel strychnine were deliberately kept to a low level. When higher currents of strychnine in the range 25–50 nA were applied to six neurones, the responses to glycine were completely suppressed while the responses to GABA were antagonized in 4 of the experiments with values of T_{50} (test-control)/T_{50} (control) up to 1.20.

Discussion

From each experiment we have derived numerical values for the antagonism of GABA and glycine by one of the test substances. This has enabled us to

make overall assessments of how consistently the antagonisms occurred with each substance and to make some objective comment on the magnitudes of the antagonisms observed. We have also examined the selectivity of each antagonist in distinguishing between GABA and glycine. Detailed descriptions of the analytical method we have used are given elsewhere (Hill & Simmonds, 1973; Simmonds, 1974) as, also, are previous examples of its use in evaluating the antagonism of both inhibitory and excitatory substances (Hill *et al.*, 1973a; Clarke, Forrester & Straughan, 1974). This, however, is the first time that the method has been extended to measure selectivity of antagonism.

In the interpretation of our results and drawing conclusions from them we have been well aware of the limitations of microiontophoretic methodology. The important assumptions on which our own methods were based include: (i) a progressively increasing rate of release of agonist during a constant current application, rather than a steady rate of release, resulting from dilution of the active ion in the pipette tip during prior passage of retaining currents (Hill & Simmonds, 1973; Clarke, Hill & Simmonds, 1973); (ii) equating T_{50} with concentration of agonist required to produce a given degree of receptor occupation which, in turn, depends on the relationship between response and receptor occupation being unaffected by

Table 1 The selectivity of some convulsants as antagonists of γ-aminobutyric acid (GABA) or glycine

	Numbers of experiments in which the difference between T_{50} (test-control)/T_{50} (control) values for GABA and glycine were:							Overall selective antagonist of:
	GABA > glycine				*glycine > GABA*			
	>2.0	*1.0–2.0*	*0.5–1.0*	*<0.5**	*0.5–1.0*	*1.0–2.0*	*>2.0*	
(+)-Bicuculline methochloride (25–50 nA)	3	5	10	9	1	1	0	GABA ($P<0.001$)
(+)-Bicuculline (50–100 nA)	3	1	1	12	3	0	0	N.S.
Picrotoxin (50–100 nA)	0	2	3	13	2	0	0	N.S.
Strychnine (0–2 nA)	0	0	0	0	1	3	16	glycine ($P<0.001$)
(+)-Tubocurarine (25–50 nA)	0	0	1	18	0	3	0	N.S.
(–)-Bicuculline methochloride (25–50 nA)	0	0	0	8	0	0	0	N.S.
Leptazol/bemegride (75–150 nA)	0	0	0	11	1	0	0	N.S.
Penicillin (75–100 nA)	0	0	3	9	0	0	0	N.S.

* Includes all experiments where both GABA and glycine values were less than 0.25.

Table 2 Comparison of the frequency of γ-aminobutyric acid (GABA) antagonism by some convulsant substances applied by microiontophoresis onto single neurones in the cuneate nucleus and cerebral cortex

	*Cuneate nucleus**	*Cerebral cortex†*
(+)-Tubocurarine	59%	85%
Picrotoxin	35%	68%
Penicillin	33%	48%
(+)-Bicuculline	30%	31%
Leptazol/bemegride	0	20%

* Present results † Taken from Hill *et al.*, 1973a.

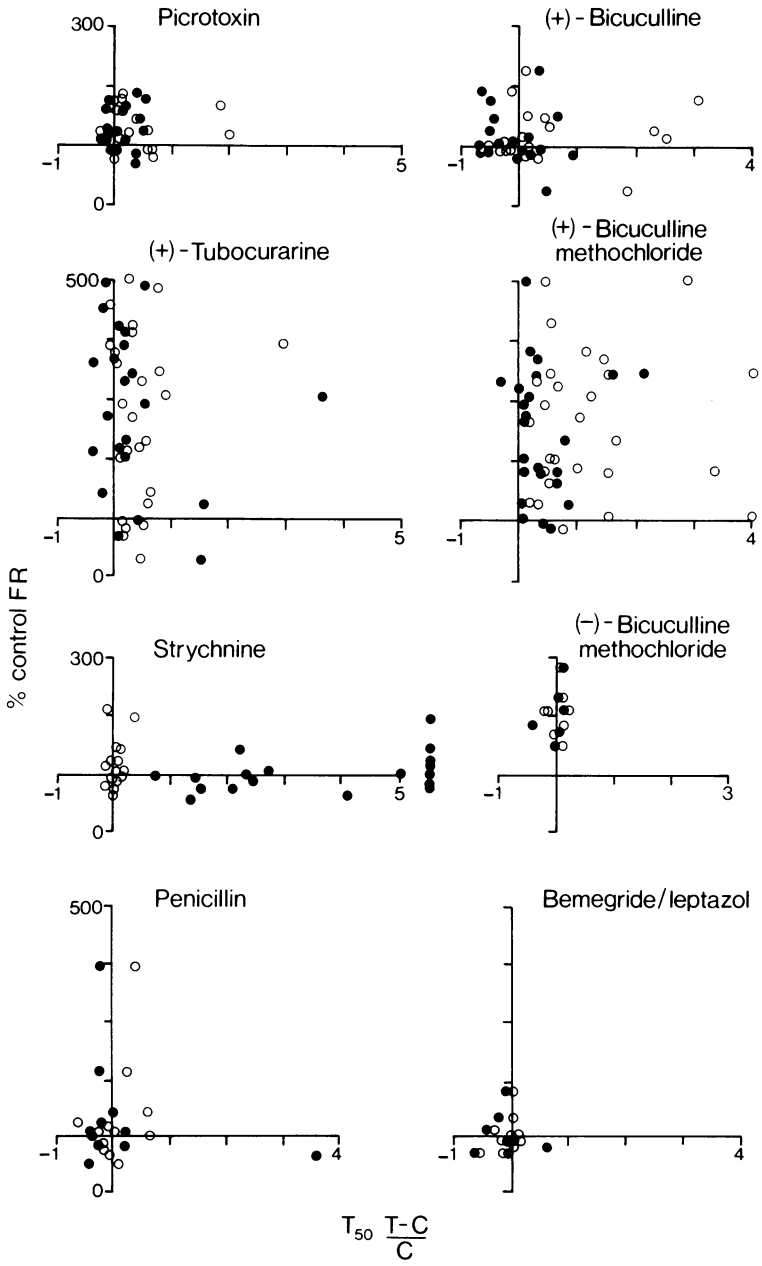

Figure 5 Relationship between effects on responses to γ-aminobutyric acid (GABA) and glycine and effects on background firing rate (FR) of some convulsant substances applied to neurones in the cuneate nucleus. The data are drawn from the same experiments contributing to Figure 4 and the iontophoretic currents used to apply the convulsants are the same as in that figure. The effects of the convulsants on the responses to GABA (○) and glycine (●) are expressed as T_{50} (test-control)/T_{50} (control) on the horizontal axes. The effects of the convulsants on background firing rate are indicated on the vertical axes as % control and were calculated from the mean values of neuronal firing during the 30 s prior to each application of GABA or glycine. With none of the convulsants was there any significant correlation between effects on background firing rate and effects on responses to GABA or glycine.

desensitization over different time intervals; (iii) minimal dependence of the numerical values of antagonism on the precise sizes of the agonist expelling and retaining current (Hill & Simmonds, 1973). Although we believe these to be reasonable assumptions, there is a possibility that they may not always hold good. When comparing the interpretations and conclusions of different authors, therefore, it is important to take account of the assumptions on which their respective methodologies are based.

Six of the substances tested as antagonists in the present experiments had also been tested previously on cortical neurones (Hill *et al.*, 1973a). In terms of how consistently they antagonized GABA with values of T_{50} (test-control)/T_{50} (control) in excess of 0.25, all six substances appeared in the same rank order in both the cuneate nucleus and the cerebral cortex (in descending order of consistency: (+)-tubocurarine, picrotoxin, penicillin, (+)-bicuculline, leptazol and bemegride) (Table 2). Nevertheless, with all except (+)-bicuculline, there was a greater percentage of 'no effects' in the cuneate nucleus. A similar difference between cuneate nucleus and cortex is apparent in terms of the magnitude of the antagonisms observed; thus, (+)-bicuculline was approximately equally effective as a GABA antagonist in both areas of brain while the other five substances caused generally smaller antagonisms in the cuneate nucleus, this being particularly marked with (+)-tubocurarine.

To what extent these differences may be due to less efficient iontophoretic release of substances in the present experiments is not clear. There is, certainly, no support for such an explanation from a comparison of the incidence of direct effects of the test substances on neuronal firing rate in the two series of experiments. An alternative explanation could involve the possibly different distributions of GABA receptors at pre- and post-synaptic sites in cuneate nucleus and cerebral cortex. Both pre- and post-synaptic inhibition have been demonstrated in the cuneate nucleus (Andersen, Etholm & Gordon, 1970) and GABA has been suggested as the transmitter for both mechanisms (Banna & Jabbur, 1969; Davidson & Reisine, 1971; Kelly & Renaud, 1971; Banna, Naccache & Jabbur, 1972; Kelly & Renaud, 1973c). In the cortex, however, only a post-synaptic action of GABA has been shown to occur, as yet (Krnjević & Schwartz, 1968).

It may be relevant that all the substances found capable of antagonizing GABA have been shown to reduce the P-wave or dorsal root potential correlates of pre-synaptic inhibition (Banna & Jabbur, 1969; Banna *et al.*, 1972; Davidoff, 1972; Hill, Simmonds & Straughan, 1973c, 1974). But other substances such as leptazol and bemegride can reduce pre-synaptic inhibition (Banna & Jabbur, 1970; Hill *et al.*, 1974) although we have been unable to show that either substance can antagonize GABA in the cuneate nucleus or the cerebral cortex (Hill *et al.*, 1973a). Even in concentrations up to 5×10^{-3} M, neither leptazol nor bemegride will antagonize the actions of GABA on the isolated superior cervical ganglion of the rat (N.G. Bowery, personal communication; see Bowery & Brown, 1974). Thus, the ability of a substance to reduce pre-synaptic inhibition does not necessarily indicate that it is a GABA antagonist. Similarly, the convulsant properties of leptazol and bemegride cannot yet be related to antagonism of GABA.

An important part of the present work has been concerned with the assessment of selectivity of an antagonist. Although numerical analyses are innately more objective than the simple visual inspection of neuronal firing rate records, care must still be exercised in the interpretation of selectivity measurements. For instance, a high degree of selectivity can only be demonstrated if the antagonist is sufficiently potent to cause a large displacement of the agonist-response curve. On the other hand, a low degree of selectivity which occurs very consistently may prove to be statistically significant but the selectivity might be too low to make practical use of the antagonist in identifying a specific receptor. When an antagonist is consistently effective, potent and its selectivity is clearcut, as in the antagonism of glycine by strychnine, no difficulty arises and there is virtually no disagreement between different authors. Descriptions attached to the antagonists of GABA by different authors, however, are more controversial, e.g. compare Curtis, Duggan, Felix & Johnston (1970a, b), Curtis *et al.* (1971a) and Dreifuss & Matthews (1972) with Godfraind, Krnjević & Pumain (1970), Bruggencate & Sonnhof (1972), Tebecis (1973), Hill, Simmonds & Straughan (1973b) and the present results. These differences are probably due to the antagonism not being sufficiently potent and selective to be seen clearly in all experimental situations.

In the present experiments, (+)-bicuculline methochloride was the only GABA antagonist that could be shown to distinguish overall between responses to GABA and those to glycine in a statistical test. Even so, while several of the individual experimental results showed clearcut selectivity, in several other experiments the responses to GABA were only slightly more antagonized than responses to glycine and some showed a reversed selectivity. (+)-Bicuculline and picrotoxin did not prove to be selective overall as GABA antagonists in the statistical test, even though, in the several individual experiments where GABA was substantially antagonized, the antagonism was clearly selective. It is possible that the failure of these two substances to show statistically significant selectivity overall was due to the small size of many of the GABA antagonisms rather than to an intrinsically poor selectivity. Similarly, the high proportion of small antagonisms of GABA by (+)-tubocurarine did not prove to be significant overall; indeed, there were occasional clearcut selective antagonisms of glycine.

This is in keeping with a report that (+)-tubocurarine can antagonize both GABA and glycine on spinal cord neurones (Curtis, Game & McCulloch, 1974).

Rather different conclusions about the selectivity of (+)-bicuculline and picrotoxin as GABA antagonists in the cuneate nucleus were reached by Kelly & Renaud (1973a, b). Their experimental design and method of quantitative analysis were different from our own and may have allowed the reported selectivity towards GABA to become more apparent. For example, Kelly & Renaud (1973a) observed that 'Although in general, bicuculline seldom caused a significant shift of the glycine log-current response curve, many of our records showed bicuculline to slow the onset of the response to glycine'. This was interpreted by the authors to mean that (+)-bicuculline did not antagonize glycine. In contrast, the method of analysis we have used attempts to quantify such slowing of responses as an essential characteristic of antagonism. Thus, the apparent selectivity of these substances as GABA antagonists depends upon the precise way in which the experiments are performed and the results analysed and interpreted.

From all these considerations of our present results, we conclude that (+)-bicuculline methochloride is the substance most likely to antagonize GABA substantially and selectively when the antagonists are applied by microiontophoresis. Its greater solubility compared with (+)-bicuculline or picrotoxin and easier ejection by iontophoresis may, in part, account for its superiority over these substances. It does, however, have some different properties from (+)-bicuculline and should not, therefore, be regarded simply as a more soluble form of (+)-bicuculline. In particular, the excitant properties of (+)-bicuculline methochloride may cause complications in its use. However, in the present experiments there was no correlation between excitation and GABA antagonism and, as a further control, the (−)-isomer can be compared since it does not antagonize GABA but retains an ability to excite neurones. Nevertheless, when this minor reservation is coupled with the larger problem of (+)-bicuculline methochloride causing some antagonisms of glycine, it becomes clear that the best means of separating GABA-operated from glycine-operated pathways at present is the use of strychnine. The currently available GABA antagonists may provide corroborative evidence but their use cannot stand alone. Despite this limitation, careful measurement of the effectiveness and selectivity of all substances capable of antagonizing GABA or glycine contributes to our understanding of inhibitory processes and to more detailed characterization of the GABA and glycine receptors.

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