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On the pharmacology of the γ-aminobutyric acid receptors on the cuneo-thalamic relay cells of the cat

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Summary

1. γ-Aminobutyric acid (GABA) and glycine applied by iontophoresis were equipotent depressants of cuneo-thalamic relay neurones isolated from the middle third of the cuneate nucleus of cats either decerebrated or anaesthetized with sodium pentobarbitone.

2. Glycine 13±2 nA and GABA 20±2 nA were equipotent depressors of hair cells (n=22) and, bicuculline applied by iontophoresis caused a parallel shift to the right of the GABA but not the glycine log-current response curves. The GABA equipotent dose-ratio was 2·0±0·2 for bicuculline currents of approximately 144 nA lasting about 11 min in cells excited either transynaptically by peripheral stimulation or postsynaptically by glutamate.

3. Although a maximal bicuculline current seldom caused a significant shift of the glycine-log current response curve, many of our records show the onset of the glycine response to be slowed by doses in excess of 84 nA.

4. Bicuculline also antagonized depressions by β-guanidinopropionic acid, and δ-aminovaleric acid which mimicked the action of GABA.

5. When tested on the same neurone, bicuculline and picrotoxin applied by iontophoresis were equipotent and their effects appear to be additive.

6. The GABA sensitivity was not modified by repetitive (5 or 6) doses of i.v. bicuculline (0·2 mg/kg).

7. The antagonism of GABA by bicuculline and picrotoxin appears to be of sufficient specificity to enable the separate roles of GABA and glycine as putative inhibitory transmitters of cuneo-thalamic relay cells to be determined.

Introduction

Afferent inhibition of cuneo-thalamic relay cells was shown by Andersen, Etholm & Gordon (1970) to have both pre- and postsynaptic components (Andersen, Eccles, Schmidt & Yokota, 1964b). Postsynaptic hyperpolarization is invariably accompanied by an increased excitability of the appropriate primary afferents. Pre- and postsynaptic mechanisms operating concurrently may well account for the inhibition of cuneate relay cells by a recurrent input from the ipsilateral medial lemniscus (Gordon & Paine, 1960 ; Gordon & Seed, 1961 ; Gordon & Jukes, 1964), contralateral inputs from the medial and sural nerves (Jabbur & Banna, 1968, 1970) and the pyramidal tract (Magni, Melzack, Moruzzi & Smith, 1959 ; Jabbur &

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Towe, 1961; Levitt, Carreras, Liu & Chambers, 1964; Andersen *et al.*, 1964b; Andersen, Eccles, Oshima & Schmidt, 1964a).

The introduction of bicuculline by Curtis, Duggan, Felix & Johnston (1970, 1971), an antagonist of γ-aminobutyric acid (GABA) and synaptic inhibition in the spinal cord, gave us an opportunity to re-examine the hypothesis of Galindo (1969) who suggested that pre- and postsynaptic inhibition in the cuneate are both mediated by GABA. In addition, he suggested that the action of GABA at both sites was specifically blocked by picrotoxin.

In this paper, the first of three companion papers, GABA and glycine are confirmed to be equipotent inhibitors of cuneo-thalamic relay cells (cf. Galindo, Krnjević & Schwartz, 1967) and the depressant action of GABA as opposed to glycine is shown to be blocked specifically by bicuculline applied by iontophoresis. Similar degrees of block occurred whether the cells were excited postsynaptically by glutamate or transynaptically by peripheral stimulation of hair, touch or joint cells. In the second paper (Kelly & Renaud, 1973a) glycine is shown to be selectively blocked by extremely small and short iontophoretic applications of strychnine. The third paper (Kelly & Renaud, 1973b) describes the influence of bicuculline, picrotoxin and strychnine on the duration and intensity of inhibition of cuneo-thalamic relay cells. Some of our results have been briefly reported (Kelly & Renaud, 1971).

Methods

Experiments, essentially similar to those described earlier by Galindo *et al.* (1967) were performed on 15 adult cats of either sex anaesthetized with 30 mg/kg of sodium pentobarbitone and on 3 cats that were surgically decerebrated by mid-collicular section under initial anaesthesia with halothane and nitrous oxide.

Surgical preparation The cervical musculature was separated in the mid-line and the foramen magnum exposed by removing the alanto-occipital membrane. The dura and arachnoid were removed after resecting the posterior arch of the atlas and removing the occiput overlying the cerebellar vermis. All exposed areas were protected by strips of opaque polythene and a continuous perfusion of mammalian Ringer solution at 37° C. This solution was also used for superfusing the surface of the cuneate with drug-containing solutions (cf. Galindo, 1969). Experiments were discontinued when the blood pressure, monitored continuously from the femoral artery, fell below 90 mmHg. A thermostatically regulated heating pad maintained the cat's rectal temperature between 36 and 38° C.

Stimulation Under direct vision, bipolar silver wire electrodes were placed on the contralateral pyramid exposed at the level of the pons by a ventral surgical approach. The sural and median nerves of the contralateral forelimb were exposed, placed on insulated electrodes and buried in the sutured tissues. The ipsilateral forepaw was stimulated by two insulated hypodermic needles inserted into the soft tissues of the central paw. In 6 experiments, an electrode array of insulated needles was placed stereotactically into the medial lemniscus (F 6, L 4·5 & H-1, cf. Jasper & Ajmone-Marsan, 1960) and the two electrodes were used which, during stimulation with 0·1 ms pulses, gave the largest antidromic response in the cuneate.

Recording micropipettes These were inserted into the cuneate through a hole in the centre of a transluscent disc placed lightly on the surface of the brain to limit

cardiovascular pulsations. Five barrelled glass micropipettes with tip diameters less than 5 μm were prepared as described by Krnjević & Phillis (1963). One barrel filled with 2·7 M NaCl was used to record action potentials by conventional techniques.

Individual action potentials These were converted by an adjustable voltage gate into standard pulses monitored by an audio-amplifier and used to brighten selected spikes with respect to the background activity by modulating the oscilloscope Z-axis input. The frequency of the output pulses of the gate was registered by a resettable counter connected to a strip chart recorder.

Solutions for iontophoresis The outer barrels of the pipettes contained: γ-aminobutyric acid (GABA). 1 M, pH 4; glycine: 1 M, pH 3·5; Na L-glutamate: 1 M, pH 6–7; bicuculline hydrochloride (a gift to Professor K. Krnjević from Dr. Manske, University of Waterloo, Canada), 5 mM in 165 mM saline, pH 3·5; picrotoxin (BDH): 5 mM in 165 mM saline, pH 7·5 (cf. Davidoff & Aprison, 1969); strychnine sulphate (BDH): 5 mM in 165 mM saline, pH 5·5; β-alanine: 1 M, pH 3·5; β-guanidino-propionic acid: 1 M pH 4·0; δ-aminovaleric acid: 1 M, pH 4·0.

Results

Selection of the cuneo-thalamic relay cells

Iontophoretic GABA and glycine were tested on the discharges of neurones recorded in the middle third of the cuneate nucleus and shown to be (a) excited by mechanical stimulation of the ipsilateral forelimb and (b) inhibited by shocks to the contralateral pyramidal tract or forelimb nerves. The majority of cuneo-thalamic relay cells, i.e. neurones projecting to the contralateral thalamus have been shown to be inhibited by stimulation of the contralateral cerebral cortex, whereas non-relay cells are excited (Andersen *et al.*, 1964b; Gordon & Jukes, 1964; Rosén, 1969). Eighty-five of the 141 cells were indisputably hair cells, that were readily excited by a small jet of air applied directly to tufts of hairs on the forelimb or neck. The other cells were also spontaneously active and when slowly adapting, their discharge could be maintained at an accelerated level by placing a joint of the limb in a critical position (20 cells) or simply applying pressure to the limb with a weight (36 cells). In 6 cats in which electrodes were placed stereotactically in the medial lemniscus, all of the cells found to be excited by peripheral stimulation or inhibited by stimulation of the pyramid or the forelimb nerves were identified by antidromic invasion as cuneo-thalamic relay cells. The antidromic nature of the action potential evoked at constant latency by lemniscal stimulation was confirmed when it was observed to be cancelled by another occurring at random (Bishop, Burke & Davis, 1962). Both the spontaneous and glutamate-evoked discharge of all identified cells was rapidly blocked by short applications of GABA and glycine with relatively low iontophoretic currents.

Bicuculline-sensitive and insensitive cells

In three preliminary experiments, iontophoretic bicuculline selectively antagonized the depressant action of GABA on 18 of 24 neurones identified as principal relay cells of the cuneate nucleus. Before, throughout and after each trial of bicuculline, GABA and glycine were released alternately with currents evoking in the absence of bicuculline equal depressions of the background neuronal discharge. The degree

of antagonism was then simply graded by inspection of ratemeter records, as either partial or complete.

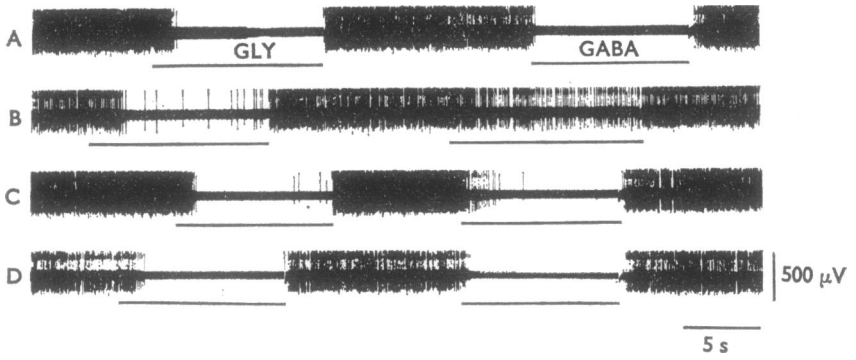

FIG. 1. The selective action of bicuculline on the response of cuneo-thalamic relay neurones to γ-aminobutyric acid (GABA). Continuous film records of a touch cell whose discharge was maintained by the constant release of glutamate. The responses of the cell to glycine (GLY) and GABA were tested alternately and the responses to 14 nA of glycine or GABA in (A) were only just maximal. Bicuculline released with 168 nA began approximately 75 s before the start of record B and terminated between records B and C which are continuous with each other and D. (Calibration=5 s and 500 μV).

When the spike discharge was recorded on moving film the effect of bicuculline on the inhibitory response evoked by GABA was often very striking. In Fig. 1, the background discharge of a cuneate relay neurone excited by light pressure to the ventral surface of the ipsilateral forelimb, was maintained at an elevated level by the continuous release of glutamate. The discharge was interrupted alternately by 20 s equipotent applications of GABA and glycine (Fig. 1A). In the presence of bicuculline (168 nA) whose release began approximately 75 s earlier, interruption of the background discharge by GABA was markedly reduced. Continuous records B, C and D show the influence of bicuculline to last at least 30 s after its release was terminated at the origin of record C. Commonly, equal depressions were evoked when the GABA releasing current was approximately 1·7 times greater than that applied through the glycine barrel. On the 17 occasions in which the response to GABA was completely blocked by bicuculline the GABA releasing current was only $24 \pm 2{\cdot}5$ (S.D.) nA, distinctly smaller than 34 ± 14 nA, the GABA response was unaltered, bicuculline-sensitive and insensitive cells lay adjacent within the same microelectrode penetration. On 4 of these occasions, the bicuculline currents exceeded 139 nA, the average used during the successful trials. The bicuculline-insensitive cells were perhaps less sensitive to GABA than the others since the average GABA current was $38{\cdot}5 \pm 13{\cdot}8$ nA. The insensitivity to GABA of the bicuculline-resistant cells is also reflected by their higher ratios for equipotent doses of GABA and glycine. This was 3·5, almost twice that found for bicuculline-sensitive cells. Alternatively these cells could be regarded as belonging to a population of cells rather more sensitive than average to glycine. In the spinal cord, Curtis *et al.* (1971a) also found that the neurones apparently unaffected by bicuculline were relatively insensitive to GABA.

Bicuculline seldom altered either the degree or the duration of the inhibitory response evoked by glycine. Twice, however, and on the same cell bicuculline

completely blocked the glycine-evoked depression of the background discharge. Smaller reductions of the inhibition evoked by glycine are discussed below.

In Fig. 2, depressions of approximately 50% were caused by 19·6 nA of GABA and glycine. They cannot, however, be regarded as equipotent, since a doubling of the GABA current caused a much smaller increase in the depression than the supramaximal response elicited by a mere 50% increase in the glycine current. Similarly, in Fig. 4 a graded series of depressions were evoked by GABA currents which required between 5·6 and 14 nA, in contrast maximal and minimal responses occurred with glycine currents of 7 and 8·4 nA. More critical studies of the relative

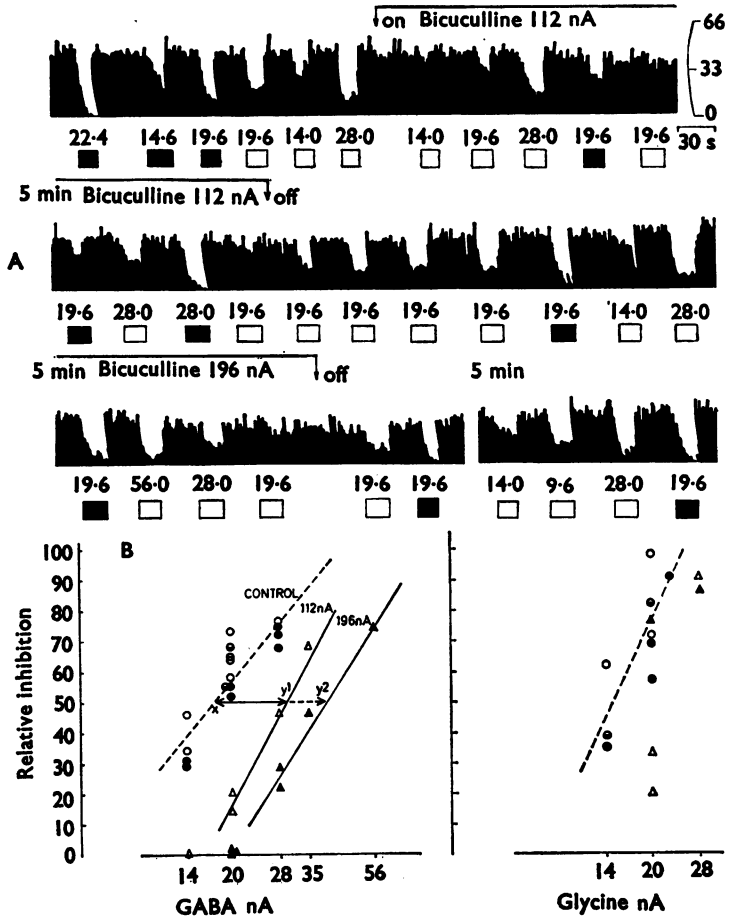

FIG. 2. The action of bicuculline on the γ-aminobutyric acid (GABA) and glycine log-current response curves. The ratemeter records separated by 5 min intervals, show the response of a hair cell, excited at a constant level by a turbulent air jet applied to the ipsilateral forepaw, to a range of GABA and glycine currents selected to cause 20–80% inhibition in the presence and absence of two doses of bicuculline. (In this and subsequent figures, the closed and open horizontal bars identify glycine and GABA currents respectively and indicate both the duration and the intensity of the current in nA. Vertical curvi-linear calibration=spikes/bin.) The appropriate responses are plotted on the graphs below to show the percentage inhibition as function of the GABA and glycine currents on a logarithmic scale. Controls; ○, before; ●, after first trial of bicuculline; ◒, after second trial of bicuculline; △ and ▲, during ejection of 112 and 196 nA of bicuculline respectively. The lines drawn through the points on the GABA curves and glycine control curve are regression lines calculated by the method of least squares. The GABA 'equipotent dose-ratio' during bicuculline application of 112 and 196 nA was determined from the measurements xy^1 and xy^2 respectively.

potencies of GABA and glycine and the influence of bicuculline were therefore only possible by a comparison of log-current response curves from individual cuneate neurones.

Log-current response curves to γ-aminobutyric acid and glycine

The ratemeter records selected for Fig. 2A show GABA and glycine evoked depressions of a cell continuously excited by a jet of air directed at a small tuft of hairs near a claw of the ipsilateral forepaw. These depressions of the background discharge are plotted as a function of the log of the currents used to release the amino acid.

The percentage depression was calculated from the mean of two estimates of the average firing frequency, read by eye from the ratemeter record, for the 15 s intervals immediately before and after the amino acid application and the mean of two estimates of the depression ; one made at the peak of the response the other 7·5 s earlier.

In most instances these two measurements were made 12·5 and 20 s after the onset of the inhibition and can, therefore, be regarded as estimates of the average level of neuronal firing during the plateau of the response. Earlier Curtis, Duggan & Johnson (1971c) suggested that the concentration of agonist in the vicinity of the receptors will approach a steady state during the plateau of the response. In other experiments, for example Fig. 3, the background discharge was not maintained at a stable level but accelerated intermittently by applying standard pulses of glutamate. The percentage depressions were estimated by comparing the maximal frequency attained during the second of the two responses to glutamate pulses applied during the release of depressant amino acid, with the mean of the peak discharges evoked by glutamate immediately before and after.

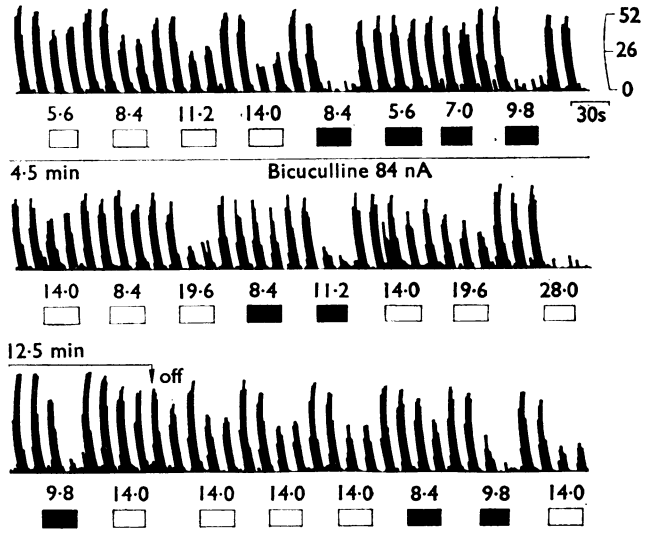

FIG. 3. Effect of bicuculline on the γ-aminobutyric acid (GABA) sensitivity of a hair cell excited intermittently with pulses of glutamate. The ratemeter records are separated by intervals of 4·5 and 12·5 minutes. During the bicuculline application, the amplitude of the responses to individual pulses of glutamate was maintained constant by increasing the current from 7 to 14 nA. Log-current response curves drawn from these results showed 7·8 nA of glycine and 13·5 nA of GABA to depress the response to glutamate by 50% and the equipotent dose-ratios for glycine and GABA to be 1·11 and 1·66 respectively.

N.B. % depression was determined from the response to the second trial of glutamate made during each drug application. The exceptional steepness of the glycine log-current response curves causes quite modest reductions in glycine sensitivity measured at less than 2 nA for 50% depression from log-current response curves to appear as dramatic changes of the ratemeter record.

TABLE 1. *Parameters calculated from γ-aminobutyric acid (GABA), glycine (GLY) and β-guanidino-propionic acid (βGP) log-current response curves in the presence and absence of bicuculline and picrotoxin*

No. cells	Type	Excitation	Dose in nA for 50% depression mean±S.E.M. GABA	GLY	βGP	Slope of the log-current response curve* (% inhibition/0·3 log nA) GABA	GLY	βGP	Slope ratio	Dose nA Bicuculline	Duration min Bicuculline	Equipotent dose-ratio GABA	GLY	βGP
13	Hair	Air jet	19±2	13±1		61±9	109±15		1·9±0·2	146±13	9±1	1·8±0·3	1·08±0·04	
9	Hair	Glutamate (glut)	21±4	14±3		58±16	95±27		1·5±0·3	138±18	13±4	2·1±0·2	1·12±0·09	
10	Non-hair	Peripheral and glut	15±2	10±2		67±16	126±23		2·1±0·5	123±16	8±1	2·0±0·3	1·31±0·18	
7	Hair and non-hair	Peripheral	18±4		22±5	63±8		59±10	1·0±0·1	112±7	9±1	1·6±0·1		1·6±0·1
										Picrotoxin				
6	Hair and non-hair	Peripheral and glut	22±1	16±3		131±18	130±26		0·9±0·1	72±3	18±4	1·6±0·2	1·19±0·13	
			(1)	(2)	(3)	(4)	(5)	(6)	(7)			(8)	(9)	(10)

The straight line log-current response curves were characterized by the dose required for 50% depression in columns (1) and (3) and the slopes given in columns (4) to (6). The slope of each line was determined by extrapolation and expressed as a % increase in the depression that would be anticipated from a doubling of the amino acid releasing current required for 50% depression. The 'slope ratio' compares the slopes for glycine or βGP curves with those for GABA and the 'equipotent dose' ratio compares two doses of agonist read from calculated regression lines to cause 50% depression in the presence and absence of the antagonist as illustrated in Figure 2 (1·0=no effect).

Iontophoretic applications of bicuculline were continued until the maximal effect was attained and stable responses were obtained repeatedly over a range of GABA currents. The recovery of GABA sensitivity was always monitored as shown in Figs. 2, 3 and 7, and only bicuculline applications lasting at least twice the duration of the recovery period were considered adequate. The responses to GABA and glycine were only considered valid when the spike discharge remained at the same level in the presence and absence of bicuculline. On a few occasions, bicuculline caused a slow acceleration of cell discharge similar to that which occurs in the spinal cord (Godfraind, Krnjević & Pumain, 1970; Curtis *et al.*, 1971a). However, this excitation was not associated with 'bursts' of high frequency firing (Curtis, Duggan, Felix, Johnston & McLennan, 1971b).

Declines of the firing frequency coinciding with the onset of the positive bicuculline expelling current (greater than 100 nA), were usually countered by altering the glutamate releasing current or adjusting the intensity of the peripheral stimulation.

The results given in Table 1 are from experiments where at least 3 responses at different GABA or glycine currents were on a straight line. In the majority of experiments, glycine was distinctly more potent than GABA. Glycine currents of 13 ± 2 nA caused 50% inhibition of 22 identified hair cells whereas the equipotent dose of GABA was 20 ± 2 nA. The currents were not corrected for a 14 nA backing current. The slope ratios in column (7) obtained by comparing columns (4) and (5) show the glycine log-current response curve to be almost twice as steep as that of GABA. The slope was measured by extrapolating the straight line part of the response curves to determine the increase in depression that would be anticipated for a doubling of the glycine or GABA currents. Comparable results were obtained from cells excited by glutamate and peripheral stimulation. Similar log-current response curves fitted the GABA and glycine depressions of 9 other relay cells also excited from the periphery but unlikely to be hair cells.

Effect of bicuculline on log-current response curves

The log-current response curves for GABA but not glycine were shifted to the right by iontophoretic bicuculline. In 19 experiments on hair cells, the equipotent dose-ratio calculated from the displacement of the log-current response curves was $2{\cdot}0 \pm 0{\cdot}2$ for bicuculline applications with currents of 146 ± 13 nA. Similar dose-ratios were obtained from cells excited either transynaptically by peripheral stimulation of postsynaptically by glutamate (Table 1, columns (7) and (8)). In a number of experiments where positive results were obtained with two or more separate applications of bicuculline, the increase in the bicuculline current and the GABA equipotent dose-ratios were linearly related as predicted by competitive antagonism. However, regression analysis of all the results showed that neither the bicuculline current, nor the GABA current required for 50% inhibition of the background discharge, to be correlated with the equipotent dose-ratio. Since the potency of GABA varied little, the lack of correlation between the bicuculline dose (log) and the equipotent dose-ratio (log DR-1) probably reflects variations of the bicuculline transport number from application to application rather than evidence for antagonism by a non-competitive mechanism (Waud, 1968). Although on the one occasion, when bicuculline caused a shift of the glycine log-current

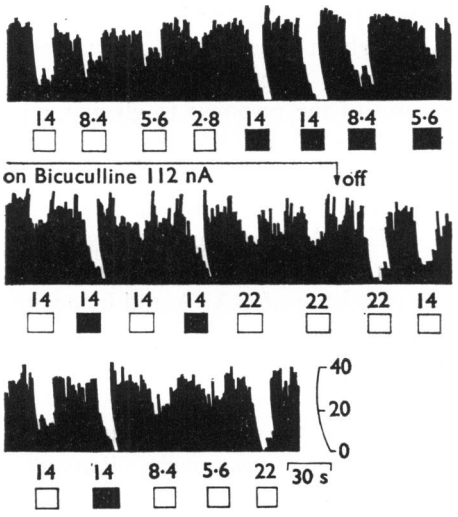

FIG. 4. Bicuculline slowing the onset of the glycine response. Ratemeter records from a spontaneously active joint cell. Subsequently log-current response curves showed 7·2 nA of glycine and 10 nA of GABA to cause 50% depression of the discharge and the equipotent dose-ratios were calculated as described earlier to be 1·84 and 3·02 for glycine and GABA respectively. Note the unusually rapid and powerful action of bicuculline 112 nA on GABA 22 nA.

response curve of a hair cell to the right, the equipotent dose-ratio was 1·7; the average value was not significantly different from 1·0 ($1·10 \pm 0·04$).

The influence of bicuculline on non-hair relay cells was less selective however, since the average equipotent dose-ratio for glycine was 1·31. For instance, in Fig. 4 the onset of the glycine effect was clearly slowed by bicuculline. Although the peak depression was little altered, the overall depression was sufficiently reduced to cause a parallel shift of the log-current response curve and the equipotent dose-

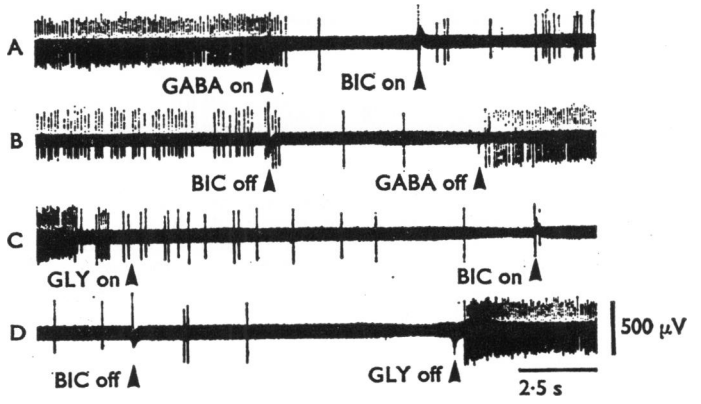

FIG. 5. The greater specificity of smaller doses of bicuculline for γ-aminobutyric acid (GABA) rather than glycine. In continuous film records A and B, separated by an interval of 17 s, arrows mark the start and finish of a GABA application which very nearly abolished the glutamate-evoked discharge. The depressant action of GABA was interrupted when the cell's excitability was restored by releasing 60 nA of bicuculline (BIC) simultaneously with the GABA during the period marked by the intervening pair of arrows. When the experiment was repeated with an equipotent dose of glycine (GLY) instead of GABA the specificity of the bicuculline/GABA interaction was confirmed. Records C and D are interrupted by a gap of 30 s during the superimposition of a much longer application of bicuculline which failed to restore the cell's discharge.

ratio was 1·87. The accompanying change in GABA sensitivity, however, was at least twice as great. 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, e.g. Figures 1–4.

The specificity of bicuculline for GABA as opposed to glycine, was never in doubt when the dose of bicuculline was restricted to about 84 nA. The spontaneously active joint cell whose discharge is shown in Fig. 5 to be almost abolished by a continuous release of GABA, began to discharge within a few seconds of the onset of a 60 nA application of bicuculline. The discharge continued to accelerate throughout the simultaneous release of GABA and bicuculline for 30 s, until silence was restored by terminating the release of bicuculline. Depression of the cells activity by glycine was not impaired by a similar bicuculline release which lasted nearly twice as long.

An effort was also made to test the possibility that low doses of bicuculline may potentiate the action of GABA on cuneate cells. Bicuculline has been reported to enhance the action of GABA on cortical neurones (Straughan, Neal, Simmonds, Collins & Hill, 1971). This test was made on extremely brief depressions of the spike discharge lasting less than 300 ms, evoked by GABA pulses 5–10 ms in duration. In Fig. 6, the photographic records of the spike discharge which began immediately after the onset of the bicuculline application, show the duration of the GABA-evoked depressions to be progressively reduced without the occurrence of a phase of GABA potentiation. Similar depressions evoked by glycine were unaffected by very much larger doses of bicuculline (cf. Fig. 2 in Kelly & Renaud, 1973b).

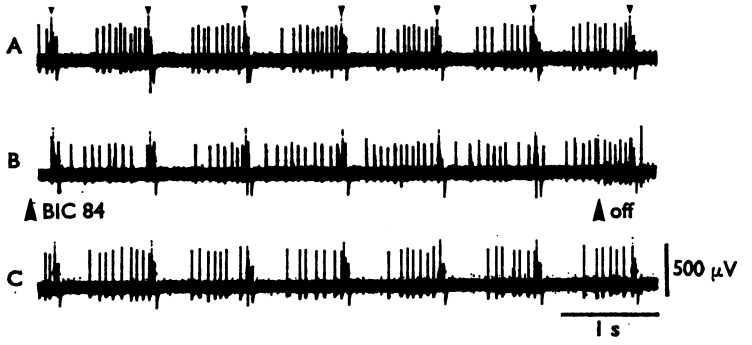

FIG. 6. In the cuneate, bicuculline blockade was not preceded by a period of enhanced γ-aminobutyric acid (GABA) sensitivity. Film records A, B and C were continuous and show the response of a glutamate-excited hair cell to pulses of GABA 10 ms in duration (marked by arrows) repeated at 1 s intervals. Within 2 s of the onset of bicuculline (BIC) 84 nA at beginning of record B the 'silent period' which followed the GABA pulse is reduced in length without any obvious potentiation occurring beforehand.

Intravenous bicuculline

During the last hour of each experiment, bicuculline was administered intravenously in 5 or 6 repeated doses of about 0·2 mg/kg. On no occasion was the GABA sensitivity modified until the animal began convulsing and alterations of the spike amplitude made reliable recording impossible. When the convulsions were controlled by paralysing the animal with intravenous succinylcholine or additional sodium pentobarbitone, the cells appear to have retained their GABA sensitivity. Intravenous picrotoxin (4 mg/kg) and strychnine (0·2 mg/kg) injected between bicuculline doses, were effective blockers of GABA and glycine respectively.

Perfusion of the surface of the cuneate with solutions containing 0·5 mM of bicuculline were also ineffective although similar concentrations of picrotoxin and strychnine proved active (cf. Galindo, 1968, 1969; Hill, Simmonds & Straughan, 1972).

β-Guanidino-propionic acid

On cuneate neurones as in the cerebral cortex (Krnjević & Phillis, 1963) β-guanidino-propionic acid (βGP), a competitive antagonist of GABA at the invertebrate inhibitory neuromuscular junction (Feltz, 1971), was found to mimic the action of GABA. Indeed the results in Table 1, on 7 cuneate neurones excited from the periphery (4 hair cells), suggest that the responses to GABA and βGP are indistinguishable. Although βGP might be slightly less potent than GABA, the log-current response curves not only have similar slopes but are shifted to the right by comparable distances by the same dose of bicuculline. In three trials, similar but qualitative results were obtained with δ-aminovaleric acid.

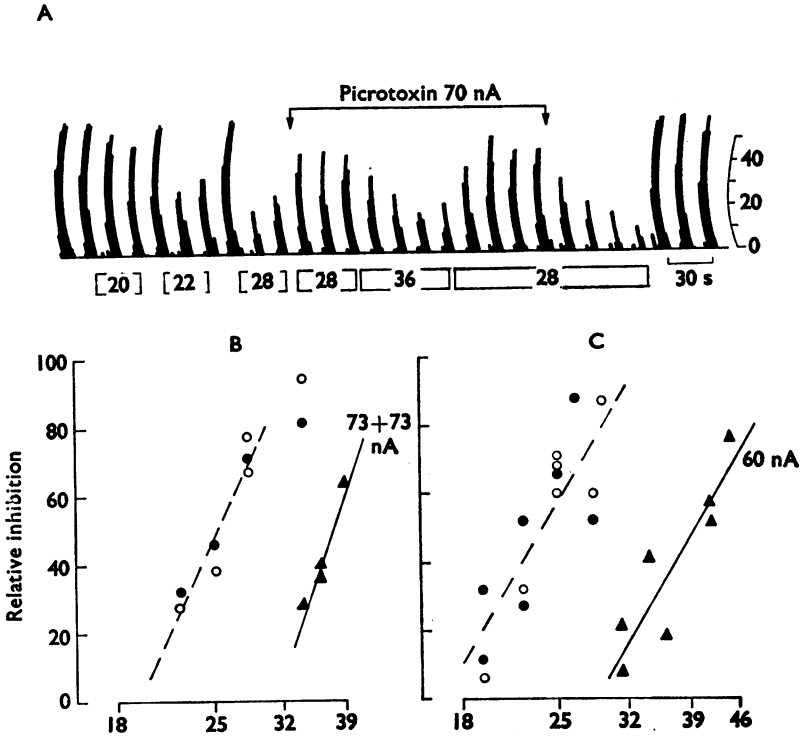

FIG. 7. Ratemeter records and γ-aminobutyric acid (GABA) log-current response curves to show picrotoxin and GABA interactions. In the ratemeter record (A) an iontophoretic picrotoxin current of 70 nA completely abolished the action of GABA 28 nA on the excitation of a joint cell by intermittent pulses of glutamate. Increasing the GABA current to 36 nA overcame the action of picrotoxin. GABA log-current response curves from the same cell are shown in C on the right below before (●), after (○) and during a 60 nA application of picrotoxin (▲). The separation of the regression lines, calculated by the method of least squares, gave an equipotent dose ratio of 1·78. The GABA log-current response curves in B are from a spontaneously active joint cell isolated from the same cat. The displacement of the curves, caused by simultaneous bicuculline and picrotoxin currents of 73 nA, was equivalent to an equipotent dose ratio of 1·48. Earlier the equipotent dose-ratios of 1·27 were measured when similar currents were administered separately. Selected parts of the ratemeter records are shown in Figure 8.

Picrotoxin

Our results, in Table 1, also confirm the finding by Galindo (1969) that picrotoxin applied to cuneate neurones in the absence of glutamate preferentially antagonizes GABA rather than glycine. Iontophoretic applications of picrotoxin caused many of the glutamate-excited cells to become hyperactive and uncontrollable. On only three cells excited intermittently by pulses of glutamate was it possible to test the action of picrotoxin. An example of the reversal by picrotoxin 70 nA of the depression evoked by GABA 28 nA is shown in Fig. 7A. Log-dose response curves for the same experiment (Fig. 7C) show a parallel shift to the right of the GABA curve equivalent to an equipotent dose-ratio of 1·8. The shift of the GABA log-current response curve shown in Fig. 7B occurred when picrotoxin and bicuculline were applied simultaneously to a spontaneously active joint cell. Earlier, separate applications of picrotoxin 73 nA and bicuculline 73 nA lasting 12·5 min had caused changes in the GABA equipotent dose-ratio of about 1·3, Later simultaneous applications of the same doses of picrotoxin and bicuculline caused the shift illustrated (Fig. 7). Since the equipotent dose-ratio was now 1·48, the effects of picrotoxin and bicuculline appear to have been additive, suggesting not only that they are equipotent but that they may react with the same receptor. Throughout this experiment the ratemeter records show the glycine response to be unaltered (Fig. 8).

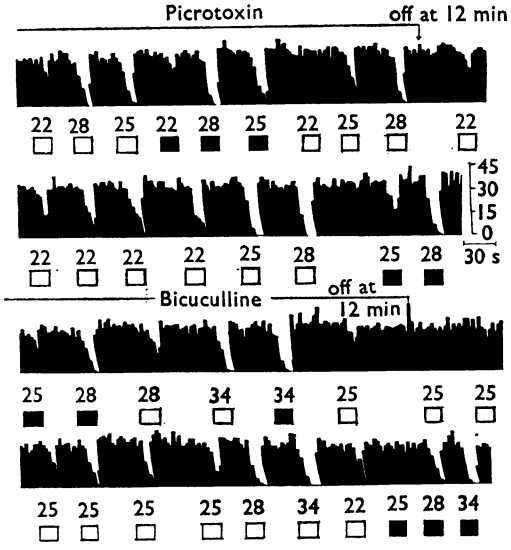

FIG. 8. Recovery of γ-aminobutyric acid (GABA) sensitivity following equipotent applications of picrotoxin and bicuculline. The upper and lower pairs of ratemeter records are continuous and show the response of a spontaneously active joint cell to repeated trials of the same equipotent dose of GABA following 12 min applications of picrotoxin and bicuculline with currents of 73 nA. Similar shifts of the GABA log-current response curve were caused by both picrotoxin and bicuculline ; the equipotent dose-ratios were about 1·27.

On withdrawal of the antagonists in the experiments described in the last paragraph (Fig. 8) a further similarity between the actions of picrotoxin and bicuculline became apparent. At the end of the 73 nA picrotoxin application, which totally blocked the action of GABA 28 nA after 12·5 min, gradual recovery of the GABA-

sensitivity occurred over a period of at least 8 minutes. Later in the same experiment, full recovery of the response to an equipotent dose of GABA followed a similar time-course when the bicuculline application was terminated. Two independent features of our data, therefore, both suggest that picrotoxin and bicuculline block the action of GABA by a similar if not identical mechanism. Although at the crayfish inhibitory neuromuscular junction, picrotoxin has been shown to be rather more potent than bicuculline as a depressant of the increase in membrane conductance produced by GABA, Takeuchi & Onodera (1972) suggested that they have a common mode of action.

Discussion

In their original study, which showed GABA and glycine to be much more potent and consistent blockers of cell activity in the cuneate nucleus than catecholamines, 5-hydroxytryptamine and histamine, Galindo *et al.* (1967) concluded that GABA and glycine appear equally capable of assuming the role of inhibitory transmitters. In this study equipotent doses of GABA and glycine were found to be released when somewhat larger currents were applied to the GABA electrode. Similar results were obtained both in experiments on every relay cell encountered, in which the currents used to expel alternate doses of GABA and glycine were adjusted until their responses matched, and in more careful experiments by comparing the GABA and glycine log-current response curves from the same cell. The glycine log-current response curves always lay to the left of the GABA curves and were almost twice as steep.

From the parameters given in Table 1, it can be calculated that the average hair cells will show a threshold response to equal GABA and glycine currents of about 11 nA. Responses of 50 and 100% respectively occur with GABA currents of 20 and 44 nA or glycine currents of 14 and 20 nA. The real ratio of the GABA to glycine potency must however, be corrected for a possible difference in their iontophoretic transport number which Werman, Davidoff & Aprison (1966) estimated as 0·3 and 0·5 respectively. Transformation of the log-current response curves to log-*dose* response curves is further complicated by the 14 nA releasing current used to offset the 'backing current' necessary to prevent leakage from the electrode by diffusion. It is possible, therefore, that the log-dose response curves for GABA and glycine are identical.

Even if the doses of GABA and glycine released from the electrode appear equipotent their concentration at the postsynaptic membrane may well differ. The GABA and glycine receptors although on the same cell membrane, may lie at differing distances from the tip of the electrode or one or other of the amino acids may be preferentially taken up by the surrounding tissue. For example GABA is preferentially taken up by the cerebral cortex (Iversen & Neal, 1968) and glycine by the spinal cord (Neal, 1971). Although the relevance of this uptake mechanism to potency is still controversial, the potency of GABA in the cortex has been shown by Gottesfeld, Kelly & Renaud (1972) to be enhanced by pretreatment with amino-oxyacetic acid, perhaps as a consequence of an impaired GABA uptake (Snodgrass & Iversen, 1973).

Cells equally sensitive to GABA and glycine are of special interest since they may resemble motoneurones where both GABA and glycine can mimic the action of the inhibitory transmitter by hyperpolarizing the membrane potential towards the same equilibrium level by selectively increasing the membrane conductance to

certain ions (Curtis, Hösli, Johnston & Johnston, 1968; Werman, Davidoff & Aprison, 1968). Indeed glycine may be a more potent depressant of spinal motoneurones than GABA (Curtis, Hösli & Johnston, 1968; Curtis *et al.*, 1968b). Similarly, Hösli, Tebécis & Filias (1969) found brain stem neurones lying deep to the cuneate nucleus to resemble motoneurones and suggested that glycine may be the inhibitory transmitter of medullary reticular neurones. Only the specific action of strychnine allowed Curtis *et al.* (1968a) to discriminate between the action of GABA and glycine and show the postsynaptic inhibitory transmitter of motoneurones to by glycine. Subsequent studies of medullary reticular neurones (Tebécis & Di Maria, 1972) have shown synaptic inhibition and glycine-evoked depression of the same neurone to be reduced or completely blocked by iontophoretically administered strychnine. More recently, following the introduction of bicuculline as a specific GABA antagonist, Curtis and his colleagues (Curtis & Felix, 1971) have extended their studies to show that motoneurones are inhibited by at least two separate pathways terminating on two distinct populations of interneurones capable of releasing either GABA or glycine. In the cortex, however, where GABA is about 4 times more potent than glycine (Krnjević & Phillis, 1963; Curtis *et al.*, 1968a; Kelly & Krnjević, 1969; Johnson, Roberts & Straughan, 1970), Kelly & Krnjević (1969) found that only GABA mimicked the effect of the inhibitory transmitter on the cell membrane. GABA was also considered a more effective depressant than glycine on the spike-discharge of Deiters' neurones (Bruggencate & Engberg, 1969, 1971; Obata, Takeda & Shinozaki, 1970), Purkinje cells of the cerebellum (Kawamura & Provini, 1970; Curtis *et al.*, 1971b) and the mitral cells of the olfactory bulb (Nicoll, 1971), three situations where the inhibitory transmitter may well be GABA. Since GABA and glycine are equipotent depressants of cuneate relay cells the transmitter released by the final interneurones of the separate inhibitory pathways which impinge on cuneo-thalamic relay cells, could be GABA or glycine.

Iontophoretic bicuculline

Although the responses of cuneate relay cells to GABA and glycine may be indistinguishable in terms of potency, bicuculline was found to discriminate between their actions. Iontophoretic bicuculline markedly reduced the sensitivity to GABA without significantly modifying the response to glycine. By an analysis of GABA and glycine log-current response curves obtained from a fairly large sample of identified neurones excited by either glutamate or peripheral stimulation, bicuculline was found to cause a reversible parallel shift of only the GABA curve. The average GABA equipotent current ratio was 2·0. Except on a few occasions where different doses of bicuculline were tested on the same cell, no correlation was found between the GABA equipotent current ratio (log DR$-$1) and the bicuculline current (log) or the sensitivity of the cells to GABA, and indeed, the results are best explained by assuming that all the electrodes released approximately the same amount of bicuculline. This may well be the case since the iontophoretic transport number may vary with the ability of the electrode to pass current (cf. Zieglgansberger, Herz & Teschemacher, 1969). The currents used were often the highest the electrode would tolerate without generating electrical 'noise' or 'blocking'. With cuneate neurones the potency of iontophoretic bicuculline as an antagonist of GABA shown in Table 1 is similar to that of spinal neurones. Curtis *et al.* (1971a) found that

bicuculline ejecting currents in excess of 100 nA were required to block the response to GABA released with a current of 20 nA. On a number of spinal neurones where the analysis was also made from log-current response curves, large iontophoretic applications of bicuculline and intravenous injections led to a two-fold increase in the GABA current required for 50% depression of the firing rate.

Comparable studies of the influence of bicuculline on GABA log-current response curves can, therefore, prove a useful method of showing cuneate and spinal interneurones to be equally sensitive to the action of bicuculline even though this type of analysis cannot define the drug-receptor interaction (Curtis *et al.*, 1971c). These experiments also showed that the selectivity of bicuculline as a reversible blocker in the cuneate also encompasses a number of structurally related GABA-like amino acids (Curtis *et al.*, 1971a). In particular δ-aminovaleric acid and βGP were found to be of comparable potency to GABA as agonists and their bicuculline equipotent dose-ratios differed little from that of GABA (cf. Table 1). These results provide evidence that GABA, βGP, δ-aminovaleric acid and bicuculline all interact with a receptor which closely resembles the GABA receptor of spinal neurones.

Usefulness and validity of log-current response curves

The construction of straight line log-current response curves allows the relative potencies of inhibitory amino acids to be expressed rather more precisely (Table 1) than can normally be achieved by comparing the currents used to eject equipotent doses. Indeed the latter approach can be rather misleading when, as in the case of GABA and glycine on cuneate neurones, the slopes of the log-current response curves differ greatly. In this situation the relative potencies of the two amino acids are dependent on the level of inhibition regarded as significant.

Our attempt to show bicuculline to be a more selective antagonist of GABA than glycine would probably have failed without the use of log-current response curves obtained in the presence and absence of the antagonist. For instance, it is not difficult to select isolated parts of the ratemeter records used to illustrate this paper which show bicuculline to antagonize some of the responses to glycine. However, log-current response curves show the maximum effect on the equipotent dose-ratio to be insignificant and equivalent to a change in glycine-sensitivity of only a few additional nA being required for 50% inhibition. The steepness of the glycine log-current response curve accounts for these anomalies.

The equipotent dose-ratio calculated from the log-current response curves also proved a useful index when used to compare the sensitivity of the individual cells to antagonists. In the cuneate for instance the effect of bicuculline and picrotoxin look similar and their effects may be additive. A comparison of our results with those of Curtis *et al.* (1971a) also allows us to suggest that the cells of the cuneate are no less sensitive to iontophoretic bicuculline than those of the spinal cord.

Like Curtis *et al.* (1971c) we have made no attempt to validate our use of log-current response curves and simply draw attention to their usefulness. Further interpretation of these curves is difficult as the reduction in neuronal firing which accompanies an increased ejection of the agonist can be attributed to an increased density of drug receptor interactions caused by exposure of the same receptors to higher concentrations of the agonist or to an involvement of a larger population of receptors by further diffusion of the agonist.

Picrotoxin

Picrotoxin was confirmed to be a selective antagonist of GABA both on cells excited from the periphery (Galindo, 1969) and cells excited by iontophoretic glutamate. Picrotoxin and bicuculline proved to be equally effective when compared on the same cuneate relay neurone. Equal shifts of the log-current response curve occurred when the releasing currents were of the same order and often the effects could be shown to be additive. Although picrotoxin may also be an effective GABA antagonist at a number of sites in the mammalian C.N.S. (Galindo, 1969 ; Bruggencate & Engberg, 1969 ; 1971 ; Engberg & Thaller, 1970 ; Obata & Highstein, 1970 ; Obata, Takeda & Shinozaki, 1970 ; Nicoll, 1971), Curtis *et al.* (1971a) have suggested that the relative potency of picrotoxin on spinal interneurones is one quarter that of bicuculline. This difference in sensitivity to picrotoxin may reflect a real difference in the GABA receptors of cuneate and spinal cord neurones. However, picrotoxin and bicuculline ejected from a microelectrode affect only a small area of the postsynaptic membrane which need not be coincident and the degree of overlap could differ in the cuneate and spinal cord.

Presynaptic interactions

Several attempts have been made to reveal a presynaptic component of GABA's action on cuneate neurones (Galindo, 1969 ; Davidson & Southwick, 1971). Although GABA but not glycine has been shown to increase the excitability of the afferent terminals in the cuneate nucleus, the relative potencies of GABA and glycine appeared in our experiments to be the same on cells excited either postsynaptically by glutamic acid or transynaptically by peripheral stimulation. In addition, the potency of bicuculline and picrotoxin as depressants, of the GABA sensitivity also appear to be indifferent to the way in which the cells were induced to discharge. If GABA receptors residing on both the pre- and postsynaptic membrane contribute to the actions of iontophoretic GABA witnessed in our experiments, iontophoretic bicuculline and picrotoxin are clearly unable to discriminate between the two populations of receptors.

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