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On the pharmacology of ascending, descending and recurrent postsynatic inhibition of the cuneo-thalamic relay cells in the cat

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Summary

1. In cats decerebrated or anaesthetized with pentobarbitone, cells of the middle third of the cuneate nucleus that were excited by tactile stimulation of the ipsilateral forelimb (responding to displacement of hairs, skin or joints) and inhibited by electrical stimulation of the contralateral pyramid, were invariably inhibited by electrical stimulation of the ipsilateral forepaw and the contralateral forelimb nerves.

2. In 50% of the cats, the cells were more fully identified by placing electrodes stereotaxically in the contralateral medial lemniscus. Recurrent inhibition was always a concomitant of the antidromic action potential.

3. The intensity and the duration of inhibition evoked by all of these pathways was totally resistant to iontophoretic and intravenous strychnine in doses at least 5 times that required to block completely the response of the same cells to iontophoretic glycine and was extremely sensitive to either iontophoretic bicuculline or picrotoxin.

4. Although the inhibition was invariably sensitive to intravenous picrotoxin, no significant change occurred in the duration or intensity of the inhibition when bicuculline was administered intravenously (5 or 6 times) as repeated doses of 0·2 mg/kg.

5. Postsynaptic inhibition in the cuneate may be mediated by γ-aminobutyric acid released from the nerve terminals of a common pool of interneurones shared by ascending, descending and recurrent pathways. Since the receptors involved in this pathway are resistant to intravenous bicuculline, they may well be distinct from those responsible for changes in the primary afferent terminal excitability, usually believed to be associated with presynaptic inhibition.

Introduction

While exploring the possibility that afferent 'surround' inhibition can be modulated by the cerebral cortex, Gordon & Jukes (1964a, b) drew attention to two distinct populations of neurones within the dorsal column nuclei. The first, inhibited by stimulation of the contralateral sensorimotor cortex (Scherrer & Harnández-Peón, 1955; Dawson, 1958; Magni, Melzack, Moruzzi & Smith, 1959; Jabbur & Towe, 1961; Dawson, Podachin & Schatz, 1963; Levitt, Carreras, Liu & Chambers,

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1964; Rosén, 1969), were exquisitely sensitive to inhibition initiated by cutaneous stimulation of the ipsilateral limb (Gordon & Paine, 1960; Pearl, Whitlock & Gentry, 1962; Dawson *et al.*, 1963; McComas, 1963); and the second excited by cortical stimulus (Levitt *et al.*, 1964; Towe & Jabbur, 1961) never showed inhibition during peripheral stimulation (Gordon & Jukes, 1964a; Andersen, Eccles, Oshima & Schmidt, 1964). The cells characterized by cortical and afferent inhibition are also the recipients of inhibition originating in the contralateral limbs (Jabbur & Banna, 1968, 1970) and recurrently following antidromic invasions of their axons (Gordon & Paine, 1960; Gordon & Seed, 1961; Gordon & Jukes, 1964b; Andersen *et al.*, 1964a). Independently Gordon & Jukes (1964b) and Andersen *et al.* (1964a) concluded that all of the inhibitory pathways share the same cortically excited cells which therefore belong to a common pool of inhibitory interneurones. The hypothesis received pharmacological support from the work of Banna & Jabbur (1969). The manifestations of presynaptic inhibition evoked by all of the pathways were found to be equally sensitive to blockade by intravenous picrotoxin and bicuculline (Banna, Naccache & Jabbur, 1972) and to be facilitated by pentobarbitone and unaltered by strychnine.

However, the attributes of presynaptic inhibition in the nucleus evoked by afferent volleys (Andersen *et al.*, 1964a) and light tactile stimulation (Andersen, Etholm & Gordon, 1970) are accompanied by postsynaptic inhibition of individual cuneate relay neurones (cf. Galindo, Krnjević & Schwartz, 1967). It is possible therefore that pre- and post-synaptic inhibitory pathways traverse different interneurones. Further subdivision may also be present since inhibition of motoneurones has recently been shown to involve at least two distinct populations of interneurones which can be differentiated by the sensitivity of their transmitters to strychnine and bicuculline respectively (Curtis & Felix, 1971a). In this study inhibitions evoked by all inputs proved resistant to strychnine and equally sensitive to either iontophoretic bicuculline or picrotoxin. In the two preceding papers (Kelly & Renaud, 1973a, b) strychnine and both bicuculline and picrotoxin were shown on the same cells to discriminate between glycine and γ-aminobutyric acid (GABA) respectively. Inhibition in the cuneate is therefore likely to be mediated by GABA rather than glycine even though both have been shown to be almost equally effective depressants of cuneate relay neurones (Galindo *et al.*, 1967; Kelly & Renaud, 1973a).

Methods

The results to be presented in this paper were obtained from the same series of experiments on cats anaesthetized with sodium pentobarbitone or decerebrated under initial halothane/nitrous oxide anaesthesia, described in two companion papers (Kelly & Renaud, 1973a, b). Action potentials from cells identified as cuneothalamic relay cells, were recorded extracellularly through one channel of a multibarrelled glass pipette (tip outer diameter >5 μm) filled with 2·7 M NaCl while the other channels were used for iontophoresis.

Iontophoretic solutions

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 (B.D.H.): 5 mM in 165 mM saline, pH 7·5 (cf. Davidoff & Aprison, 1969) ; strychnine sulphate (B.D.H.): 5 mM in 165 mM saline, pH 5·5 ; β-alanine: 1M, pH 3·5 ; β-guanidino-propionic acid: 1 M, pH 4·0 ; δ-aminovaleric acid: 1 &, pH 4·0.

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 output pulses of the gate were also available for analysis on-line by a LINC-8 computer.

LINC-8 computer

The computer was programmed to display post-stimulus latency histograms (PSLH) on line. Every second, synchronized with the start of each oscilloscope sweep, a pulse from the Digitimer (Devices) reset a 2 kHz crystal clock to zero. The number of 1 s periods or clock resets was usually limited by the programme to 128. Each action potential interrupted the display and caused one of the 1,000 memory registers, determined by reading the clock, to be incremented. The data were kept in 1,000 registers but displayed as histograms of 128, 8 ms bins. The contents of each bin were shown normalized as a percentage of the average frequency of discharge in the immediate pre-stimulus period 100–300 milliseconds.

Results

Only cells of the cuneate nucleus inhibited by stimulation of the pyramids, were selected for the studies reported in this and the two companion papers (Kelly & Renaud, 1973a, b). Inhibition was revealed by means of post-stimulus latency histograms as a stimulus linked depression of their discharge maintained at an adequate frequency either postsynaptically by iontophoretic glutamate (cf. Galindo *et al.*, 1967) or presynaptically by sustained peripheral stimulation. Although the majority of cells were indisputably hair cells (cf. Gordon & Jukes, 1964a) a number of the inhibited cells showed quite different characteristics. Like hair cells they were spontaneously active but could not be excited by a jet of air applied to the forelimb and neck and required light or firm pressure to the limb or fine or coarse movement of a joint.

When the background synaptic activity or the glutamate-evoked discharge of a relay cell was found to be inhibited by either a single shock or a three shock train to the pyramids it was invariably found to be inhibited by single shocks applied in turn to the contralateral median and sural nerves, the ipsilateral forepaw and the medial lemniscus. By design, each experiment was confined to the middle third of the cuneate nucleus and most of the cells tested lay in the immediate vicinity of the first cell of that experiment found to be inhibited by the inputs tested. The majority of the cells in these regions were undoubtedly hair cells and all of the cells found to be excitable by stimulation of the periphery were inhibited by all of the inputs tested. No cells were selectively inhibited by only a single input. Single cells inhibited by ascending, descending and recurrent pathways, seemed equally easy to locate regardless of how the background discharge was initiated. Earlier, Gordon & Jukes (1964a) had also found hair cells inhibited by afferent stimulation,

to predominate in the middle third of the dorsal column nuclei. In addition, they found the axons of these cells to project within the contralateral medial lemniscus (Andersen *et al.*, 1964a).

The records in Fig. 1 are from a cuneate cell found to respond to light pressure applied to the dorsum of the forearm and identified as a relay cell when stimulation of the medial lemniscus was found to evoke an antidromic action potential. Stimulation of the medial lemniscus was also found to be followed by the inhibitory period shown in Fig. 1 by photographic records of both multiple superimposed oscilloscope traces and post-stimulus histograms. Almost identical inhibitions were evoked by single shock stimulation by all of the alternative inputs tested, ipsilateral forepaw, contralateral pyramid and the contralateral median and sural nerves. Synaptic inhibition could be mimicked by interrupting the glutamate-evoked discharge for periods of about 500 ms, by short pulses of glycine only 5 to 25 ms in duration. The first column of records in Fig. 1 show the recovery of the cell's sensitivity to glycine which followed termination of a short application of strychnine 14 nA for 40 s which was sufficient to block completely the cell's response to glycine but not to GABA. During a longer application of strychnine 28 nA lasting nearly 9 min there was a reversible parallel shift of the glycine log-dose response to the right, equivalent to an equipotent dose-ratio of 3·2 (see Fig. 3, Kelly & Renaud, 1973b) and recovery of full glycine sensitivity took at least 6 minutes. Two columns of post-stimulus histograms recorded before and near the end of the strychnine application show the synaptically evoked inhibition not to be impaired during the marked reduction in glycine sensitivity. Although in the previous paper (Kelly & Renaud, 1973b) glycine was shown to be invariably blocked by small doses of strychnine, on no occasion was the synaptic inhibition seen to be modified by strychnine in maximal doses applied by either iontophoresis 28 to 112 nA, topical application

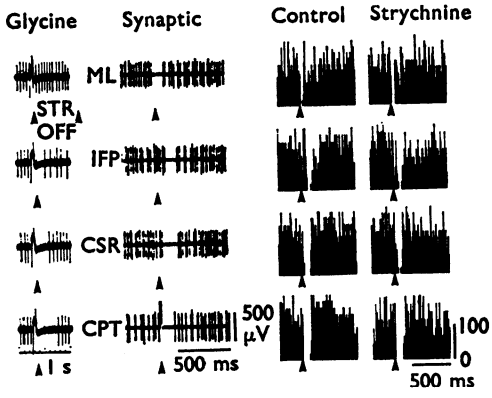

FIG. 1. The influence of iontophoretic strychnine on a cuneo-thalamic relay cell inhibited by pulses of glycine and transynaptically. Action potentials and histograms from a touch cell identified by antidromic invasion from the medial lemniscus. In the first column, the arrowheads mark on single oscilloscope traces, the end of a 20 ms, 30nA pulse of glycine. The records were made 1 s before and 30, 40 and 60 s after the end of a 40 s, 14nA iontophoretic application of strychnine (STR). In the subsequent columns, the arrowheads mark electrical stimulation with 0·1 ms pulses to the medial lemniscus (ML) 10V, the ipsilateral forepaw (IFP) 10V, contralateral sural nerve (CSR), 2V and the contralateral pyramidal tract (CPT), three shock train at 400 Hz of 6V. In the second column, a selection of superimposed traces show the synaptic inhibition as an interruption of the spike discharge. Post-stimulus latency histograms in the last two columns, were compiled on-line to show the synaptic inhibition to be unaffected near the end of a 28nA application of strychnine lasting 8·8 minutes Each histogram was compiled from 64 stimulus trials at 1 Hz and the bins were normalized about the mean discharge level in the 300 ms period prior to each stimulus. (In the first column where the gain was twice that in the second, the tops of the action potentials are masked.)

0·5 mM in mammalian Ringer solution or intravenous injections of 2·0 mg/kg. It appears, therefore, that the inhibitory transmitter of the pathways tested is unlikely to be glycine rather than GABA even though both proved equally potent depressants of cuneate neurones (Galindo *et al.*, 1967 ; Kelly & Renaud, 1973a).

Synaptic inhibition in the cuneate could also be mimicked by iontophoretic GABA applied by short current pulses lasting less than 30 ms which interrupted the background activity or glutamate-evoked discharge for periods of less than 500 milliseconds. Almost invariably GABA but never glycine-evoked inhibition was blocked by quite moderate applications of bicuculline. In Fig. 2 the selective influence of

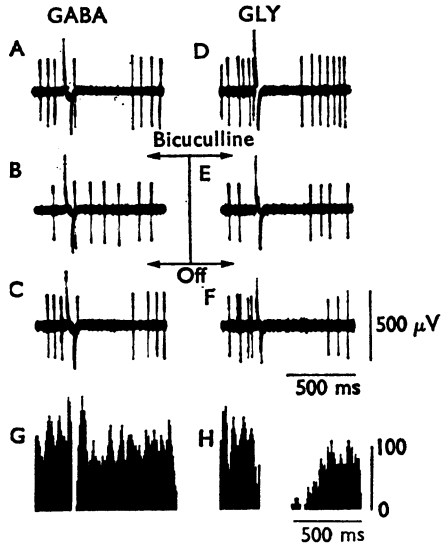

FIG. 2. The influence of bicuculline on synaptic inhibition mimicked by γ-aminobutyric acid (GABA) and glycine pulses. Photographic records of selected single oscilloscope traces show the responses of a touch cell excited by iontophoretic glutamate 11nA, to a 30 ms pulse of GABA; A, before, B, 4 min after the application of bicuculline 112nA and C, after the recovery. Glycine pulses only 15 ms in duration caused a similar interruption of the discharge and D–F show selected traces before, during and after the same application of bicuculline. (Approximately 300 ms from the origin of each trace, stimulus artifacts mark the GABA or glycine pulses; in this experiment the Z-axis intensification circuit intermittently failed to brighten in part the later components of the action potentials.) The post-stimulus latency histogram G–H were compiled during the bicuculline application from 64 GABA and glycine pulses respectively tested at frequencies of 1 Hz.

bicuculline on GABA-induced inhibition as opposed to glycine is demonstrated by photographic records of single oscilloscope traces and post-stimulus latency histograms compiled during the bicuculline application. In Fig. 3, histograms A–C show a joint cell, excited by iontophoretic glutamate, and inhibited by stimulation of the contralateral pyramid by a three shock 400 Hz train of 2 V intensity repeated at 1 s intervals. The inhibition was very much curtailed during applications of a bicuculline of 112 nA (B) and almost complete recovery is shown in C. Histograms D–F show the inhibition evoked by a single 1 V shock to the contralateral medial nerve. Again the inhibitory response virtually disappeared during the 112 nA application of bicuculline. During similar experiments on 15 of the cells whose GABA sensitivity was reported in our companion paper (Kelly & Renaud, 1973a) to be reduced approximately two-fold by bicuculline, inhibition evoked by two or more of the ascending and descending pathways was also found to be greatly impaired during the same or a similar application of bicuculline. Although by

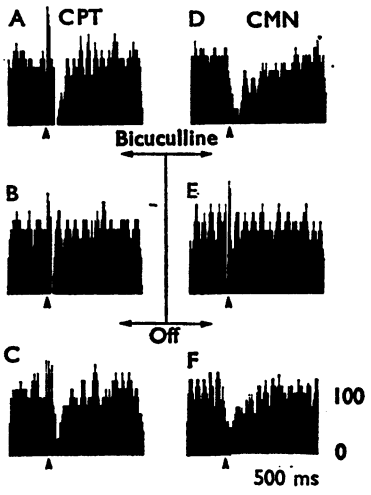

FIG. 3. The influence of bicuculline on transynaptic inhibition of a cuneate relay cell. Smoothed post-stimulus latency histograms (PSLH) photographed from an oscilloscope display computed from the discharge of a joint cell excited by iontophoretic glutamate. Stimulation at 1 s intervals by either a 3 shock train at 400 Hz to the contralateral pyramid (CPT) of 2V intensity (A, B and C) or a single 1V shock to the contralateral median nerve (CMN) (D, E and F). A and D were controls, B and E were recorded 4 and 3 min after beginning of a bicuculline application of 112nA, and C and F were recorded 6 and 3 min after the bicuculline was turned off. (Each PSLH was recorded from 64 stimuli, arrows mark the stimulus artifact. The ordinate value of each bin was derived by averaging the contents of 4 adjacent bins which were then normalized about the mean content of the initial 27 bins of the histograms (15–19 spikes/second).

the end of the bicuculline application, the inhibition evoked by all pathways appeared in most experiments to be equally impaired, the recurrent inhibition from the medial lemniscus or the surround inhibition from the ipsilateral forepaw often seemed to 'disappear' rather more rapidly or more completely than that from the other inputs onto the same cell (see row C, Fig. 4).

The synaptic inhibitions could also be modified by iontophoretic applications of picrotoxin which were no less effective than bicuculline. Intravenous bicuculline however proved rather ineffective even in cumulative doses which exceeded 12 mg/kg. On the other hand in the same experiments intravenous picrotoxin completely abolished the inhibitory responses for several hours when the dose was just less than 4 mg/kg.

The PSLHs in Fig 4 are from a hair cell excited by iontophoretic glutamate, whose discharge was depressed to 50% of its initial frequency by 20 s applications of GABA 10·2 nA and glycine 7·3 nA. Towards the end of the 9·6 min, 84 nA bicuculline the GABA equipotent current ratio was 1·6 and the glycine log-current response curve remained unaltered. Synaptic inhibition evoked by shocks to either the contralateral medial lemniscus, ipsilateral forepaw, the contralateral sural and median nerves or the contralateral pyramidal tract was virtually unaltered on two occasions by intravenous injections of bicuculline (Fig. 4 rows B & D). Bicuculline 84 nA, the maximum current tolerated by the electrode, caused a rather mild but distinct, reduction in the duration and intensity of the inhibition evoked by all inputs (row C). During complete or partial blockade no change occurred in the duration of the inhibitory pause when the stimulus strength was increased more than two-fold. Finally (row D) an injection of 4 mg/kg of picrotoxin completely abolished inhibition for at least one hour. The GABA receptors involved in

synaptic inhibition in the cerebellum appear to display a similar, although perhaps less extreme, differential sensitivity to intravenous antagonists. Bisti, Iosif, Marchesi & Strata (1972) found the blockade of synaptic inhibition of Purkinje cells by bicuculline to be rather transitory (less than 2 min) and that of picrotoxin to be extremely long lasting.

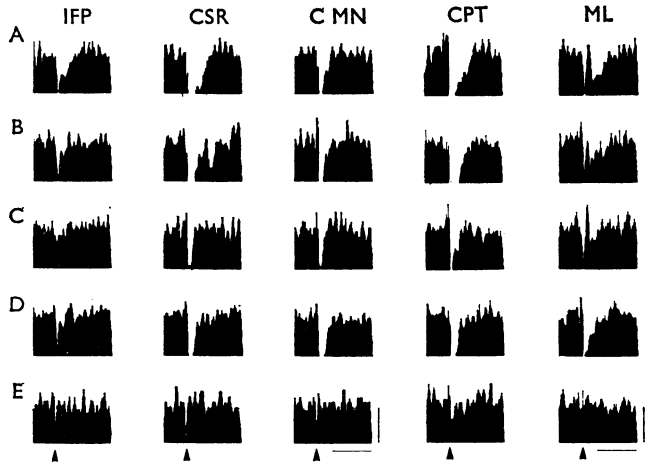

FIG. 4. The susceptibility of transynaptic inhibition of cuneo-thalamic relay neurones to intravenous picrotoxin. Five columns of post-stimulus latency histograms (PSLH) compiled and annotated as described in the legend of Fig. 3, show the inhibition of a glutamate excited hair cell by shocks of 0·5 ms in duration to the ipsilateral forepaw (IFP), 10V, contralateral sural nerve (CSR), 0·4V, contralateral median nerve (CMN), 0·4V, contralateral pyramid (CPT), 3 shock train of 13V at 400 Hz, and medial lemniscus (ML), 13V. The PSLHs of row A were compiled during a 1 h control period, B and D in the period following two intravenous injections of bicuculline, 2 mg/kg, C during an iontophoretic application of bicuculline restricted to a maximum of 84nA by the characteristics of the electrode and E following a single intravenous injection of 4 mg/kg picrotoxin. (Calibrations 100 per cent and 500 milliseconds.)

Inhibition of synaptically evoked excitation

Inhibition of cuneo-thalamic cells excited transynaptically by a single shock to the ipsilateral forepaw by conditioning shocks to the same or other pathways, also proved sensitive to bicuculline and picrotoxin administered iontophoretically. It is, therefore, possible that pre- and post-synaptic inhibition in the cuneate are equally sensitive to iontophoretic applications of bicuculline and picrotoxin (cf. Galindo, 1969).

The inhibitory curves in Fig. 5A show the percentage of shocks to the ipsilateral forepaw which, in 20 trials, successfully evoked an orthodromic spike, as a function of the interval between the testing shock to the forepaw and the conditioning inhibitory shock to the contralateral median nerve. During iontophoretic picrotoxin 112 nA, the inhibitory curve disappeared and similar but much less complete changes accompanied a 100 nA application of bicuculline. The time-course of the effects of each antagonist is shown below by plotting the probability of shocks to the forepaw causing excitation when the test conditioning interval was 30 ms i.e., the shock to the contralateral median nerve reduced the probability of discharge to near zero. Shortly after the onset of picrotoxin release, the probability of discharge was restored to 100% where it remained for several minutes after the application was terminated. Recovery was delayed for at least 20 minutes. Bicuculline was

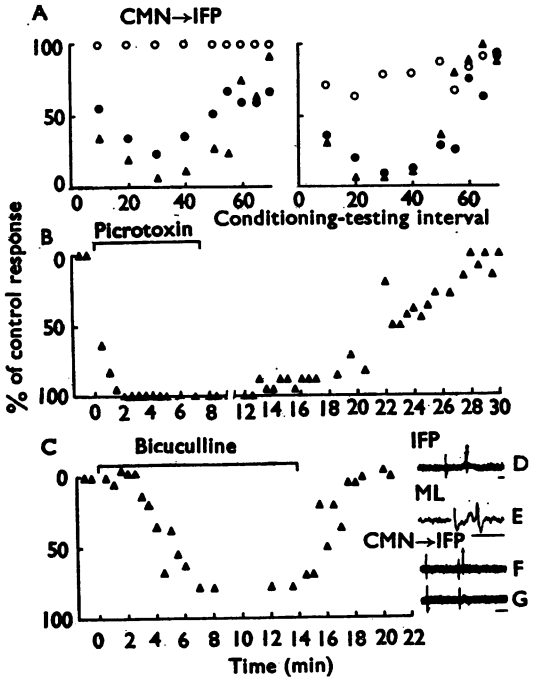

FIG. 5. Comparison of iontophoretic picrotoxin and bicuculline on inhibitory curves from a joint cell. The inhibitory curves were drawn from counts made directly from the oscilloscope face of the number of shocks to the ipsilateral forepaw (IFP) in 20 trials which successfully evoked an orthodromic action potential when preceded by a conditioning pulse to the contralateral median nerve at different test intervals. Sample records are shown in the insert; F and G show the response to a IFP stimulus in the presence and absence of a shock to the contralateral median nerve (CMN) (cal=10 ms), D displays the orthodromic response on an expanded sweep (cal=1 ms) and E the antidromic spike initiated in the medial lemniscus (ML) (cal=1 ms). The percentages of successful trials are plotted in A as a function of the conditioning test intervals before (▲), during (○) and after (●) iontophoretic applications of picrotoxin 112nA on the left and bicuculline 100nA on the right. The recovery times are shown in D and C by plotting inversely the percentage of successful trials when inhibition was maximal at a condition test interval of 30 milliseconds.

clearly less effective since the maximum probability of discharge was not attained for 4 min, was never maximal and recovery was complete within 5 min of the bicuculline release ending. In similar experiments bicuculline, although never as effective as comparable dose of picrotoxin, was found to antagonize the inhibition of orthodromically evoked action potentials conditioned by shocks to either the contralateral sural nerve, contralateral pyramid, ipsilateral medial lemniscus and the ipsilateral forepaw.

Discussion

Our observations show that the synaptic inhibition of cuneate relay neurones can be reduced or even abolished by iontophoretic applications of picrotoxin and bicuculline known to cause a two-fold reduction in the cell's sensitivity to GABA. Although the potency of glycine as a depressant of the cells spike discharge is unaltered in the presence of picrotoxin and bicuculline, synaptic inhibition by ascending, descending and recurrent pathways are all equally affected. Since the mechanisms responsible for generating the inhibitory currents can still be activated by glycine, bicuculline and picrotoxin must interrupt synaptic inhibition at the

level of the GABA receptor rather than by a non-specific change in either the cell membrane permeability or the trans-membrane ionic gradients. Therefore, synaptic inhibition regardless of the pathway involved, appears to be mediated through the same group of receptors all equally accessible to bicuculline and picrotoxin released from the micropipette. The hypothesis that a common pool of inhibitory pathways is the destination of the inhibitory pathways entering the cuneate (Gordon & Jukes, 1964b; Andersen *et al.*, 1964a) demands that inhibition of cuneate relay cells is mediated by a single transmitter and that the receptors should behave as if they all belonged to the same population and lie equidistant from the tip of the micropipettes.

The shape and size of cuneate relay cells may well explain the ease with which bicuculline and picrotoxin administered by iontophoresis block synaptic inhibition. Alternatively Curtis & Felix (1971b) have argued that blockade of inhibition by iontophoretic administration of GABA and glycine antagonists can only be demonstrated on projecting cells inhibited through relatively simple pathways. If, for example, the endings of the inhibitory interneurones terminate solely on the projecting cells and the inhibitory interneurones themselves receive only excitatory fibres, the action of the antagonist will be restricted to inhibitory synapses situated on the projecting cells. If, however, the inhibitory interneurones also receive inhibitory fibres where the transmitter is also sensitive to the antagonist, the result will be less certain, since the blockade of inhibitory synapses on interneurones will release the projecting cells from disinhibition causing the apparent potency of the inhibitory pathways to increase. We can, therefore, conclude that the sensitivity of the synaptic inhibition in the cuneate to bicuculline and picrotoxin is in accord with the hypothesis that there is a common pool of interneurones which receive only excitatory fibres (Gordon & Jukes, 1964b; Andersen *et al.*, 1964a).

Two alternate schemes in Fig. 6 show the possible relationships that may exist between the inhibitory interneurones and the excitatory nerve endings from ascending (IFP, CMN & CSR), descending (PT) and recurrent (ML) pathways. In the first scheme, the separate inhibitory interneurones are excited by each input and their endings terminate independently on the cuneo-thalamic (C-T) neurones). In

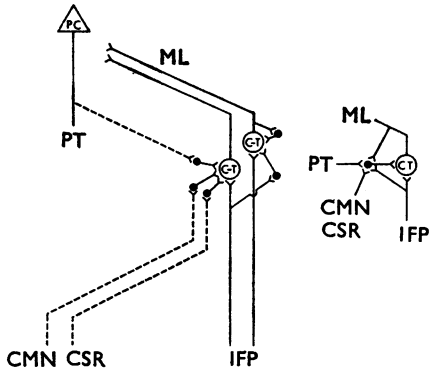

FIG. 6. Schematic diagram illustrating two alternative suggestions for the relationship between the inhibitory interneurones, which mediate postsynaptic inhibition when excited by ascending ipsilateral forepaw (IFP), contralateral median nerve (CMN) and contralateral sural nerve (CSR), descending (pyramidal tract—PT) and recurrent (medial lemniscus—ML) pathways and the cuneo-thalamic (C–T) relay cells.

the other a single population of interneurones are excited by all inputs and inhibition is mediated through their axons. Presynaptic inhibition may be mediated by another population of interneurones or by the same neurones either as a direct consequence of the transmitter released from strategically placed synapses (Walberg, 1965) or the indirect result of potassium release co-incident with their excitation.

Inhibition revealed as an interruption of the amino acid evoked discharge, is usually regarded as postsynaptic (Krnjević, Randić & Straughan, 1966; Galindo *et al.*, 1967; Curtis & Felix, 1971b; Gottesfeld, Kelly & Renaud, 1972). Krnjević *et al.* (1966) tested this idea indirectly by showing that the threshold current required to evoke inhibition of cerebral cortical neurones by a single shock to the pial surface, and the durations of the inhibitions evoked by higher currents, were indistinguishable when measured from intracellular records of inhibitory postsynaptic potentials and from extracellular records of the stimulus linked depression of the glutamate-evoked discharge. Although intracellular recordings from cuneate relay cells have shown inhibitory postsynaptic potentials to be generated in response to afferent stimulation (Andersen, Eccles, Schmidt & Yokota, 1964b; Andersen *et al.*, 1970), the records were only transitory and quite unsuitable for testing the action of GABA antagonists.

In the absence of further evidence from intracellular recordings results based on the extracellular studies of the stimulus linked depression of the glutamate-evoked discharge must be interpreted with caution. If, for instance, presynaptic inhibition is an essential feature of surround inhibition in the cuneate nucleus, the extracellular approach is too crude to detect any difference between the inhibitions evoked in the presence and absence of activity evoked presynaptically by peripheral stimulation.

Since iontophoresis cannot limit the spread of glutamate to the surface of a single neurone (Herz, Zieglgansberger & Farber, 1969), glutamate could change the excitability of primary afferent nerve terminals either directly by combining with receptors on the presynaptic membrane or more indirectly by exciting adjacent neurones. Activity in neighbouring neurones could change the membrane current of the nerve endings through morphologically identifiable axo-axonal synapses (Walberg, 1965) or as a result of a transient increase in the extracellular potassium ion concentration (Barron & Matthews, 1938; Eccles & Krnjević, 1959) caused by the close proximity of the excited cells to the nerve terminals. It is unlikely, however, that glutamate is a potent excitant of primary afferent nerve terminals. In the cuneate nucleus of the cat, Galindo (1968) was unable to repeat the experiments of Curtis & Ryall (1966) in the spinal cord which showed iontophoretic glutamate to increase the excitability of the primary afferent nerve terminals. When the surface of the rat cuneate is bathed with glutamate it causes an increase in primary afferent terminal excitability and enhances presynaptic inhibition (Davidson & Southwick, 1971) however, it is unlikely that these changes are a result of a specific action of glutamate on the nerve endings.

In the past, pre- and post-synaptic inhibition have also been differentiated on pharmacological grounds. Relatively small intravenous doses of picrotoxin (0·2 to 1·0 mg/kg) were found to reduce presynaptic inhibition in the spinal cord (Eccles, Schmidt & Willis, 1963) in the trigeminal nucleus (Shende & King, 1967) and in the cuneate nucleus (Jabbur & Banna, 1968; Banna & Jabbur, 1969). However, much larger doses of picrotoxin in excess of 2 mg/kg, similar to those found in

the present series of experiments to block the synaptically evoked depression of the glutamate-evoked discharge, have been found to block postsynaptic inhibition in occulomotor neurones (Ito, Highstein & Tsuchiya, 1970), vestibular nuclei (Ito, Highstein & Fukada, 1970), spinal motoneurones (Engberg & Thaller, 1970) and Deiters' neurones (Obata, Takeda & Shinozaki, 1970 ; Ten Bruggencate & Engberg, 1971). Indeed, iontophoretic picrotoxin has been repeatedly shown to interact specifically with GABA receptors of the postsynaptic membrane (Ten Bruggencate & Engberg, 1969 ; Obata & Highstein, 1970 ; Nicoll, 1971). The location of the interaction was found indisputably to be postsynaptic in Dieters' neurones when intracellular recording showed iontophoretic picrotoxin to block the hyperpolarizing response to GABA and inhibitory postsynaptic potentials (Obata *et al.*, 1970 ; Ten Bruggencate & Engberg, 1971). Relatively small intravenous doses of bicuculline (0·5 to 1·0 mg/kg) have been found not only to block the increase in excitability of the primary afferent nerve terminals which follows a conditioning stimulus in the cuneate (Davidson & Southwick, 1971 ; Banna *et al.*, 1972) and in the spinal cord (Levy, Repkin & Andersen, 1971) but also the dorsal column and dorsal root reflexes. On the other hand, in this paper inhibition believed to be postsynaptic was resistant to intravenous bicuculline even when injections of 2 mg/kg were repeated 5 or 6 times. Inhibition in the cuneate must, therefore, be mediated by at least two groups of GABA receptors which can be distinguished by their sensitivity to intravenous bicuculline.

Neither the duration nor the intensity of the synaptically evoked inhibition was ever modified by strychnine administered in fairly massive doses both iontophoretically and intravenously (Curtis, 1962). In view of our finding that iontophoretic glycine was exquisitely sensitive to strychnine (Fig. 1, this paper ; Kelly & Renaud, 1973b) when the dose was approximately one fifth to one tenth of that which failed to influence synaptic inhibition, it is unlikely that glycine can play even a minor role in the inhibitory pathway which impinges on relay cells of the cuneate nucleus. The possibility remains, however, that the failure of strychnine to block synaptic inhibition is a consequence of much higher concentrations of glycine occurring in the synaptic cleft than can be mimicked by iontophoresis. At other sites however relatively small doses of intravenous strychnine (cf. Curtis, 1962) are usually sufficient to abolish strychnine-sensitive inhibition in the spinal cord (Bradley, Easton & Eccles, 1953) and medulla (Kidokora, Kubota, Shuto & Sumino, 1968 ; Morimoto, Takata & Kawamura, 1968). Since large doses of strychnine can be less specific and interact with GABA and glycine in the spinal cord (Davidoff, Aprison & Werman, 1969) and not infrequently in the cuneate nucleus (Kelly & Renaud, 1973b), it must be of significance that synaptic inhibition was quite unaffected by iontophoretic applications of strychnine in excess of 100 nA and repeated intravenous injections, whose cumulative dose often exceeded 2 mg/kg. The GABA/strychnine interaction which was reported in the preceding paper (Kelly & Renaud, 1973b) must therefore only occur rarely or at extrajunctional sites where the GABA receptors are not involved in synaptic transmission. Whether the receptors which are susceptible to strychnine, are less dedicated to GABA and perhaps more sensitive to glycine than their counterparts involved in synaptic transmission cannot be determined.

Although all the glycine receptors can be regarded as extrajunctional in the sense that they are not involved in synaptic transmission, it is a matter of speculation as to how many of the GABA receptors shown to interact with bicuculline and

picrotoxin in the companion paper (Kelly & Renaud, 1973a) can be described as extrajunctional. Even when inhibition was all but abolished by iontophoretic bicuculline, the blockade could be overcome by iontophoretic GABA in doses only two or three times that found to be equipotent in the absence of bicuculline. Since our experience was similar when the synaptic inhibition was blocked by intravenous picrotoxin, the ability of GABA to overcome the blockade by ionotophoretic bicuculline may well reflect a competitive interaction with receptors involved in synaptic transmission rather than the spread of GABA to extrajunctional receptors (cf. Curtis, Duggan & Johnston, 1971).

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