J. Physiol. (1976), **256**, *pp.* 651–662
With 6 text-figures
Printed in Great Britain

GLYCINE-MEDIATED INHIBITORY TRANSMISSION OF GROUP 1A-EXCITED INHIBITORY INTERNEURONES BY RENSHAW CELLS

By G. BELCHER, J. DAVIES and R. W. RYALL

From the Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD

(*Received 26 August 1975*)

SUMMARY

1. Electrophysiological and micro-electrophoretic studies were carried out in anaesthetized cats on spinal interneurones which were monosynaptically activated from group 1 muscle afferents and recurrently inhibited by Renshaw cells.

2. The recurrent inhibition was blocked by the iontophoretic administration of strychnine, which also blocked the action of glycine.

3. The time course of the block of synaptic inhibition by strychnine was slower than the block of glycine evoked inhibition. The significance of this observation in terms of the location of the inhibitory synapses is discussed.

4. The observation that recurrent inhibition of motoneurones and of interneurones is blocked by strychnine whereas the mutual inhibition of Renshaw cells is not is discussed in relation to the proposal that the nature of the post-synaptic inhibitory receptor is not determined solely by the innervating neurone, which is the Renshaw cell in all three instances.

5. The recurrent inhibition of the interneurones was not blocked by the iontophoretic administration of bicuculline, but difficulties were encountered in demonstrating that this agent consistently antagonized the inhibitory effects of GABA.

INTRODUCTION

It is generally accepted that a given neurone liberates the same transmitter from all of its terminals. Although there are no known exceptions, there are few clear examples in the mammalian central nervous system. The cholinergic excitation of Renshaw cells and of skeletal muscle by motoneurones provides one example. However, Renshaw cells themselves are inhibitory to at least three different types of central neurone and this situation lends itself to an examination of the hypothesis that not only

is the same transmitter liberated at all of the terminals but that the nature of the post-synaptic receptor is determined by the innervating neurone.

It has long been known that strychnine blocks recurrent inhibition of motoneurones (Bradley, Easton & Eccles, 1953) and the demonstration that strychnine is a selective antagonist of the spinal actions of glycine (Curtis, Hösli & Johnston, 1968) clearly pointed to this amino acid as the inhibitory transmitter liberated by the Renshaw cells. Renshaw cells also inhibit the interneurones which mediate reciprocal inhibition (Hultborn, Jankowska & Lindström, 1968). It is therefore assumed that the transmitter is glycine and the purpose of the investigation was to determine whether the inhibition of the group 1 excited interneurones was blocked by strychnine.

METHODS

The experiments were carried out on forty-eight interneurones excited at a central latency of 1·5 msec or less on electrical stimulation of low threshold afferents in the biceps–semitendinosus (BST) or gastrocnemius–soleus (GS) nerve in six cats and on sixteen motoneurones in three cats. Anaesthesia was induced with halothane and maintained with chloralose (50–60 mg/kg I.V.). The body temperature was maintained at about 38° C.

The spinal cord was exposed by a laminectomy extending from L2 to L7 vertebrae and was transected at lower L1 segment. Ventral roots S1, L7 and L6 were severed ipsilaterally and mounted upon bipolar platinum electrodes for stimulation. The ipsilateral BST and GS nerves were also dissected free and mounted for stimulation. The exposed spinal cord and limb nerves were covered by a pool of paraffin oil. The dorsal root volleys were recorded by means of a silver ball electrode placed upon the L7 dorsal root near its point of entry into the spinal cord and central latencies of cell firing were measured from the beginning of the negative-going deflexion of the dorsal root volley to the shortest latency of firing of action potentials recorded extracellularly by means of a micro-electrode containing 4 M-NaCl inserted at the level of L7 segment.

Amplified action potentials were led into a window-discriminator and the frequency of the output pulses was analysed by a rate-meter and displayed as an analogue wave form on a pen-recorder. Synaptically evoked discharges were similarly detected by the window discriminator and the output was fed through a transistorized gate into a digital counter. The gate was positioned and its duration adjusted so that only the synaptically-evoked discharges were counted by the analyser. Between ten and fifty repetitions of the discharge were integrated and the integral was converted into an analogue signal which was continuously monitored on the chart recorder. In this way it was possible to obtain automatically a continuous record of the firing index of the cell. Some micro-electrode recordings were photographed from the oscilloscope for illustrative purposes.

The stimuli were rectangular, constant voltage pulses of 0·05 msec duration. The interval between stimuli to the ventral root and those to the afferent nerves was varied in order to obtain the time course of inhibition.

Drugs were administered iontophoretically from 5-barrel micropipettes. The drugs used were glycine hydrochloride (0·5 M), γ-aminobutyric acid (GABA, 0·5 M), strychnine hydrochloride (0·01 M dissolved in 0·16 M-NaCl) and bicuculline hydro-

chloride (0·01 M dissolved in 0·16 M-NaCl). A 'retaining' voltage of 0·5 V was applied routinely to all drug-containing barrels.

The technique is shown diagrammatically in Fig. 1.

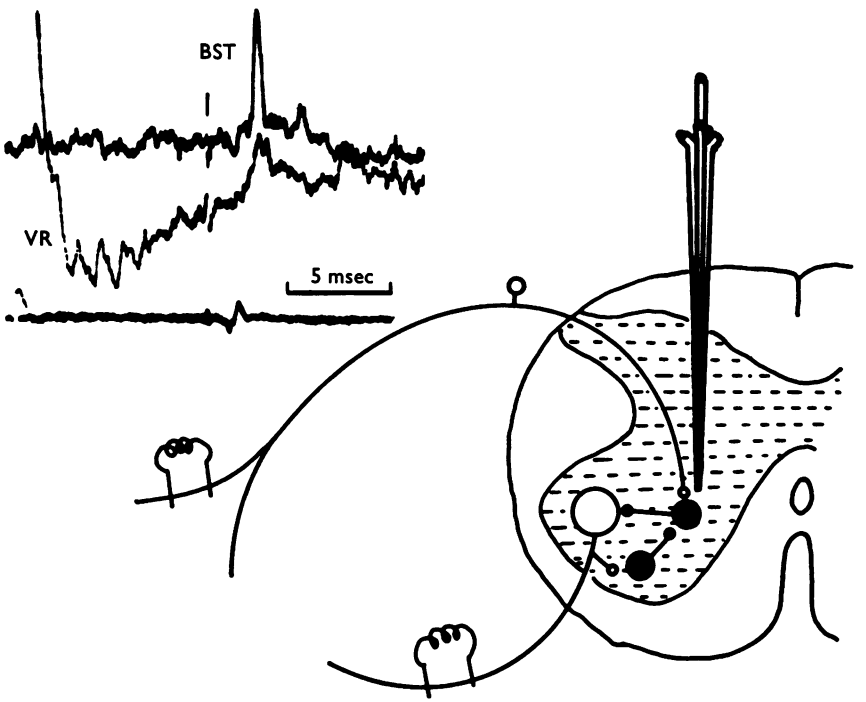

Fig. 1. Diagram of experimental technique, showing stimulating electrodes on a peripheral muscle afferent nerve and on transected ventral root and a multibarrelled micropipette situated in proximity to an interneurone which is both monosynaptically activated via dorsal root fibres and recurrently inhibited via Renshaw cells. The inset traces display extracellular microelectrode recordings from an interneurone (upper and middle records) which was monosynaptically activated from BST in the upper record but was not activated when the BST stimulus was preceded by an antidromic volley in the ventral root (middle record). The bottom record shows superimposed dorsal root volleys.

RESULTS

Characterization of interneurones

It was particularly important in this study that the experiments with strychnine were carried out on interneurones and not on motoneurones on which it is already established that recurrent inhibition is blocked by strychnine. In general, the criteria used to identify the interneurones were similar to those used by Hultborn *et al.* (1968).

It seems self-evident that the interneurones should not be invaded antidromically when L7 ventral root was stimulated. Although this seems rather obvious, it is in fact not possible to be certain from an examination of an extracellular recording that a given neurone is not antidromically invaded because a small extracellular unitary antidromic action potential is not readily discerned when superimposed on a relatively large antidromic field potential, unless the neurone is activated at a low stimulus strength when the field is small. In contrast, it was possible to positively identify motoneurones in this way, although it was easier to make observation on motoneurones by intracellular recording techniques.

Motoneurones are localized within sharply defined nuclei in the ventral horn. It was possible first to locate the motoneurones and then to move the micro-electrode dorsally and medially to these nuclei in order to record from the group 1 excited interneurones. All of the interneurones selected for this study were therefore located in regions where positively identified motoneurones could not be found.

The interneurones always fired after a single volley in the muscle afferent nerve. In contrast, many but not all motoneurones fired action potentials orthodromically only when there was temporal summation of two monosynaptically evoked excitatory post-synaptic potentials (e.p.s.p.s). Moreover, in some motoneurones monosynaptic, e.p.s.p.s but not action potentials were recorded even when double volleys were employed for excitation. In addition, the interneurones followed frequencies of orthodromic stimulation in excess of 100 Hz whereas motoneurones did not (see also Lloyd, 1957; Decandia, Provini & Taborikova, 1967).

Only interneurones which were monosynaptically excited from BST or GS with central latencies of 1·5 msec or less and with a threshold for excitation of less than twice the threshold for activation of 1A fibres in the dorsal root were chosen for investigation. The data in Table 1 indicate that monosynaptically activated interneurones which did not show evidence of recurrent inhibition had significantly higher thresholds for excitation than did the monosynaptically activated interneurones which were recurrently inhibited. There was probably therefore an additional component of excitation from group 1B fibres in the former group, which were located more superficially than the latter (Table 1). All of the interneurones which were recurrently inhibited were monosynaptically activated from BST only, although interneurones which were not recurrently inhibited were distributed between both those which were excited by BST and those which were excited by GS. The mean central latency of excitation was $1{\cdot}05 \pm 0{\cdot}06$ (S.E.) msec, and the mean threshold for excitation was $1{\cdot}23 \pm 0{\cdot}05$ times 1A threshold for recurrently inhibited

TABLE 1. Characteristics of group 1 excited interneurones

	BST		GS
	a	*b*	*c*
Number of cells	20	15	13
Ventral root stimulation	Inhibition	No inhibition	No inhibition
Depth mm ± S.E.	3·04 ± 0·09	2·70 ± 0·28	2·03 ± 0·09
P	—	$bc < 0{\cdot}05$	$ac < 0{\cdot}001$
Latency msec ± S.E.	1·05 ± 0·06	1·15 ± 0·07	1·09 ± 0·08
Threshold ± S.E.	1·23 ± 0·05	1·50 ± 0·09	1·43 ± 0·09
P	$ab < 0{\cdot}01$	—	$ac < 0{\cdot}05$

P values shown determined by Student *t* tests. Other differences between means were not significant ($P > 0{\cdot}05$). Depth measured from the dorsal surface of the spinal cord.

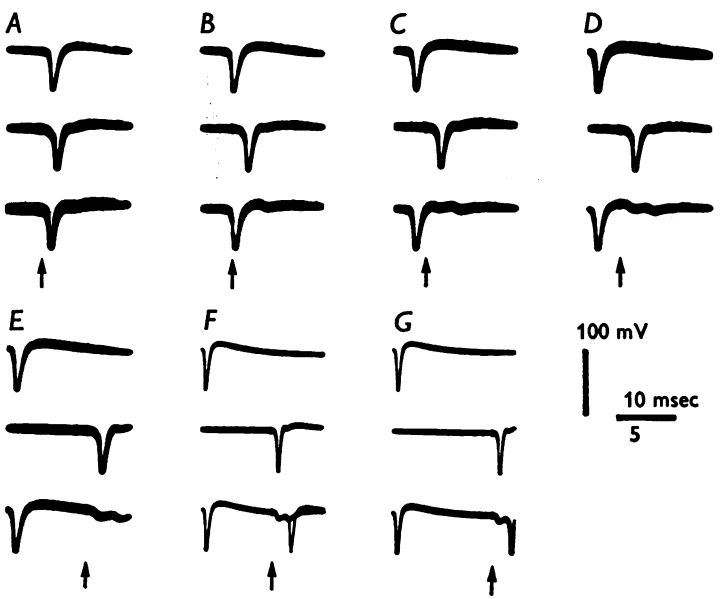

Fig. 2. Intracellular recordings from a motoneurone in L7 segment in cat anaesthetized with chloralose. Upper record in *A–G* is response to an antidromic volley in the ventral root. The middle record is the action potential evoked by an orthodromic volley in the nerve to GS and the time of arrival of the orthodromic volley at the spinal cord is indicated by the arrow below each set of records. The bottom records in each set show the abolition of the orthodromically evoked discharge at all conditioning-test intervals from very brief intervals to 5 msec. Resting membrane potential 80 mV, voltage calibration 100 mV, time scale 5 msec for records *A–E* and 10 msec for records *F–G*. Every record consists of about ten superimposed traces.

interneurones. This threshold is less than that for 1B fibres in nerves to thigh muscles (Bradley & Eccles, 1953) although the value is not as high for the nerve to soleus muscle (Coppin, Jack & MacLennan, 1970; Jack & MacLennan, 1971).

On presumed interneurones which showed recurrent inhibition there was a marked difference in the time course of the inhibition compared with the depression which followed an antidromic volley in motoneurones. In Fig. 2, the interval between the orthodromic and antidromic excitation of a motoneurone was systematically varied. The orthodromic action

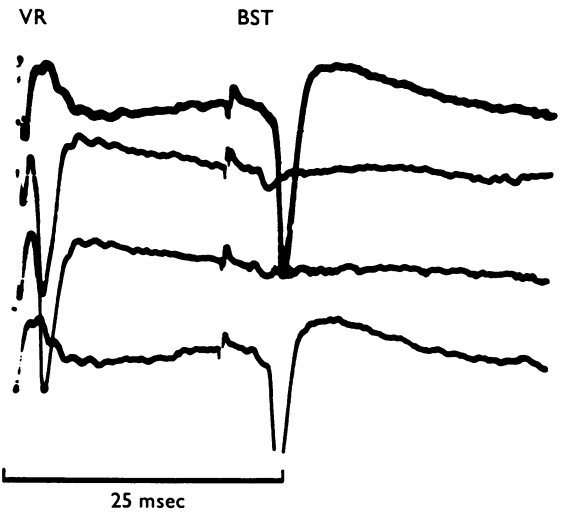

Fig. 3. Just extracellular recordings from a motoneurone in L7 segment (no membrane potential recorded). An antidromic volley occurred at the beginning of every sweep but the strength of the stimulus to the ventral root was such that the cell only fired antidromically in two of the four sweeps shown. The orthodromic volley in BST occurred near the middle of every sweep. Orthodromic responses were blocked only when the cell was invaded antidromically. Each trace is of a single sweep.

potential was completely suppressed at all intervals to 5 msec. At longer intervals of 10–15 msec there was occasional firing only when there was temporal summation of two orthodromic e.p.s.p.s and firing to the first volley only occurred at intervals of 20 msec or more (not illustrated). The depression is due to refractoriness after the antidromic action potential, with a probable superimposition of a recurrent inhibition. The short-latency depression was typical of all motoneurones examined.

The refractoriness probably accounted for a large part of the depression after an antidromic action potential, at least in some motoneurones. An example is illustrated in Fig. 3 in which the antidromic stimulus was set

at such a value that the motoneurone was invaded in only a proportion of the trials. It may be seen that the orthodromically evoked action potential was suppressed only when the cell was invaded antidromically, and that the relative refractoriness was evident even at the long conditioning-test interval of 20 msec.

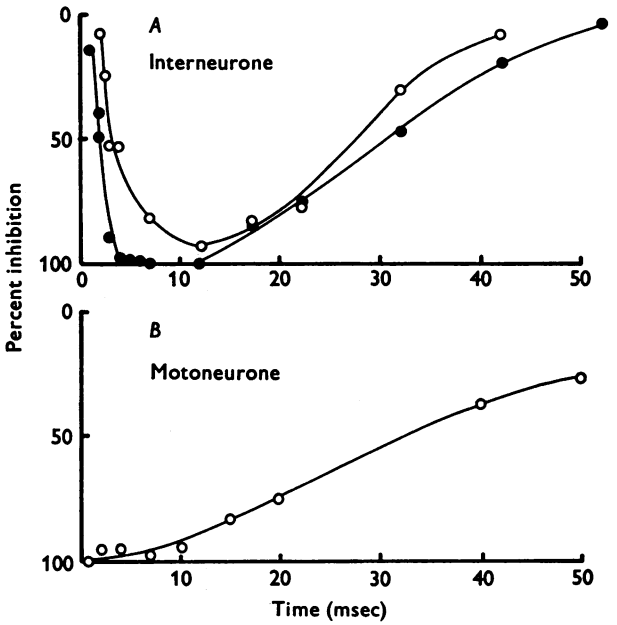

Fig. 4. Time course of Renshaw inhibition of two interneurones (*A*) and of a motoneurone (*B*). Every point represents the percentage inhibition of the firing index for the orthodromic volley determined from at least 100 sweeps. The control firing indices were 70% for the cell shown as filled circles in *A*, 72% for the open circles in *A* and 100% for the motoneurone in *B*. The ordinate shows percent inhibition of the firing index and the interval between conditioning antidromic volley and evoked orthodromic action potential is shown on the abscissae.

In contrast with these results on motoneurones, the recurrent inhibition of interneurones was only slight or absent at short conditioning-test intervals. This is illustrated in Fig. 4 where it is shown that the inhibition was weak at brief intervals, reached a maximum in 5–10 msec and thereafter waned over the next 40 msec. In general, this time course is similar to that of the discharge of Renshaw cells to an antidromic ventral root volley, except that the maximum rate of discharge with a supramaximal shock decreases progressively after the onset of excitation (Eccles, Fatt & Koketsu, 1954; Curtis & Ryall, 1966; Ryall, 1970). Since the recurrent

inhibition of the interneurone is thought to be monosynaptic, the relatively long time to peak inhibition may represent temporal summation of repetitive i.p.s.p.s generated by the Renshaw cell discharges.

The action of strychnine on recurrently inhibited interneurones

Strychnine was administered iontophoretically to nine of the interneurones inhibited by stimulation of L7 ventral root. Strychnine blocked the inhibition on every cell and Fig. 5 illustrates some typical results. These results were obtained from a neurone monosynaptically excited at 1 msec central latency by a single volley in BST nerve. Every stimulus to the BST nerve evoked a single action potential, i.e. the firing index was 100. Gated counts of the evoked action potentials were integrated over

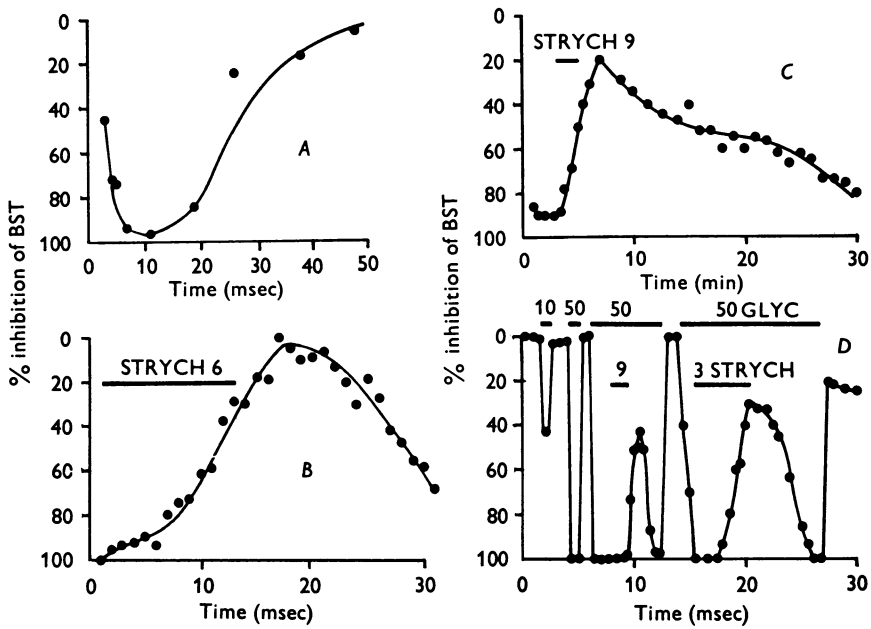

Fig. 5. Action of micro-electrophoretically administered strychnine on recurrent inhibition of 1 A excited interneurone. *A*, shows the time course of Renshaw cell inhibition of this neurone (firing index from BST, 100%). Strychnine (STRYCH) was administered with electrophoretic currents of 3, 6 or 9×10^{-9} A, for the periods indicated by horizontal bars in *B*, *C* and *D*. *B* and *C* show the action of strychnine on inhibition evoked via the Renshaw cells whereas in *D* inhibition was produced by the micro-electrophoretic administration of glycine (GLYC) administered with currents of 10 or 50×10^{-9}A for periods indicated by the upper horizontal bars. In all records the ordinate is the percentage inhibition of the response to orthodromic activation. In *A*, the conditioning-test interval was varied but in *B* and *C* it was fixed at 10 msec. Time scale: *A* (msec), *B–D* (min).

fifty sweeps and the results were plotted continuously on a chart recorder. Several consecutive values were pooled for each of the points shown in Fig. 5. In Fig. 5*A* is shown the characteristic time course of the recurrent inhibition, with a maximum at 10 msec interval between conditioning volley and evoked action potential. In Fig. 5*B*, *C* and *D* the conditioning-test interval was maintained at 10 msec. Strychnine, administered with an electrophoretic current of 6×10^{-9} A for 12 min (Fig. 5*B*), caused a slow and progressive block of the inhibition. The strychnine current was terminated before the inhibition had been completely blocked and the inhibition decreased further for a few minutes before slowly recovering over the next 15 min. When strychnine was administered with a slightly larger ejecting current (9×10^{-9} A, Fig. 5*C*), the block of inhibition occurred more rapidly, but recovery was as prolonged as in Fig. 5*B*, despite the shorter period of administration.

On the same cell, the excitation evoked by stimulation of BST was completely inhibited by glycine administered with a current of 50×10^{-9} A as shown by the horizontal bars in Fig. 5*D*.

In contrast with the slow onset and recovery from strychnine when tested on synaptically evoked inhibition (Fig. 5*B* and *C*), the onset and recovery from strychnine tested upon glycine-evoked inhibitions was relatively fast (Fig. 5*D*), when the strychnine was administered with a current the same as that used in Fig. 5*C* or only 3×10^{-9} A, half of that used in Fig. 5*B*.

The action of bicuculline

Bicuculline is claimed (Curtis, Duggan, Felix & Johnston, 1971) to be a selective antagonist of the central actions of GABA. It was therefore of interest to test the effects of this antagonist on the recurrent inhibition of the group 1 activated interneurones.

Bicuculline was administered with iontophoretic currents of 70–200 $\times$ 10^{-9} A on three interneurones which were monosynaptically excited by group 1 volleys in BST and inhibited by volleys in L7 ventral root. On each of these cells, the inhibition was attenuated by the iontophoretic administration of strychnine ($20–30 \times 10^{-9}$ A) but was unaffected by bicuculline. However, on only one of these cells did bicuculline (200×10^{-9} A) antagonize the inhibitory effect of iontophoretically applied GABA (100×10^{-9} A) and the effect of GABA was reduced by only 50 %, in contrast to the very effective and complete block of glycine evoked inhibitions by strychnine. On the other two cells, inhibition was produced by 70 or 100×10^{-9} A GABA respectively but no antagonism was observed with bicuculline (100 or 150×10^{9} A respectively).

It seemed possible that the effects of GABA on these cells may have

been unusually resistant to the action of bicuculline. The antagonist was therefore tested on glycine and GABA evoked inhibitions of four other cells, two of which were identified as Renshaw cells and two of which were unidentified interneurones. On only one neurone, a Renshaw cell, did bicuculline produce a clear and unambiguous selective antagonism of the response to GABA (6×10^{-9}A). On this cell a current of 55×10^{-9}A ejecting bicuculline caused a 50% reversal of the action of GABA, whereas 20×10^{-9}A had no effect. Larger currents were not tested. On one other cell there was possibly a slight (less than 30%) reduction of the response to GABA (25×10^{-9}A) with a current of 70×10^{-9}A of bicuculline, but on the remaining two cells no antagonism was observed with 100×10^{-9}A (currents ejecting GABA were 30 and 40×10^{-9}A respectively).

Thus, in these experiments bicuculline did not prove to be the reliable, potent and selective antagonist of GABA that was expected, and no conclusions can be drawn from the negative results. It is possible that a selective block of the responses to GABA could have been obtained with larger concentrations of bicuculline, but the iontophoretic currents used were limited by the current carrying capacity of the micropipettes. It is perhaps of some significance that the currents ejecting GABA required to inhibit the recurrently inhibited interneurones were in the region of 100×10^{-9}A. Under such conditions it may not be possible to attain a sufficiently high concentration of bicuculline at the receptors with these techniques (Curtis *et al.* 1971).

DISCUSSION

There was a very clear antagonism by strychnine of the recurrent inhibition of the monosynaptically excited inhibitory interneurones. The pharmacology of transmission is therefore similar to that between Renshaw cells and motoneurones. This supports the concept that the same inhibitory transmitter is liberated from all terminals and could be used to support an argument that the nature of the post-synaptic receptor is determined by the innervating neurone. However, the argument is invalidated by the observation (Ryall, Piercey & Polosa, 1972) that mutual inhibition of Renshaw cells is resistant to the action of strychnine which indicates that, even though the transmitter may be the same at all three terminations of Renshaw cells, the post-synaptic receptors on Renshaw cells differ from those on motoneurones and inhibitory interneurones. Alternative proposals that either Dale's principle is invalid or that there are different populations of Renshaw cells liberating different transmitters have been considered unlikely (Ryall *et al.* 1972). The postulated connexions of Renshaw cells are summarized in Fig. 6.

It was curious that the time course of the block of glycine inhibition by strychnine and the recovery was faster than the block and recovery of synaptically induced recurrent inhibition, when identical electrophoretic currents were used to expel strychnine. Under such conditions the distribution of strychnine should have been similar for similar durations of administration. The rapid reversal of glycine inhibition therefore may be

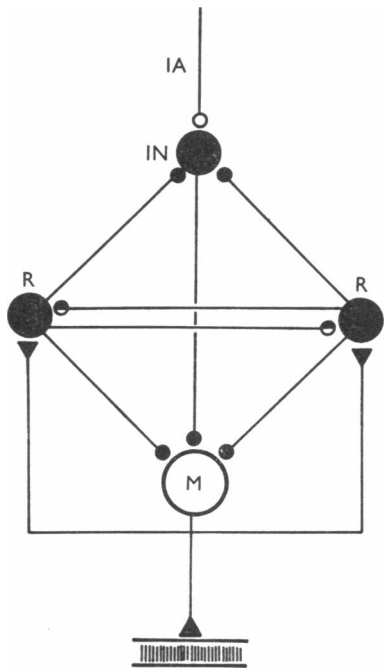

Fig. 6. Diagrammatic representation of the connexions between motoneurones (M), Renshaw cells (R) and interneurones mediating reciprocal inhibition (I). Cholinergic terminals are represented by filled triangles. Synapses at which glycine functions as a transmitter are indicated by filled circles (strychnine-sensitive) or by half-filled circles (strychnine-resistant).

attributed to a limited effective distribution of the glycine. This could arise because the glycine is taken up by neuronal or non-neuronal elements as it diffuses away from the site of application. Such removal would effectively increase the rate at which glycine concentration decreased with distance from micropipette. Alternatively, it may be that an action of glycine on the neuronal soma is dominant in causing inhibition and that effects at distant sites exerted by micro-electrophoretically administered glycine are relatively unimportant. In this event, the failure to achieve a rapid block of synaptic inhibition with amounts of strychnine sufficient

to cause a rapid reversal of the action of glycine indicates that the synapses mediating inhibition by the Renshaw cells on the interneurones might be located largely at sites distant from the soma, so requiring long periods for strychnine to achieve maximal concentrations by diffusion.

REFERENCES

BRADLEY, K., EASTON, D. M. & ECCLES, J. C. (1953). An investigation of primary or direct inhibition. *J. Physiol.* **122**, 474–488.

BRADLEY, K. & ECCLES, J. C. (1953). Analysis of fast afferent impulses from thigh muscles. *J. Physiol.* **122**, 462–473.

COPPIN, C. M. L., JACK, J. J. B. & MACLENNAN, C. R. (1970). A method for the selective activation of tendon organ afferent fibres from the cat soleus muscle. *J. Physiol.* **210**, 18–20*P*.

CURTIS, D. R., DUGGAN, A. W., FELIX, D. & JOHNSTON, G. A. R. (1971). Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. *Brain Res.* **32**, 69–96.

CURTIS, D. R., HÖSLI, L. & JOHNSTON, G. A. R. (1968). A pharmacological study of the depression of spinal neurons by glycine and related amino acids. *Exp. Brain Res.* **6**, 1–18.

CURTIS, D. R. & RYALL, R. W. (1966). The synaptic excitation of Renshaw cells. *Exp. Brain Res.* **2**, 81–96.

DECANDIA, M., PROVINI, L. & TABORIKOVA, H. (1967). Mechanisms of the reflex discharge depression in the spinal motoneurone during repetitive orthodromic activation. *Brain Res.* **4**, 284–291.

ECCLES, J. C., FATT, P. & KOKETSU, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *J. Physiol.* **126**, 524–562.

HULTBORN, H., JANKOWSKA, E. & LINDSTRÖM, S. (1968). Recurrent inhibition from motor axon collaterals in interneurones monosynaptically activated from Ia afferents. *Brain Res.* **9**, 367–369.

JACK, J. J. B. & MCLENNAN, C. R. (1971). The lack of an electrical threshold discrimination between group Ia and group Ib fibres in the nerve to the cat peroneus longus muscle. *J. Physiol.* **212**, 35*P*.

LLOYD, D. C. P. (1957). Monosynaptic reflex response of individual motoneurones as a function of frequency. *J. gen. Physiol.* **40**, 435–450.

RYALL, R. W. (1970). Renshaw cell mediated inhibition of Renshaw cells: patterns of excitation and inhibition from impulses in motor axon collaterals. *J. Neurophysiol.* **33**, 257–270.

RYALL, R. W., PIERCEY, M. F. & POLOSA, C. (1972). Strychnine-resistant mutual inhibition of Renshaw cells. *Brain Res.* **41**, 119–129.