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TWO KINDS OF RECURRENT INHIBITION OF CAT SPINAL α-MOTONEURONES AS DIFFERENTIATED PHARMACOLOGICALLY

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SUMMARY

1. The effects of I.V. administration of the glycine-antagonist strychnine nitrate and the GABA-antagonists bicuculline hydrochloride and picrotoxin on the recurrent inhibition of lumbosacral α-motoneurones were studied in cats anaesthetized with pentobarbitone sodium.

2. As revealed from both monosynaptic reflex experiments and intracellular recordings, each of the drugs generally reduced, but rarely abolished, the recurrent inhibition. The amount of reduction was more or less identical for bicuculline and picrotoxin.

3. By applying de- and hyperpolarizing currents intracellularly it could be shown that both the strychnine-resistant and bicuculline/picrotoxin-resistant recurrent inhibitory potentials were genuinely post-synaptic in nature.

4. The strychnine-resistant part of the recurrent inhibition had a later maximum and a longer duration than the part which was resistant to bicuculline/picrotoxin.

5. The time course of the strychnine-resistant recurrent inhibition was more or less identical to that of the bicuculline/picrotoxin-sensitive recurrent inhibition.

6. The bicuculline/picrotoxin-resistant recurrent inhibition was blocked by strychnine and, vice versa, the strychnine-resistant recurrent inhibition was blocked by bicuculline/picrotoxin. The combined administration of strychnine and bicuculline/picrotoxin always resulted in a virtual abolition of the recurrent inhibitory effects.

7. The values for central delay suggested that both the strychnine-resistant and bicuculline/picrotoxin-resistant inhibitions were mediated via disynaptic pathways.

8. The results suggest that both glycine and GABA act as transmitter substances of Renshaw cells in mediating recurrent inhibition to α-motoneurones.

9. No organizational pattern of the two types of recurrent inhibition based on motor pool category or motor unit type could be detected.

INTRODUCTION

It is generally accepted that the inhibitory action of the neurotransmitters glycine and γ-aminobutyric acid (GABA) is fairly selectively antagonized by strychnine and

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bicuculline, respectively (Krnjević, 1974; de Feudis, 1975; Johnston, 1978). The recurrent inhibition of spinal α-motoneurones which is mediated via the motor axon collaterals and the Renshaw cells, has been claimed to be abolished by strychnine (Eccles, Fatt & Koketsu, 1954; Brooks & Wilson, 1959; Curtis, 1962; Curtis, Game, Lodge & McCulloch, 1976), suggesting that glycine could be the transmitter of the inhibitory Renshaw cells. In contrast both Kellerth (1968) and Larson (1969) noticed that part of the recurrent inhibition appeared to be resistant to I.V. administration of strychnine. Furthermore, Kellerth (1968) reported that recurrent inhibitory post-synaptic potentials (r.i.p.s.p.s) could be reduced by the GABA-antagonist picrotoxin. In recent morphological investigations, the recurrent axon collaterals of α-motoneurones have been demonstrated to make synaptic contacts not only with small neurones located in the Renshaw cell area but also directly with synergic α-motoneurones (Cullheim, Kellerth & Conradi, 1977; Lagerbäck, Ronnevi, Cullheim & Kellerth, 1978). Since it is generally held that the same transmitter is released at all synaptic terminals of a neurone and since there is overwhelming evidence for acetylcholine to be the transmitter substance of α-motoneurones (cf. Dale, 1938; Eccles *et al.* 1954; del Castillo & Katz, 1956; Krnjević, 1974), the existence of direct cholinergic interconnexions between α-motoneurones via axon collaterals could provide a basis for recurrent synaptic effects remaining after the administration of strychnine. Against this background it was considered necessary to reinvestigate the pharmacological properties of the recurrent inhibition of α-motoneurones. It will be demonstrated that this inhibition can be separated into two parts, one part being sensitive to strychnine and the other part being sensitive to the GABA-antagonists bicuculline and picrotoxin. The results indicate that both glycine and GABA act as transmitter substances within the recurrent inhibitory loop.

METHODS

Adult cats (2·0–4·0 kg) were anaesthetized with intraabdominal injections of pentobarbitone sodium (40 mg/kg). Small additional doses (5 mg/kg) were given I.V. when needed during the course of the experiment.

The left hind limb was dissected and a number of muscle nerves were cut distally and mounted on stimulating or recording electrodes. The nerves used were the posterior biceps + semitendinosus, the medial gastrocnemius, the lateral gastrocnemius-soleus, and the plantaris nerves. In some experiments the lateral gastrocnemius-soleus nerve was separated into the lateral gastrocnemius and the soleus nerves. In a few instances the entire posterior tibial nerve was used for stimulation.

The spinal cord was exposed by a lumbar laminectomy and all ipsilateral dorsal roots below segment L4 were cut. Usually the spinal cord was transected at the lower thoracic level. The animals were paralysed by intravenous administration of gallamine triethiodide or tubocurarine chloride and artificially ventilated. During the course of an experiment, the paralysis was allowed to wear off at least every second hour to assess the depth of anaesthesia of the animal. Expired CO_2 concentration was monitored continuously (Beckman Gas Analyser LB I). Body temperature was kept around 38 °C by means of infra-red light.

Monosynaptic test reflexes were elicited by stimulating the central ends of the cut dorsal roots L7 and S1 at a frequency of 0·5 Hz. The monosynaptic responses were recorded from one of the prepared peripheral nerves at a time, the remaining muscle nerves being used for conditioning antidromic single-shock stimulation. A number of combinations were tested in this way in each animal. The monosynaptic responses were recorded at different time intervals between the antidromic conditioning stimulation and the dorsal root test stimulation. At each interval the size of the monosynaptic reflex was taken as the mean value of ten subsequent measurements, and plotted graphically in percentage of the size of the unconditioned test reflex.

Intracellular recordings were obtained with single-barrelled micropipettes filled with 2-M-

potassium citrate and having resistances ranging from 3 to 10 MΩ. A bridge circuit allowed the passage of polarizing currents through the recording micro-electrode. Identification of the impaled motoneurones as well as generation of recurrent inhibitory post-synaptic potentials (r.i.p.s.p.s) were obtained by single-shock antidromic stimulation of the various muscle nerves. Usually the recorded r.i.p.s.p.s were collected through a signal averager (Neurolog), giving the mean of 32–128 sequential potentials.

Solutions of strychnine nitrate (0·2 mg/ml.), picrotoxin (Sigma Co.: 1·0–2·0 mg/ml.) and bicuculline hydrochloride (K & K Labs., Calif.; 0·5–1·0 mg/ml.) in Ringer were administered I.V. in various doses indicated in the text.

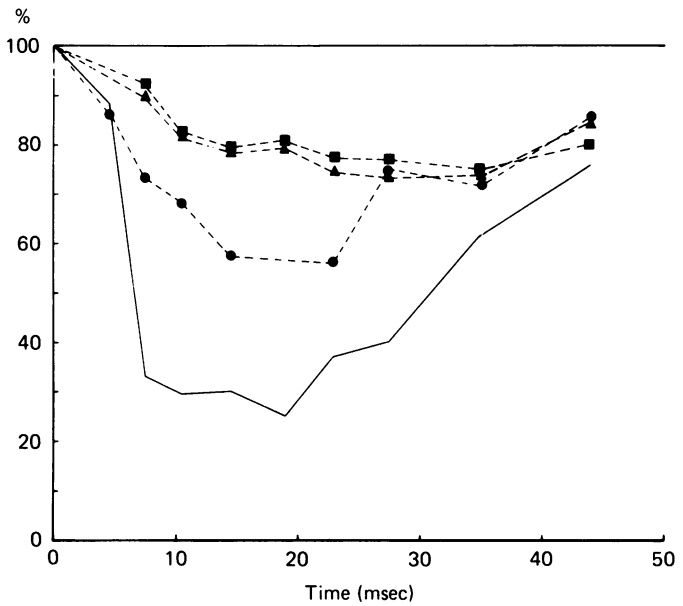

Fig. 1. The effects of increasing I.V. doses of strychnine nitrate on the recurrent inhibition of α-motoneurones. The monosynaptic lateral gastrocnemius-soleus reflex was conditioned by antidromic stimulation of the medial gastrocnemius nerve. Abscissa gives the time interval between the conditioning and test stimuli. Ordinate gives the size of the recorded monosynaptic reflex in percentage of the unconditioned control value. The continuous line indicates the inhibition present before the administration of strychnine. The dashed lines show the inhibition remaining after giving strychnine I.V. in doses of 0·10 mg/kg (--●--), 0·20 mg/kg (--▲--) and 0·30 mg/kg (--■--).

RESULTS

A. Effects of strychnine on the recurrent inhibition

Demonstration of strychnine-resistant recurrent inhibition. Fig. 1 shows the effects of antidromic stimulation of the medial gastrocnemius nerve on the size of the monosynaptic reflex of the lateral gastrocnemius-soleus nerve, after gradually increasing intravenous doses of strychnine. This experiment exemplifies the usual observation that the maximal effect of strychnine is achieved at an intravenous dose of 0·15–0·20 mg/kg and that additional doses do not further reduce the remaining inhibitions. This finding was confirmed also with intracellular recordings. It should be emphasized, however, that in the experiments using monosynaptic testing it was often difficult or impossible to visualize any recurrent inhibition remaining after

strychnine when maximal stimulation was applied to the dorsal roots. In the present experiments the amplitude of the unconditioned monosynaptic test reflex was therefore maintained at about 50 % of its maximum value, which was empirically found to allow quite reproducible and stable measurements of the inhibitory effects.

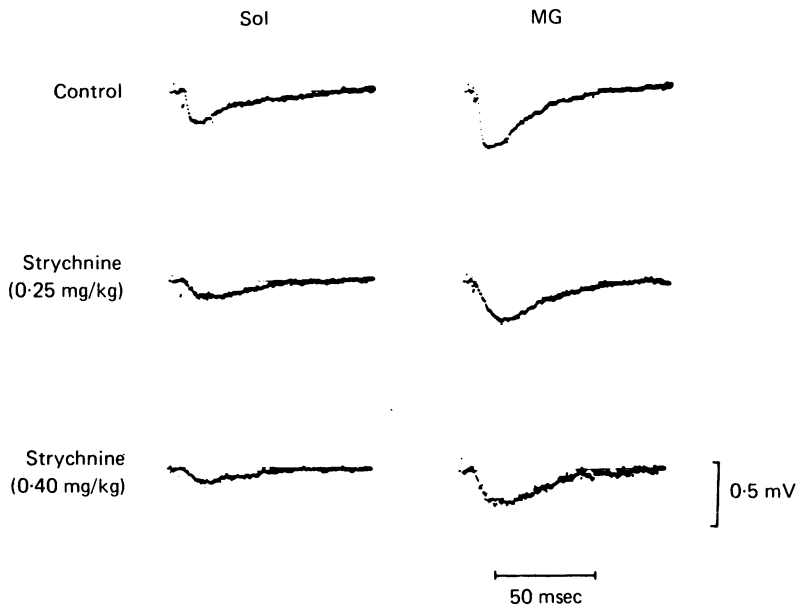

Fig. 2. Intracellular recordings from a lateral gastrocnemius α-motoneurones in the L7 segment. Recurrent i.p.s.p.s were elicited by antidromic stimulation of the soleus (Sol) and medial gastrocnemius (MG) nerves. The i.p.s.p.s were recorded before and shortly after the I.V. administration of 0·25 mg/kg and 0·40 mg/kg of strychnine nitrate. Each i.p.s.p. was averaged from 64 sequential recordings.

In Fig. 2 are shown intracellular recordings from a lateral gastrocnemius α-motoneurone which received r.i.p.s.p.s from the soleus and medial gastrocnemius motor pools. Both of these r.i.p.s.p.s were reduced by strychnine in a dose of 0·25 mg/kg I.V. Even if a minor further reduction could not be excluded when more strychnine was added (0·40 mg/kg), prominent r.i.p.s.p.s from both sources were still present. Among the large number of cells ($n > 100$) investigated in this way it was found that in all neurones which received recurrent inhibition, part of the inhibition always remained after strychninization. The amount of remaining inhibition was quite variable, but we never observed a cell in which all the r.i.p.s.p.s were either entirely resistant or completely abolished by strychnine.

Time course of the strychnine-resistant recurrent inhibition. With regard to the time course of the recurrent inhibition, it was noticed that the maximum of the inhibition occurred later in the strychninized animal than in the non-strychninized animal (Figs. 1 and 2). In the 'normal' situation the inhibitory peak usually occurred less than 10 msec after the onset of the inhibition, while after strychnine the peak was delayed to about 20 msec. This is exemplified also in Fig. 3 which shows the effects of antidromic stimulation of the medial gastrocnemius nerve on the size of the lateral

gastrocnemius-soleus monosynaptic reflex before and after strychninization. The curves respresent the average result of ten experiments. By subtracting the two experimentally obtained curves (continuous lines) an inhibitory curve could be reconstructed (dashed curve), which should grossly mirror the time course of the part of the recurrent inhibition which had been removed by strychnine. It is obvious that

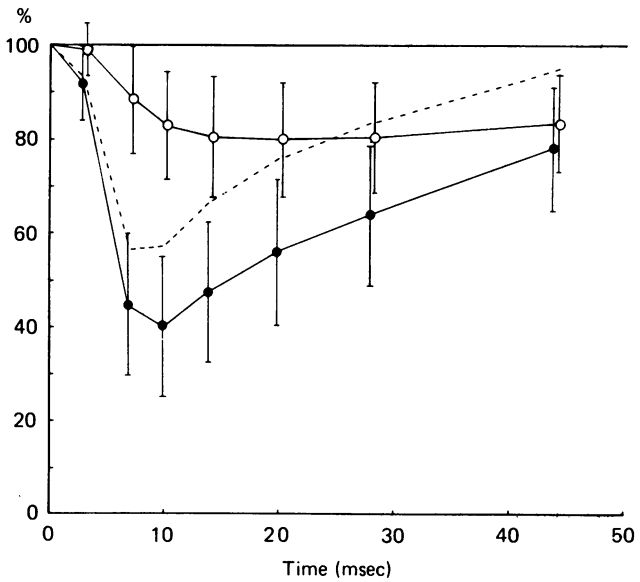

Fig. 3. The effects of strychnine nitrate (0·20–0·40 mg/kg I.V.) on recurrent inhibition. The monosynaptic lateral gastrocnemius-soleus reflex was conditioned by antidromic stimulation of the medial gastrocnemius nerve. Abscissa and ordinate as in Fig. 1. The inhibitory effects are indicated before (●—●) and after (○—○) the administration of strychnine. The graph shows the averaged result of ten different experiments and the vertical bars indicate the standard deviations. The dashed curve shows the component of recurrent inhibition which was removed by strychnine. It was reconstructed by subtracting the inhibitory curve obtained after strychnine from the control curve.

the peak of this constructed curve occurs significantly earlier (at about 7 msec) than is the case with the strychnine-resistant inhibition (at about 20 msec). Also in the intracellular experiments the peak of the r.i.p.s.p.s after strychninization was found to occur after about 20 msec (range 13–25 msec). From Fig. 3, and also confirmed in the intracellular experiments, it is evident that strychnine seems to affect the early part of the recurrent inhibition more than the late part.

Neuronal pathway of the strychnine-resistant recurrent inhibition. Lately, direct monosynaptic interconnexions between α-motoneurones via the recurrent axon collaterals have been demonstrated morphologically (Cullheim *et al.* 1977). Since no physiological correlate to this pathway has been established so far, the strychnine-resistant recurrent inhibition might possibly be explained by activation of these direct interconnexions. It is also known that afferent fibres may enter the spinal cord via the ventral roots (Coggeshall, Coulter & Willis, 1974), and antidromic stimulation might therefore produce 'recurrent' inhibition of motoneurones via polysynaptic

afferent pathways. To obtain further information on the source of the strychnine-resistant recurrent inhibition, a number of experiments were performed with special reference to its latency and its anatomical distribution in the spinal cord.

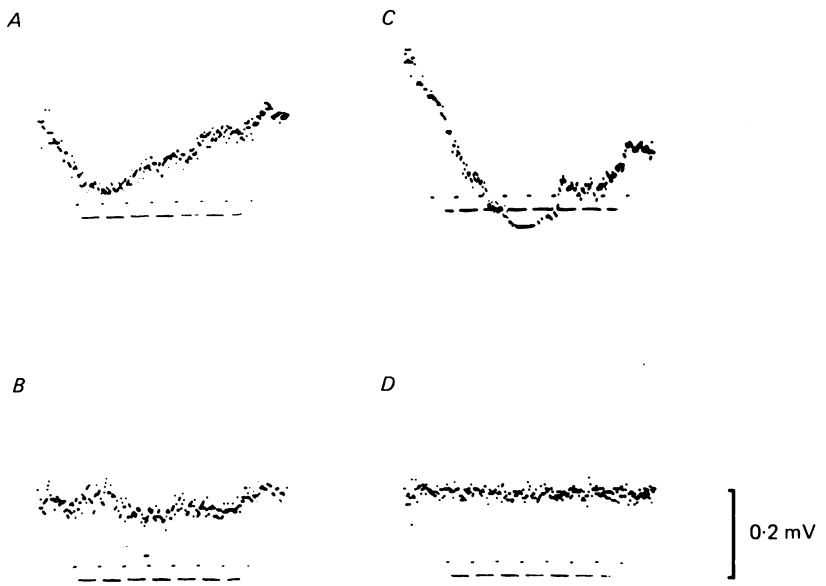

Fig. 4. Intracellular recordings from an unidentified α-motoneurone in a strychninized (0·40 mg/kg I.V.) cat. The motoneurone was located in the L7 segment 2·1 mm rostral to the most rostral part of the S1 segment. The ventral roots L6 and L7 were cut. The medial gastrocnemius and lateral gastrocnemius-soleus nerves were stimulated antidromically, thus producing a recurrent i.p.s.p. via the S1 ventral root (*A*). *B* shows the i.p.s.p. during the passage of hyperpolarizing current (40 nA) while *C* shows the i.p.s.p. during the passage of depolarizing current (40 nA). *D* is an extracellular recording obtained immediately outside the motoneurone. Each record was averaged from thirty-two sequential sweeps. Time scale is 10 msec.

In Fig. 4 are shown recurrent i.p.s.p.s recorded from an unidentified α-motoneurone located in the L7 segment during antidromic stimulation of the medial gastrocnemius and lateral gastrocnemius-soleus nerves. In this particular preparation not only the dorsal roots below the L4 segment but also the ventral roots L6 and L7 were cut. The impaled cell was located 2·1 mm rostral to the most rostral end of the antidromic S1 field potential as revealed by stimulation of the intact S1 ventral root. Knowing the maximal rostro-caudal extensions of motoneurone dendrites (Kellerth & Ulfhake, in preparation) and axon collaterals (Cullheim & Kellerth, 1978*a*, *b*), the distance between the activated recurrent collaterals in the S1 segment and the impaled motoneurone safely excludes any synaptic effects contributed by direct monosynaptic interconnexions between α-motoneurones. The recordings show an r.i.p.s.p. persisting after strychnine 0·40 mg/kg (Fig. 4 *A*). Hyperpolarization (Fig. 4 *B*) and depolarization (Fig. 4 *C*) of the motoneurone membrane caused a disappearance and an increase, respectively, of the i.p.s.p., indicating that the strychnine-resistant recurrent inhibition was truly post-synaptic in nature and not due to disfacilitation or to extracellular events.

The central latency of the strychnine-resistant part of the recurrent inhibition was often difficult to determine due to the small amplitude of the initial part of the i.p.s.p.s and the complication of extracellular field potentials. Fig. 5 shows recordings from a motoneurone in the L7 segment shortly after the I.V. administration of strychnine (0·30 mg/kg). In this neurone a strychnine-resistant r.i.p.s.p. was evoked by antidromic

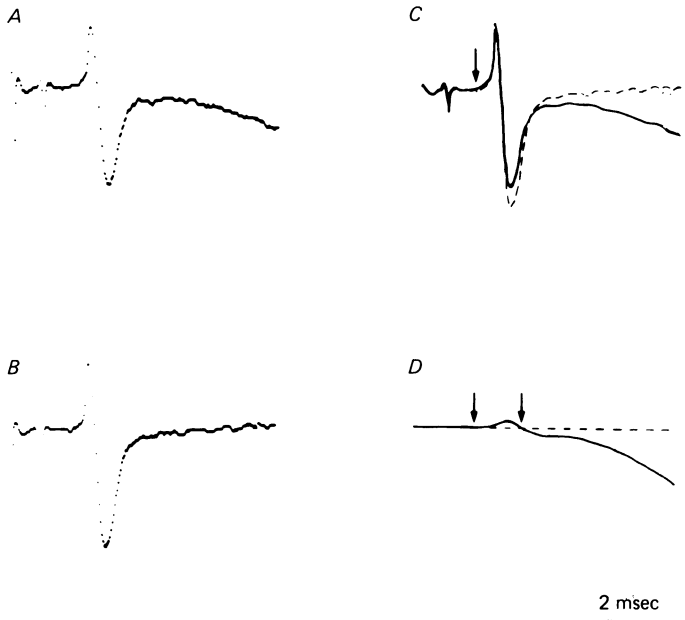

Fig. 5. In *A* is shown an intracellular recording from an unidentified α-motoneurone in the L7 segment in a strychninized (0·30 mg/kg I.V.) cat. A recurrent i.p.s.p. was elicited by antidromic stimulation of the tibial nerve. The onset of this i.p.s.p. was obscured by the diphasic electrical field potential produced by antidromically firing tibial motoneurones. Record *B* shows this field potential recorded extracellularly just outside the motoneurone. In *C* the two records *A* and *B* have been superimposed, record *B* being dashed. The arrow points at the beginning of the antidromic field potential. After subtracting the two records and compensating for a slight difference in electrode resistance, *D* could be reconstructed indicating the start of the i.p.s.p. at the second arrow. The time interval between the arrows was about 1·3 msec. Records *A* and *B* were averaged from sixty-four sequential sweeps.

stimulation of the posterior tibial nerve. After subtracting the extracellular field potential, it was noticed that the time elapsing from the onset of the field potential, representing the earliest antidromic activation of motoneuronal cell bodies, to the beginning of the r.i.p.s.p. was about 1·3 msec, which indicates that the i.p.s.p. was evoked by a disynaptic pathway. The total range of latencies for strychnine-resistant r.i.p.s.p.s measured in this way was 1·2–3·2 msec.

Distribution of the strychnine-resistant recurrent inhibition among different types of α-motoneurones. The intracellular recordings of Fig. 6 derive from a motoneurone with recurrent i.p.s.p.s evoked by stimulation of both the medial gastrocnemius and the posterior biceps-semitendinosus nerves. The inhibition from the latter nerve was particularly prominent. After an I.V. injection of strychnine 0·05 mg/kg, both inhibitions were reduced in amplitude, the one from posterior biceps-semitendinosus

relatively more so. When more strychnine was added (up to 0·15 mg/kg) the i.p.s.p. from the posterior biceps-semitendinosus nerve was completely abolished, while the one from the medial gastrocnemius nerve was not further reduced. Thus, the presence of strychnine-resistant recurrent inhibition does not seem to be directly related to the identity of the postsynaptic motoneurone.

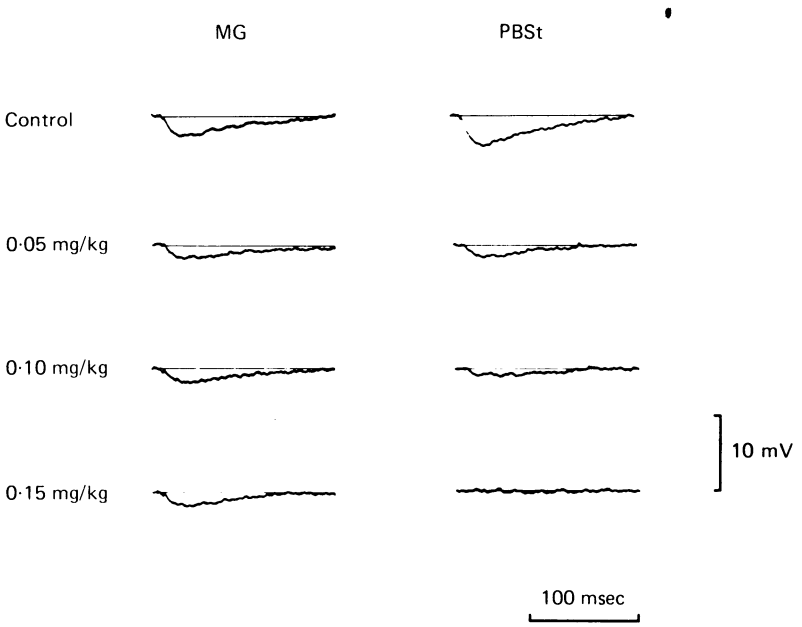

Fig. 6. Intracellular recordings from an unidentified α-motoneurone in the L7 segment. Recurrent i.p.s.p.s elicited by antidromic stimulation of the medial gastrocnemius (MG) and posterior biceps-semitendinosus (PBSt) nerves. The i.p.s.p.s were recorded before and after the administration of increasing I.V. doses of strychnine nitrate.

To test whether there was a selectivity in the motor pools producing or receiving strychnine-resistant inhibition, the effect of antidromic stimulation of one muscle nerve on the monosynaptic reflex of another was tested in a number of different combinations. Although the inhibitory effects varied in relative magnitude, there was always a mixture of strychnine-resistant and strychnine-sensitive recurrent inhibition in all combinations studied. The overall results from ten experiments are illustrated by the curves of Fig. 3.

Within each motor pool different functional types of motoneurones can be distinguished. For example, in the triceps surae motor pool, the motor units may be separated into gastrocnemius FF, FR and S as well as soleus-S types, on the basis of their functional characteristics (Burke, Levine, Zajac, Tsairis & Engel, 1971; Burke, Levine, Salcman & Tsairis, 1974). The possibility that one type of motor unit may specifically activate or receive only one of the kinds of recurrent inhibition described here seems less likely, since the soleus motoneurone pool, which contains almost entirely α-motoneurones of an extreme S-type (Burke *et al.* 1974; Hammarberg & Kellerth, 1975), was found to both produce (Fig. 2) and receive a mixture of strychnine-sensitive and strychnine-resistant recurrent inhibition.

Thus, the present results seem to suggest that the distribution of the two types of recurrent inhibition as differentiated by strychnine is not correlated to motor unit type, nor to motor pool category.

B. Effects of GABA-antagonists on the recurrent inhibition

With respect to the duration of the effects of bicuculline and picrotoxin on the recurrent inhibition of α-motoneurones, it was found that with the doses used here the effect of bicuculline started to diminish after about 20 min (see e.g. Fig. 10).

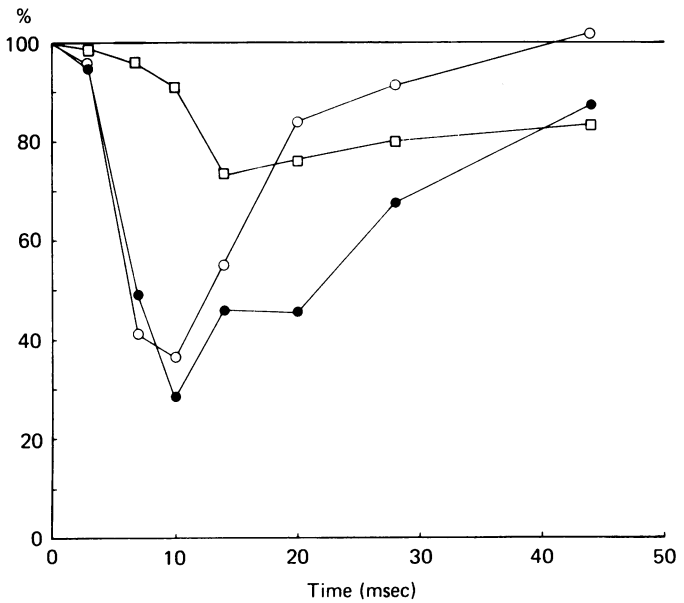

Fig. 7. The separate effects of bicuculline and strychnine on the recurrent inhibition in one and the same preparation. The monosynaptic lateral gastrocnemius-soleus reflex was conditioned by antidromic stimulation of the medial gastrocnemius nerve. ●—●, control; ○—○, inhibition remaining after the administration of bicuculline (0·25 mg/kg); □—□, inhibition remaining after giving strychnine 0·30 mg/kg 120 min later (i.e. after the effect of bicuculline had vanished).

However, if bicuculline was readministered several times, the effects seemed to become progressively more prolonged and could last for a few hours. The effects of picrotoxin remained for several hours after the first injection.

Demonstration of bicuculline-sensitive recurrent inhibition. Due to the relatively quick disappearance of the effects produced by bicuculline it was possible to demonstrate the separate effects of bicuculline and strychnine in one and the same preparation. This is exemplified in Fig. 7 where the effects of the two convulsants were tested on the medial gastrocnemius recurrent inhibition of the lateral gastrocnemius-soleus monosynaptic reflex. It is noticed that the bicuculline-resistant inhibition had an earlier peak and a shorter duration than the strychnine-resistant inhibition.

Time course of the bicuculline-sensitive recurrent inhibition. In Fig. 8 is demonstrated the effects of bicuculline (0·20–0·50 mg/kg) on the recurrent inhibition of the lateral gastrocnemius-soleus monosynaptic reflex. The inhibition was elicited by antidromic stimulation of the medial gastrocnemius nerve. The Figure shows the averaged result

of eight experiments, and illustrates that bicuculline reduces the recurrent inhibition, relatively more so in its later part. The dashed curve of Fig. 8 is a reconstruction obtained by subtraction of the two experimentally obtained curves (continuous lines), and it illustrates the time course of the inhibitory component which had been removed by bicuculline. It is evident that the bicuculline-sensitive part of the

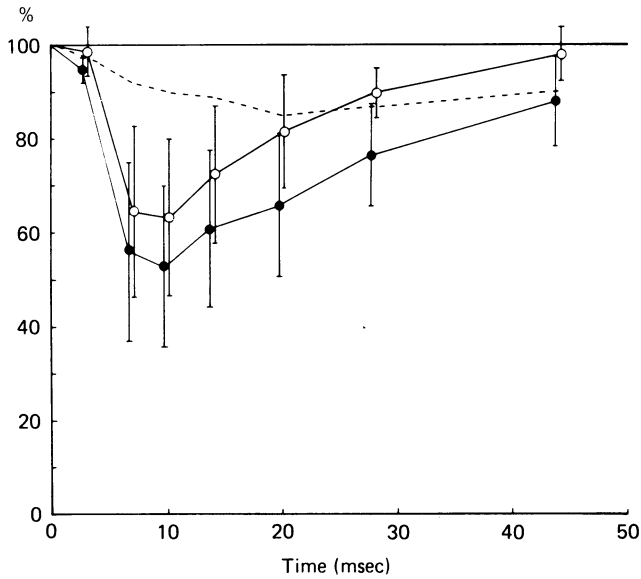

Fig. 8. The effects of bicuculline (0·25–0·50 mg/kg) on the recurrent inhibition of α-motoneurones. The monosynaptic lateral gastrocnemius-soleus reflex was conditioned by antidromic stimulation of the medial gastrocnemius nerve. The inhibitory effects are indicated before (●—●) and after (○—○) the administration of bicuculline. The graph shows the averaged result of eight different experiments. The vertical bars indicate the standard deviations. The dashed curve shows the component of recurrent inhibition which was removed by bicuculline. It was reconstructed by subtracting the inhibitory curve obtained after strychnine from the control curve.

recurrent inhibition has a slower time course with a maximum after around 20 msec compared to the bicuculline-resistant inhibition which has its peak after about 10 msec. In the intracellular experiments the peak of the r.i.p.s.p. after bicuculline administration was found to occur after about 7 msec (range 6–10 msec). When comparing Fig. 8 with Fig. 3 of the present study, it is obvious that an extremely close correspondence in time course exists between the strychnine-resistant and bicuculline-sensitive inhibitory curves on one hand, and between the strychnine-sensitive and bicuculline-resistant inhibitions on the other.

Comparison between the effects of bicuculline and picrotoxin. In Fig. 9 is shown an experiment where the separate effects of the GABA-antagonists bicuculline and picrotoxin were compared and, furthermore, the combined effects of picrotoxin and strychnine were studied. The results indicate that picrotoxin and bicuculline produce more or less identical depressions of the recurrent inhibition and, also, that a combination of antagonists to both GABA and glycine is needed to abolish all the recurrent inhibitory effects.

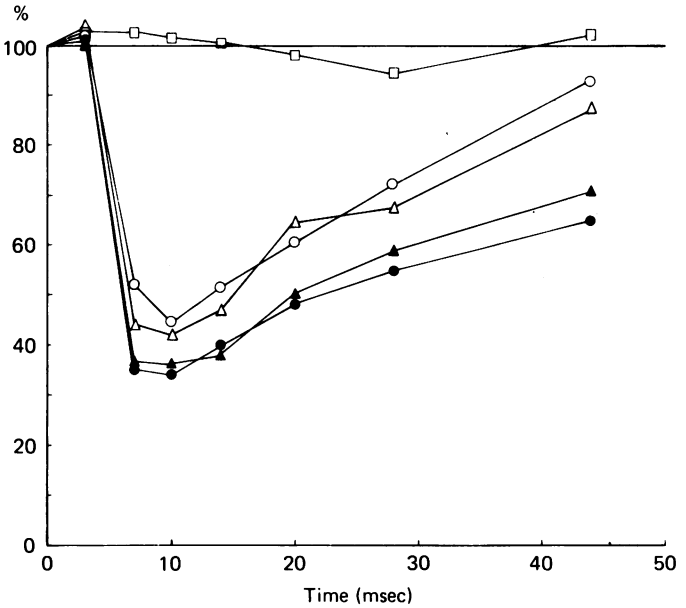

Fig. 9. The separate effects of bicuculline and picrotoxin, as well as the combined effects of picrotoxin and strychnine on the recurrent inhibition in one and the same preparation. The monosynaptic lateral gastrocnemius-soleus reflex was conditioned by antidromic stimulation of the medial gastrocnemius nerve. ●—●, Control; ○—○, inhibition remaining after the administration of bicuculline (0·50 mg/kg); ▲—▲, recovery of inhibition 90 min after giving bicuculline; △—△, inhibition remaining after 1·25 mg/kg of picrotoxin; □—□, the combined effect of picrotoxin (1·25 mg/kg) and strychnine (0·20 mg/kg).

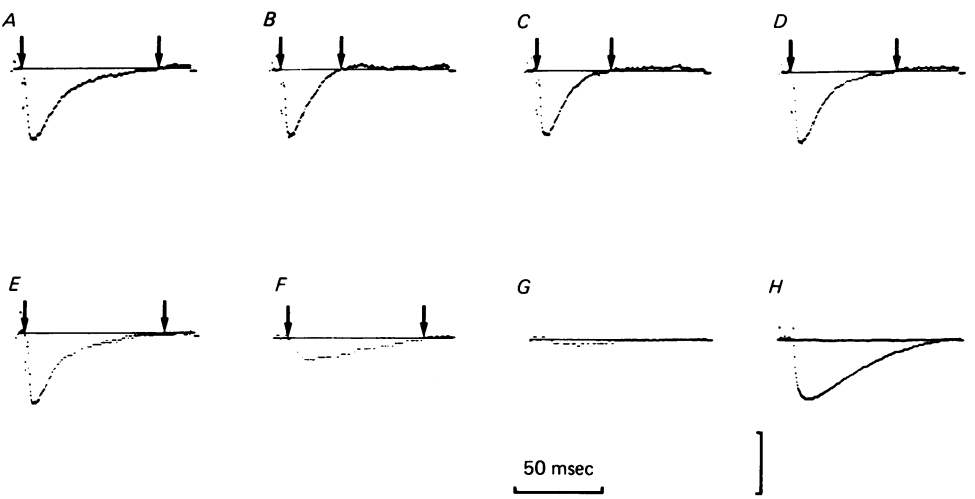

Fig. 10. Intracellular recordings from a lateral gastrocnemius-soleus α-motoneurone located in the L7 segment. A recurrent i.p.s.p. was elicited by antidromic stimulation of the medial gastrocnemius nerve (*A*). Bicuculline (0·30 mg/kg) was administered and the i.p.s.p. was recorded after 2 min (*B*), 10 min (*C*), 40 min (*D*) and 70 min (*E*). Thereafter strychnine (0·25 mg/kg) was given (*F*) and 10 min later a further dose of bicuculline (0·30 mg/kg) was added (*G*). In *H* is shown the afterhyperpolarization following the antidromically elicited spike potential. The vertical bar indicates 1 mV for records *A*–*G* and 5 mV for *H*. Each record was averaged from sixty-four sequential sweeps.

Intracellular recordings. The results described above have been confirmed in all respects by intracellular recordings from single α-motoneurones. Fig. 10 is from a lateral gastrocnemius-soleus α-motoneurone and shows an r.i.p.s.p. elicited by antidromic stimulation of the medial gastrocnemius nerve. The intravenous administration of bicuculline (0·30 mg/kg) hardly reduced the peak of the inhibition but the late part of the r.i.p.s.p. was removed (Fig. 10*B*). During the following 70 min the effects of bicuculline gradually vanished (Fig. 10*C–E*) and the i.p.s.p. finally returned to its initial shape (Fig. 10*E*). The subsequent administration of strychnine produced a substantial reduction of the early part of the r.i.p.s.p. while the later part remained fairly unchanged (Fig. 10*F*). An additional dose of bicuculline virtually abolished the i.p.s.p. (Fig. 10*G*), while the afterhyperpolarization following the antidromic spike potential was still unchanged (Fig. 10*H*), indicating an undisturbed condition of the impaled neurone. This experiment clearly shows that the bicuculline-resistant r.i.p.s.p. is sensitive to strychnine and, furthermore, that the strychnine-resistant r.i.p.s.p. is blocked by bicuculline.

Several measurements were made of the central latency of the r.i.p.s.p.s both before and after the administration of bicuculline/picrotoxin. The latency was found to be as brief as 1·0–1·2 msec, and it was not affected by the administration of the convulsants.

It should also be pointed out that on no occasion did we see any recurrent synaptic effects remaining after the combined administration of strychnine and bicuculline/picrotoxin in adequate doses. This is of relevance when discussing a possible role for the direct monosynaptic interconnexions between α-motoneurones via axon collaterals which have been described previously (Cullheim *et al.* 1977), since these presumably cholinergic synapses would not be expected to be antagonized by the convulsive drugs used here.

DISCUSSION

The present results are in conflict with the general view that the recurrent inhibition of spinal α-motoneurones is abolished by strychnine (Eccles *et al.* 1954; Brooks & Wilson, 1959; Curtis, 1962; Curtis *et al.* 1976). On the other hand, these results confirm earlier observation by Kellerth (1968) and Larson (1969) that part of the recurrent inhibition appears to be insensitive to intravenous administration of strychnine. The conflicting views may be partly explained by the fact that in the early investigations a reduction of recurrent inhibition by strychnine was interpreted as a total sensitivity, based on the assumption that the recurrent inhibition is pharmacologically homogeneous. However, Curtis *et al.* (1976) recently denied the existence of a strychnine-resistant recurrent inhibition of α-motoneurones. The reason why no such inhibition was observed in their study might possibly be that only monosynaptic reflex experiments were used, and the inhibitory effects remaining after strychnine may then not have been disclosed by the test stimulation used. As has already been mentioned above, in the present experiments the recurrent inhibitory effects persisting after strychninization were regularly seen only when the test stimulus to the dorsal root was kept at a strength submaximal for the monosynaptic reflex.

Our results suggest that bicuculline and picrotoxin block part of the recurrent

inhibition of α-motoneurones. This is in disagreement with the finding by Curtis *et al.* (1976) who reported that bicuculline had no effect on this type of inhibition. It may be argued that the effects of bicuculline and picrotoxin described here might be explained by an unspecific change in the response of the Renshaw cells to stimulation of the α-motor axon collaterals and, or, by an altered response of the α-motoneurones to the activity of the Renshaw cells. However, as far as bicuculline is concerned, no changes of either the inhibition of Renshaw cells or the early Renshaw cell discharges could be detected by Piercey, Goldfarb & Ryall (1973) after intravenous administration of bicuculline hydrochloride even at 0·75 mg/kg. It is difficult to understand why the α-motoneurones would be more susceptible to unspecific membrane effects than the Renshaw cells and, furthermore, such unspecific effects can not readily explain the characteristic and consistent time course of the inhibition remaining after the administration of bicuculline or picrotoxin.

The present findings show that the time course of the strychnine-resistant part of the recurrent inhibition is practically identical with that of the bicuculline/picrotoxin-sensitive inhibition. This suggests that the strychnine-resistant and bicuculline/picrotoxin-sensitive inhibitory components may indeed be identical. Such an interpretation is also supported by the fact that the combined administration of both strychnine and bicuculline/picrotoxin results in a virtual abolition of the recurrent inhibition and, furthermore, that in single motoneurones it could be shown that the bicuculline-resistant r.i.p.s.p.s are blocked by strychnine and, vice versa, that the strychnine-resistant r.i.p.s.p.s are abolished by bicuculline. Another observation of crucial importance in this context derives from the elegant experiments by Dr van Keulen (personal communication) who has been recording recurrent unit-i.p.s.p.s elicited in α-motoneurones from single Renshaw cells. He found that these recurrent unit-i.p.s.p.s formed two distinct groups with respect to their time courses, one group having i.p.s.p.s with a shorter duration and an earlier maximum than the other. Furthermore, each Renshaw cell gave rise to only one of these i.p.s.p. types, never a mixture.

When discussing the different recurrent effects described here, it seems pertinent to ask whether central effects elicited by activity in ventral root axons could be produced by sources other than the recurrent axon collaterals of α-motoneurones. It has been demonstrated that the L7 and S1 ventral roots contain a substantial number of unmyelinated sensory axons projecting into the spinal cord (Coggeshall *et al.* 1974) and that these axons are predominantly cutaneous nociceptive fibres and visceral afferents (Coggeshall & Ito, 1977). Due to their high stimulus thresholds and slow conduction velocities these axons should not be responsible for any of the inhibitory effects described in the present study. However, some low-threshold muscle afferent fibres with high conduction velocities have also been observed in the L7 and S1 ventral roots (Loeb, 1976; Coggeshall & Ito, 1977) and, even if their number seems to be very small, it cannot be completely excluded that they may have contributed to the recurrent effects studied here. Still, since the time course of both the strychnine-sensitive and bicuculline/picrotoxin-sensitive r.i.p.s.p. is very unlike that of the disynaptic Ia i.p.s.p., it does not seem probable that afferent fibres in the ventral roots contribute to the disynaptic recurrent effects described in the present investigation.

The hypothesis that the morphologically described monosynaptic interconnexions between α-motoneurones via axon collaterals (Cullheim *et al.* 1977; Cullheim & Kellerth, 1978*a*, *b*) could account for the strychnine-resistant inhibitory effects has not been supported by the present findings, since these effects could be demonstrated also in preparations where any influence by the direct interconnexions should be absent (see Results). Also, the minimum central delay of the strychnine-resistant part of the inhibition (1·2 msec) is similar to that reported for the entire recurrent inhibition (1·1 msec) (Eccles *et al.* 1954) and is therefore compatible with a disynaptic pathway. Furthermore, no signs of synaptic activity could be detected after the combined administration of both strychnine and bicuculline/picrotoxin, in spite of the fact that the monosynaptic interconnexions, which are most certainly cholinergic, should not have been influenced by the convulsants.

If accepting that the recurrent inhibition of spinal α-motoneurones contains two separate components as differentiated by strychnine and bicuculline/picrotoxin, and that the effects of these convulsants are due to the antagonism to glycine and GABA, respectively, both of these transmitter substances seem to be involved in the recurrent inhibitory pathway. Measurements of the central delay of both the strychnine-resistant and the bicuculline/picrotoxin-resistant inhibitions have indicated that they may exert their effects via disynaptic pathways. If also accepting the validity of Dale's principle that each neurone liberates the same transmitter substance at all its synaptic terminals, these findings seem to indicate the existence of two types of Renshaw cells using glycine and GABA, respectively, as transmitters in their synaptic contacts with motoneurones. This conclusion is also supported by the observations by Dr van Keulen mentioned above.

The reason for the presence of two pharmacologically different types of recurrent inhibition of α-motoneurones has not been clarified from the present studies. Apart from the difference in time course between the two types of inhibition, the one mediated by GABA having a later maximum and a longer duration than the one mediated by glycine, no organizational pattern based on motor pool category or motor unit type has been detected, and both inhibitions seem to be mediated via both short and fairly long (at least 2 mm) intrameduallary routes. However, the need for two recurrent inhibitory systems may not be primarily coupled with the recurrent effects on α-motoneurones, since these cells are not the only neurones in the spinal cord receiving recurrent effects. In fact, recurrent inhibition after stimulation of α-motor axons has been demonstrated also in Ia-interneurones (Hultborn, Jankowska & Lindström, 1968, 1971), γ-motoneurones (Brown, Lawrence & Matthews, 1968; Ellaway, 1971), Renshaw cells (Ryall, 1970) and neurones of the ventral spinocerebellar tract (Lindström & Schomburg, 1973). Another possible explanation for having two types of recurrent inhibition is that the different types of Renshaw cells may receive different kinds of specific synaptic inputs from other sources than the α-motor axon collaterals. In this way, one or the other of the recurrent systems could be selectively used in situations with special demands.

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