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# RETICULOSPINAL INHIBITION OF INTERNEURONES

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SUMMARY

1. The effect of electrical stimulation of the brain stem on interneurones in the dorsal horn and intermediary region has been investigated in decerebrate cats after partial transection of the spinal cord.

2. Stimuli that effectively depress reflex transmission without giving a primary afferent depolarization inhibit the discharge evoked from the flexor reflex afferents in interneurones.

3. Brain stem stimulation did not give post-synaptic potentials in the great majority of interneurones but effectively depressed the excitatory post-synaptic potentials (EPSPs) and inhibitory post-synaptic potentials (IPSPs) evoked from the flexor reflex afferents in these interneurones.

4. IPSPs were, however, evoked in five of seventy-eight intracellularly recorded interneurones. These five interneurones were monosynaptically activated from primary afferents.

5. It is tentatively postulated that a dorsal reticulospinal system inhibits reflex transmission by giving post-synaptic inhibition in first order interneurones. The results are also discussed in relation to effects on interneurones from other descending pathways.

## INTRODUCTION

Engberg, Lundberg & Ryall (1968) employed electrical stimulation of the lower brain stem to define a reticulospinal system with inhibitory effect on interneuronal transmission from the flexor reflex afferents to motoneurones, primary afferents and ascending pathways and from Ib afferents to motoneurones. The present paper reports on extra- and intracellular recording from interneurones, the aim being to find the mechanism by which interneuronal transmission is inhibited from this reticulospinal system. It will be shown that IPSPs are evoked from the brain stem in a few of the interneurones investigated. A preliminary report has been given (Engberg, Lundberg & Ryall, 1966).

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## METHODS

The experiments were performed on unanaesthetized cats decerebrated by intercollicular section. Details of the experimental methods have been given by Engberg *et al.* (1968) (cf. diagram of the preparation in their Fig. 1). The absence of antidromic invasion following stimulation of the appropriate ventral roots and of the ventrolateral funiculi in the lower thoracic cord served to identify cells as interneurones. Interneuronal recordings were obtained as described by Hongo, Jankowska & Lundberg (1966). The micro-electrodes were usually filled with a 2 M potassium citrate solution, but electrodes filled with 3 M-KCl solution were sometimes used when it was difficult to impale cells with citrate electrodes. Passage of hyper- and depolarizing current was routinely employed to ascertain that depolarizing responses were not reversed IPSPs caused by diffusion of chloride into the cell.

Abbreviations in addition to those used by Engberg *et al.* (1968): joint: posterior joint nerve, Pl: plantaris nerve.

## RESULTS

Extra- and intracellular recordings were obtained from interneurones in the dorsal horn and intermediary region in order to investigate the mechanism by which interneuronal transmission can be inhibited from the brain stem. At the beginning of each experiment a stimulating electrode was inserted into the region of the reticular formation from which reflex depression was evoked at the lowest stimulus strength when tested as described by Engberg *et al.* (1968). The threshold strength for producing reflex depression and for evoking a DRP was measured and these values were repeatedly checked during the experiments. Effects on interneuronal discharges were always tested with a stimulus strength subthreshold for evoking DRPs, but to exclude that these stimuli produced IPSPs in the interneurones stronger stimuli were used as well.

With extracellular recording from interneurones activated from the FRA it was a regular finding that discharges from the FRA could be effectively depressed; in addition there was often an inhibition of the resting discharge in these interneurones. However, when interneurones of this type were impaled it was usually found that BS stimulation did not evoke any IPSPs. This is illustrated in Fig. 1 in which extracellular (*A–F*) and intracellular (*G–J*) records were obtained from the same cell. The cell receives excitation from the FRA (*B, C* and *I*) but also monosynaptic excitation from group I afferents in the PBSt nerve (*G*) and disynaptic inhibition from group I muscle afferents from G–S (*H*). BS stimulation (*J*) does not change the membrane potential. Since the interneurone in Fig. 1 belongs to the special type which, in addition to the polysynaptic EPSP from the FRA, receives monosynaptic EPSP from group I muscle afferents (Hongo *et al.* 1966) it should be noted that the same results have been found on 'pure' FRA interneurones. This is illustrated in Fig. 2 for an interneurone in which EPSPs are evoked from group II (*B*) and III (*D, E*) muscle afferents, from cutaneous afferents (*F*) and from high threshold joint

afferents (*G*). BS stimulation clearly has no effect on the membrane potential (*H*).

A decreased excitability of the interneurone, due to the withdrawal of background excitation during BS stimulation, does not seem to be a major factor in explaining the decreased efficacy of FRA volleys in discharging

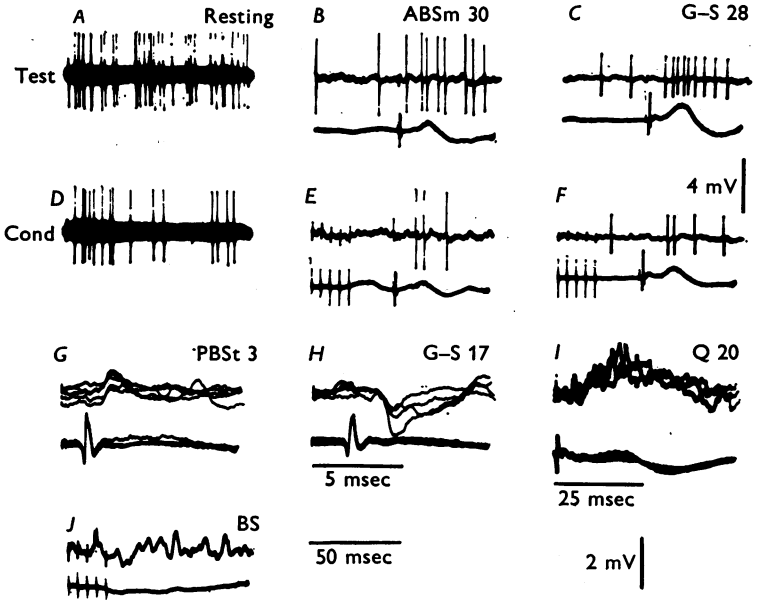


Fig. 1. Effect of BS stimulation on interneuronal discharges. Upper traces in *A–F* are extracellular, in *G–J* intracellular (citrate electrode) records from an interneurone at 2·3 mm depth. *A* shows resting discharge (10 superimposed sweeps), *D* the resting discharge conditioned at the beginning of each sweep with 5 BS stimuli. *B*, *C*, *E* and *F* show the BS action on discharges evoked from high threshold muscle afferents (*E* and *F* conditioned). The cell was monosynaptically activated by group I afferents from PBSt (*G*) and received disynaptic group I inhibition from G–S (*H*). *I* shows EPSP from high threshold muscle afferents and in *J* is a single sweep from a series of records taken with BS stimulation, none of which showed any evoked potential. The lower traces in *B*, *C* and *E–J* were recorded from the cord dorsum. Voltage calibrations: 4 mV for *C* and *F*, 2 mV for all other records. Time calibration: 5 msec for *G* and *H*, 25 msec for *I* and 50 msec for the other records. Strengths of nerve stimuli are given in multiples of threshold above the records in this and the following figures.

these cells. The most likely mechanism for the inhibition of discharges evoked by FRA volleys is a depression of the EPSPs that generate these discharges. Figure 3 *A* and *C* gives an example of this. The EPSP depression could be evoked at a strength of BS stimulation subthreshold for evoking a DRP. Figure 3 also illustrates a similar depression of the IPSPs evoked from high threshold afferents in an interneurone (*B*, *D*). This

interneurone in addition received a monosynaptic EPSP from group I muscle afferents and this EPSP was not depressed by the BS stimulation. It can be concluded that volleys in the reticulospinal system depress the activity in excitatory and inhibitory paths from the FRA to interneurones, approximately to the same extent.

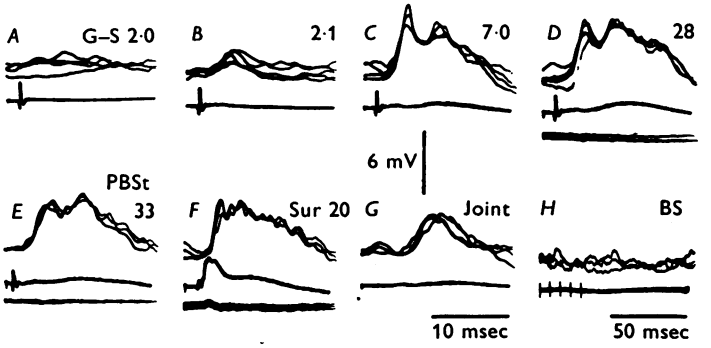


Fig. 2. Intracellular records (citrate electrode) from an FRA interneurone. Depth 1·7 mm. Upper traces in *A–D* show increasing EPSPs evoked by stimulation of G–S afferents. An EPSP only appears when the stimulus is above group II threshold (*B*) and then increases with the stimulation strength, also within the group III range to the size in *D*. In *E–G* are EPSPs from high threshold PBSt, sural and joint afferents. BS stimulation has no effect on the membrane potential (*H*). In this and the following figures the second trace in each set of records was recorded from the cord dorsum and the third trace (in some records only) by the micro-electrode just after withdrawal from the cell to show extracellular field potentials. Time calibration: 10 msec for *A–G*, 50 msec for *H*.

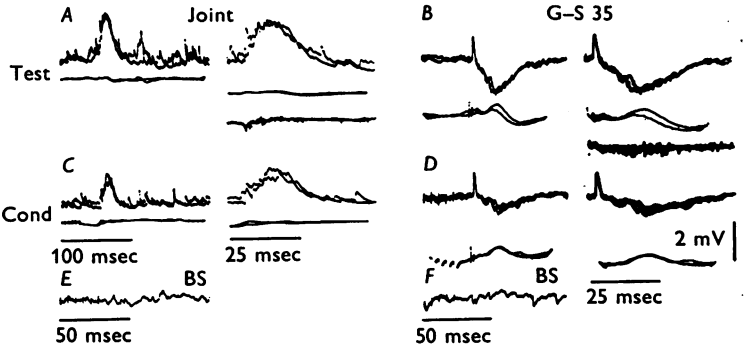


Fig. 3. BS depression of EPSP and IPSP in interneurones. *A* and *C* are intracellular records from an interneurone (KCl electrode) at 1·9 mm depth. *B* and *D* from another (citrate electrode) at 2 mm depth, each with two sweep speeds. The EPSP evoked from joint afferents (*A*) is depressed by about 30% after BS stimulation (*C*). *B* and *D* show very strong depression of the IPSP evoked from high threshold muscle afferents whereas there is no change in the early EPSP (which was found to be monosynaptic from group I afferents). BS stimulation evokes no synaptic potential in either of these cells (*E* and *F*).

It is not known why the withdrawal of excitation, indicated by the decreased frequency of the resting discharge during BS stimulation, does not show up as a hyperpolarization of the cell (*J*, Fig. 1). It does not seem reasonable, at the present stage, to suggest that impulse initiation is remote from the soma. The intracellular records were obtained from depolarized cells and it would not have been easy to detect a removal of an excitatory synaptic potential. It is probable that the resting discharge is evoked by activity in the pathway which transmits excitation from the FRA and hence that it is an indicator of inhibition of cells along this pathway.

The absence of IPSPs in the majority of the interneurones described above is a very relevant finding (cf. Discussion) but in considering the mechanism by which the reticulospinal system acts on interneuronal transmission special attention must be drawn to the finding that IPSPs were evoked from the brain stem in 5 of 78 intracellularly recorded interneurones. The results are summarized in Table 1 in relation to the pattern of convergence

TABLE 1. Classification of interneurones examined intracellularly with regard to monosynaptic excitation from cutaneous and group I muscle afferents versus polysynaptic FRA actions from the hind limb nerves. Numbers of cells with IPSPs from the brain stem and the total numbers are shown for each combination

| | Polysynaptic effects from the FRA | | | | |
|---|---|---|---|---|---|
| | EPSP + IPSP | EPSP | IPSP | None | Total |
| Monosynaptic EPSP from: | | | | | |
| Cutaneous and group I muscle | 0/2 | 0/2 | 0/2 | 0/0 | 0/6 |
| Group I muscle only | 0/3 | 0/3 | 1/15 | 0/2 | 1/23 |
| Cutaneous only | 1/5 | 1/4 | 0/9 | 2/3 | 4/21 |
| None | 0/6 | 0/9 | 0/12 | 0/1 | 0/28 |
| Total | 1/16 | 1/18 | 1/38 | 2/6 | 5/78 |

from primary afferents. This pattern from primary afferents was as complex as found in spinal cats by Hongo *et al.* (1966) but only the main effects are included. Since BS stimulation evoked IPSPs in so few cells it is not easy to classify these cells in relation to effects from primary afferents. At present we would like to draw attention mainly to the finding that all the interneurones with IPSPs from the brain stem received monosynaptic excitation from primary afferents, four of them from cutaneous afferents and one from group I muscle afferents. Furthermore, as already emphasized above, of thirty-four interneurones in which EPSPs were evoked from the FRA only two received an IPSP from the brain stem.

The cells with IPSPs from the brain stem were located at different depths between 1·3 and 2·5 mm from the dorsal surface of the cord. The records in Fig. 4 are from an interneurone 1·8 mm from the surface which received mixed inhibition and excitation from high threshold muscle afferents (*A*,

*B*) and cutaneous afferents (*C–F*). The excitation from cutaneous afferents was partly monosynaptic (*E*, *F*). The IPSP evoked from the brain stem in *G* has a rather short duration compared with that of inhibition of reflexes presented earlier (Engberg *et al.* 1968) but only three stimulus pulses were used. The IPSP was more long-lasting in other cells when four stimuli were used and it is possible that a longer tetanus may increase the

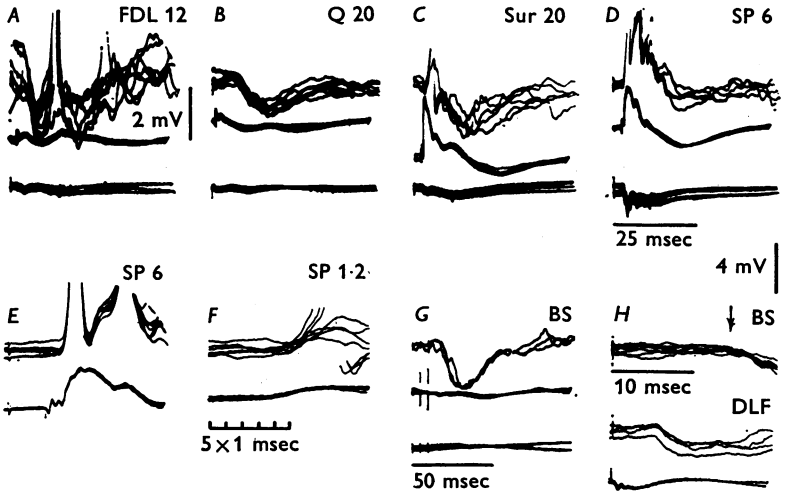


Fig. 4. Interneurone with synaptic inhibition from BS. Depth 1·8 mm. Intracellular records (citrate electrode). *A* and *B*: synaptic actions from high threshold muscle afferents. Inhibition dominates but some excitation is also evoked from FDL. *C–F*: excitatory and inhibitory actions from cutaneous afferents. The excitation from SP is partly monosynaptic. *G*: IPSP evoked by three stimulus shocks in BS. *H* shows onset of IPSP evoked by a single shock in BS (the beginning of the first deflexion is marked by an arrow); below is the IPSP evoked by a single stimulus of the contralateral DLF in the lower thoracic region. Third traces in *A–D* and *G* are extracellular records as in Fig. 2. Time calibration for *A–D* under *D*, for *E* and *F* under *F*.

duration of the effect considerably. The IPSPs were evoked at the same low strength of BS stimulation that gave inhibition of reflex transmission.

The latency of the IPSP was accurately measured for three cells as illustrated in the upper trace of record *H*, Fig. 4. In this record with many superimposed sweeps, a faint deflexion occurs (at the arrow) 14 msec after a single shock stimulation and a second larger wave about 3 msec later. Below is a record taken with stimulation of the intact DLF of the cord just above the level of the spinal transections. In this case an IPSP appears after 5·6 msec. The distances between stimulation and recording sites in the two cases were 296 mm and 86 mm respectively. Calculations based on the differences in conduction times and distances give a maximal conduction velocity from the brain stem to the lower thoracic cord of 25 m/sec

provided that the stimulation activates the descending neurones directly without any synaptic delays. (No change in latency was seen when the reticular stimulation was increased to twice the strength.) With the same conduction velocity further down about 2 msec would be left for the transmission to cross over to the side of recording and for the synaptic delay. As the conduction may be slower in the terminal parts of the fibres it seems likely that the IPSP is evoked via a disynaptic pathway but the possibility of a monosynaptic inhibition of the interneurones is not entirely excluded. In the two other cells analysed the latency from the brain stem was found to be 13–14 msec but in a few records from one of these cells (Fig. 5) latencies of 7·5–10 msec were observed. The reliability of the latter

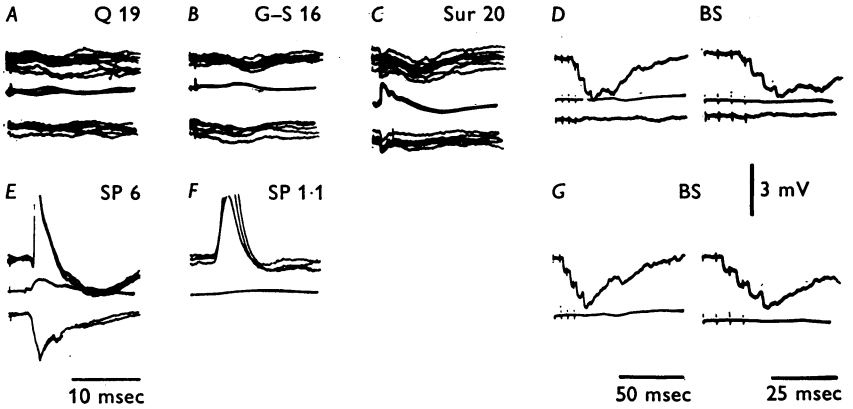


Fig. 5. Short latency and stepwise increase of BS IPSP in interneurone. Intracellular records (citrate electrode). This cell, at 1·3 mm depth, was monosynaptically activated from low threshold SP afferents (*E* and *F*) but received only inhibition, if anything, from the FRA (*A–C*). *D* and *G* are single sweep records with two sweep speeds showing IPSPs evoked by four BS stimuli. Third traces show extracellular fields as in Fig. 2. 25 msec time calibration also valid for *A–C*.

measurements may be questioned since they are based on occasional records. However, the record with the shortest latency (Fig. 5*G*) displays a regular stepwise increase in the IPSP, each step following 7·5 msec after one of the four stimulation pulses. In the record above (*D*) the same stepwise increase is seen but with latencies successively decreasing from 13 to 12·5 msec. The same unitary responses were seen in the seven other records taken from this cell with BS stimulation but with a latency of about 13 msec. Only a few superimposed sweeps were photographed from this cell with stimulation of the right DLF. The latency was then 4·5–5·0 msec. The cell could only be activated (monosynaptically) from cutaneous afferents in the SP nerve (*E*, *F*).

Of twenty-nine interneurones with monosynaptic EPSPs from group I

muscle afferents BS stimulation gave an IPSP in only one cell. Figure 6 shows an interneurone with a group Ia EPSP from the Q nerve. The maximal EPSP is evoked in *B* at a strength slightly submaximal for the Ia volley and when the main part of the Ib volley is activated at a

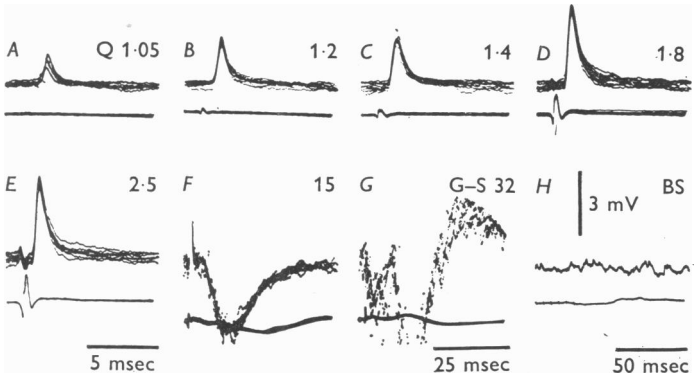


Fig. 6. Group Ia interneurone with IPSPs from FRA. Intracellular records (citrate electrode), depth 1·6 mm. *A–D* show a monosynaptic EPSP increasing with the stimulus strength within the group Ia range of the Q nerve. In *E* the stimulus has been increased to just above maximum for the Ib afferents: in *F* volleys in group II and III afferents from the same nerve give an additional IPSP. *G*: IPSP evoked from high threshold G–S afferents. *H* shows absence of effect from 6 BS stimuli. Time calibrations: 5 msec for *A–E*, 25 msec for *F* and *G*.

slightly higher strength in *E* there is no increase of the EPSPs. An IPSP is evoked from high threshold afferents in Q and G–S. BS stimulation is without effect (*H*). The monosynaptic EPSP in the interneurone of Fig. 7 *A–E* is evoked by plantaris afferents within the entire group I range tested including high threshold group I afferents but the cell also receives monosynaptic EPSPs from G–S and ABSm (*K*, *L*). The threshold of the group I afferents from which the effect was evoked in the latter two nerves was not tested. In the two records of Fig. 7 *M*, trains of respectively six and three BS stimuli are shown to evoke consecutive unitary IPSPs in this interneurone, rather similar to those of Fig. 5 but more variable and short-lasting. The convergence pattern of this interneurone is of interest not only in that it receives monosynaptic Group I EPSPs from three extensor nerves but also in that group Ib afferents to flexor nerves (*G–J*) evoke a disynaptic IPSP and that volleys in the FRA also produce an IPSP (*K*, *L*, *N–Q*) (cf. Discussion).

## DISCUSSION

It was expected that activity in the dorsal reticulospinal system (Engberg *et al.* 1968) should inhibit transmission to interneurones in the spinal cord. Another indication of this inhibition is the depression of the

negative cord dorsum potential evoked from the FRA (Engberg *et al.* 1968). This potential is assumed to represent the extracellularly recorded synaptic potential of interneurones in the dorsal part of the spinal cord. Intracellular recording from interneurones activated from the FRA

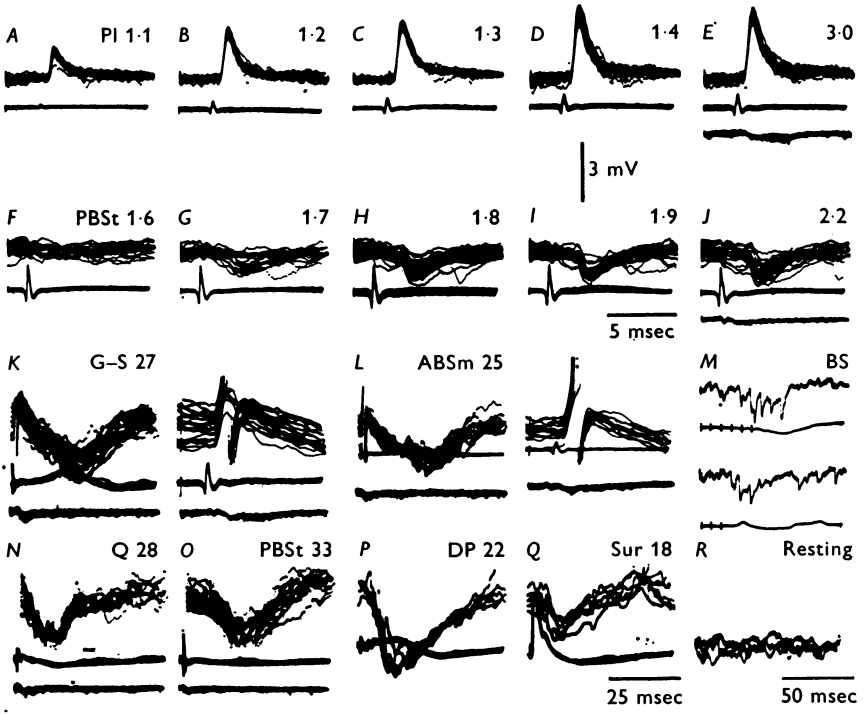


Fig. 7. Group I interneurone with IPSPs from group Ib and FRA. Intracellular records (citrate electrode), depth 2·0 mm. *A–E* show monosynaptic EPSP increasing with the strength of group I Pl afferent stimulation. *F–J* similarly show disynaptic IPSP evoked from group Ib afferents of PBSt. *K* and *L* (with two sweep speeds) show IPSPs from high threshold muscle afferents as well as monosynaptic group I excitation from G–S and ABSm. *N–Q* show other examples of FRA IPSPs. The two records under *M* are excerpts from a series of single sweeps taken with different trains of BS stimuli; in each sweep small unitary IPSPs followed the stimuli. *R* shows the resting variations of the membrane potential. Time calibrations: 5 msec for *A–J* and fast sweep of *K* and *L*, 50 msec for *M* and *R*, 25 msec for other records.

revealed that reticular stimulation does not evoke IPSPs in the great majority of these interneurones. Hence it may be suggested that the reticulospinal inhibition of transmission in reflex pathways is not caused by widely distributed IPSPs in the interneuronal network. On the other hand corresponding to inhibition of the discharge there was an effective depression of the EPSPs (and IPSPs) evoked in these interneurones from

the FRA (Fig. 3). Since this depression was evoked by reticular stimulation subthreshold for evoking a DRP it is postulated that the inhibition is exerted on the interneuronal path to the interneurone recorded from. Although IPSPs were evoked from the reticular formation in only five of the seventy-eight interneurones it is postulated that they contribute to the inhibitory effect described by Engberg *et al.* (1968). The reason is that these IPSPs have the required latency and time course and are evoked at the same low strength of the reticular stimulation that produces reflex inhibition. The profound inhibition of reflex transmission exerted from the reticulospinal system could probably be entirely explained by post-synaptic inhibition at selective interneuronal stations. However, our experiments have not been designed to exclude other mechanisms, for example, presynaptic inhibition of transmission from interneurones.

A picture therefore emerges of an inhibition of transmission from primary afferents to widely distributed interneuronal networks exerted early in the interneurone chain, possibly in the first order neurones, which receive monosynaptic effects from primary afferents. This must be so in the Ib pathways to motoneurones which are largely disynaptic (Laporte & Lloyd, 1952; Eccles, Eccles & Lundberg, 1957; cf. discussion below). It is also of interest that four of the five interneurones with IPSPs from the reticular formation received monosynaptic EPSPs from cutaneous afferents. Since cutaneous afferents are part of the FRA it is tempting to correlate this finding with the strong inhibitory control of transmission from the FRA. However, most interneurones with monosynaptic EPSPs from low threshold cutaneous afferents may not belong to FRA paths (many of them are in fact inhibited from the FRA) but to more specialized neuronal paths activated exclusively from cutaneous afferents (Hongo *et al.* 1966). There is only scant knowledge of reflex connexions of such paths but they may of course also be subject to a descending inhibitory control. One example is actually provided by the reflex path giving plantar flexion from mechanoreceptors in the central plantar cushion; this reflex is tonically inhibited in the decerebrate state (Engberg, 1964). For identification of a first order FRA interneurone a convergence of monosynaptic EPSPs from cutaneous afferents and high threshold muscle afferents would be required. To our knowledge no such records have been published and it is in fact not known if the convergence occurs already on the first order interneurones. In any case there are probably very few of these first order FRA interneurones but there may be a large divergence from a few cells leading to activation of many second order interneurones. Without this assumption it would be difficult to explain that the great majority of the interneurones in the spinal cord are influenced from the FRA (cf. Hongo *et al.* 1966).

IPSPs evoked by reticular stimulation in interneurones with mono-

synaptic EPSPs from group I muscle afferents are of particular interest. The reason is that of all known pathways from Ia and Ib afferents to motoneurones and primary afferents only the pathways from Ib afferents to motoneurones seem to be under reticular inhibitory control (Eccles & Lundberg, 1959; Carpenter, Engberg, Funkenstein & Lundberg, 1963; Engberg *et al.* 1968). In the present series reticular stimulation produced an IPSP only in one of the interneurones with monosynaptic EPSP from group I muscle afferents. It should be noted that this cell belonged to the type receiving IPSPs from Ib afferents and from the FRA. Although data from a single cell make a poor basis for speculation one might ask if Ib pathways to motoneurones are under an interactive inhibitory control from Ib afferents and from the FRA since this may explain the finding that Ib volleys often have little or no effect in motoneurones, particularly in unanaesthetized spinal cats (A. Lundberg, unpublished). Altogether it seems likely that in future attempts to identify interneurones as belonging to a certain pathway the pattern of descending actions will be as important as the effects from primary afferents.

It is interesting to compare the effects from the dorsal reticulospinal system with those evoked from the corticospinal and rubrospinal tracts. Activity in the latter two pathways facilitates transmission in reflex paths to motoneurones and primary afferents by giving excitatory action to their interneurones (Lundberg & Voorhoeve, 1962; Lundberg, Norrsell & Voorhoeve, 1962; Hongo, Jankowska & Lundberg, 1965; Lundberg, 1964, 1967). However, in many interneurones IPSPs are evoked from the two pathways, in fact much more frequently than from the dorsal reticulospinal system. The explanation of this seemingly paradoxical finding is the following: most of the interneurones in the dorsal horn and intermediary region receive excitatory and inhibitory pathways from primary afferents. Both these pathways may be facilitated from the corticospinal and rubrospinal tracts and the IPSPs evoked secondarily by this facilitatory mechanism. By contrast the dorsal reticulospinal system seems to be primarily inhibitory; transmission in excitatory and inhibitory paths from primary afferents to interneurones is inhibited to an equal degree and no trace of an EPSP was evoked in any of the seventy-eight intracellularly recorded interneurones.

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