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Characterization of a Descending System that Enables Crossed Group II Inhibitory Reflex Pathways in the Cat Spinal Cord

N. C. Aggelopoulos,[1] M. J. Burton,[1] R. W. Clarke,[2] and S. A. Edgley[1]

[1]Department of Anatomy, University of Cambridge, Cambridge CB2 3DY, United Kingdom, and [2]Department of Physiology and Environmental Science, University of Nottingham, Sutton Bonington Campus, Loughborough Leics LE12 5RD, United Kingdom

In the cat, stimulation of group II afferents from hindlimb muscles evokes different crossed reflex actions depending on the integrity of the spinal cord: with the cord intact, extensor motoneurons are inhibited by activation of contralateral group II afferents; after spinal transection, the same stimuli excite these neurons (crossed extension reflex). We have investigated the mechanisms underlying this descending control. To delimit the descending pathway, the effects of funicular lesions of the thoracic cord on the crossed actions on motoneurons were examined. Bilateral lesions of the dorsolateral funiculi abolished the crossed IPSPs as effectively as complete spinal section. If either dorsolateral funiculus was spared, the IPSPs remained. To examine whether serotonergic fibers were involved, the effects of agents selective for 5-hydroxytryptamine (5-HT)$_{1A}$ receptors were examined. After abolishing the crossed IPSPs by spinal transection, systemic administration of the 5-HT$_{1A}$ receptor agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT; 0.1–1.3 mg/kg, i.v.) restored the crossed inhibition. This effect was antagonized by the selective 5-HT$_{1A}$ receptor antagonist WAY-100135 (0.7–3.7 mg/kg, i.v.). The simplest explanation of these findings is that a serotonergic pathway, descending via the dorsolateral funiculi and acting via 5-HT$_{1A}$ receptors, is involved: with the spinal cord intact, the pathway would be tonically active and thus enable the crossed inhibition. This raises the possibility that a serotonergic pathway is involved in the selection of specific spinal reflex patterns via 5-HT$_{1A}$ receptors.

Key words: serotonin; spinal cord; spinal reflexes; muscle afferents; spinal interneurons; descending control

Stimulation of some groups of peripheral afferents can evoke spinal reflexes of different sign under different experimental conditions. The idea that the brain may use these different reflex pathways, selectively controlling them to choose a particular pattern of movement, is an old and attractive one (see Lundberg, 1979). A crucial problem has been to identify the mechanisms responsible for selection between alternative reflex patterns.

We have examined the mechanisms underlying selection between different crossed reflex actions evoked by group II afferents. A robust and unusual set of crossed actions that show different patterns under different experimental circumstances has been described in the cat spinal cord. Under chloralose anesthesia, stimulation of certain nerves at strengths sufficient to activate group II afferents evokes different patterns of crossed reflexes in extensor motoneurons depending on the integrity of the spinal cord (Arya et al., 1991). When the spinal cord is intact, stimulation of contralateral group II muscle afferents evokes crossed IPSPs in hindlimb flexor and extensor motoneurons; when the cord is transected, the same stimuli evoke a crossed extension reflex (i.e., EPSPs in extensor motoneurons). These actions are evoked via different central pathways: the crossed IPSPs have short latencies and are evoked reliably by single stimuli, whereas the crossed EPSPs seen in spinal animals usually require several stimuli and have longer latencies (Arya et al., 1991). Crossed IPSPs are evoked by nerves that activate a system of neurons in the midlumbar segments of the spinal cord (Edgley and Jankowska, 1987), and it has been demonstrated that they involve a relay in the midlumbar segments (Aggelopoulos and Edgley, 1995). The central latencies of the crossed IPSPs (minimally just over 3.0 msec) are similar to those of ipsilateral EPSPs and IPSPs evoked by the same nerves in ipsilateral motoneurons (Edgley and Jankowska, 1987; Arya et al., 1991). Taken together, the short central latency and long central pathway argue strongly for a disynaptic linkage (i.e., a single central interneuron) in the pathway (Arya et al., 1991; Aggelopoulos and Edgley, 1995). Anatomical evidence in support of this view is provided by the observation that interneurons in the contralateral ventral horn in the midlumbar segments can be labeled transneuronally from hindlimb motoneurons with wheat germ agglutinin–horseradish peroxidase (Harrison et al., 1986). Furthermore, interneurons at this location with identified axonal projections to the contralateral hindlimb motor nuclei have been examined electrophysiologically in the midlumbar segments, and they are monosynaptically excited by the appropriate group II afferents (Jankowska and Noga, 1990).

The dramatic alterations in crossed actions of group II afferents that occur on spinal transection indicate a potent descending control of the reflex pathway. In the present experiments, we have sought to characterize the descending system that controls the crossed inhibition, first with limited funicular lesions to identify the location of its axons and, second, using pharmacological manipulation to provide clues as to its chemical identity.

Preliminary accounts of some of this work have been published in abstracts (Burton and Edgley, 1993; Aggelopoulos et al., 1994).

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Correspondence should be addressed to Dr. S. A. Edgley, Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK.

MATERIALS AND METHODS

Experiments were performed on 10 adult cats. All procedures were performed under the UK Animals Scientific Procedures Act (1986) and were overseen by the UK Home Office inspectorate. In all cases, anesthesia was induced with ketamine and xylazine (15 and 1 mg/kg, i.m., respectively). Surgery was performed under halothane anesthesia, which was replaced for the recordings with α-chloralose anesthesia, using a regimen that has been described in full in recent publications from this laboratory (Arya et al., 1991). During recording, the animals were paralyzed with pancuronium bromide (0.5–1.0 mg/kg, i.v., initially) and artificially ventilated. Additional doses of pancuronium (0.25–0.35 mg/kg) were given when paralysis waned. Several precautions were taken to ensure that full anesthesia was maintained during paralysis. These precautions included ensuring that neither blood pressure nor heart rate was altered in response to noxious stimulation, that the pupils remained fully constricted, and that areflexia remained as paralysis waned. These tests were performed at 30 min intervals.

A number of hindlimb nerves were dissected free, transected, and ligated. In all experiments, the nerves to quadriceps and sartorius on both sides were mounted in bipolar tunnel electrodes for stimulation. Branches of the left sciatic nerve were stimulated via pairs of silver wire electrodes. These branches included the nerves to the hamstring flexors [posterior biceps and semitendinosus (PBSt)] and the hamstring extensors [semimembranosus and anterior biceps (SmAB)] and the nerve to gastrocnemius and soleus (GS). Intracellular recordings were made from antidromically identified motoneurons using potassium citrate-filled electrodes (impedance, 5–15 MΩ). Motoneurons with resting membrane potentials >40 mV were accepted.

Identification of the location of the descending pathway. Six experiments were performed. Limited funicular lesions were used to identify the locations of descending fibers controlling the crossed inhibition. After first recording from a sample of motoneurons, small lesions were made in the spinal white matter at the low thoracic level (T11–12). These lesions were made carefully with watchmakers' forceps. We attempted to minimize mechanical activation of axons by wiping the exposed surface of the spinal cord with cotton–wool balls soaked in 2% lidocaine before making the lesions. Recordings commenced 10 min after the lesion. Sequential lesions were made at different rostrocaudal levels (3–5 mm apart) to allow the extent of each lesion to be verified postmortem. At the end of each experiment, animals were perfused via the aorta with formol saline and the spinal cords were processed subsequently for histological reconstruction of the lesion sites.

Chemical identification of the descending pathway. Four experiments were performed. In these experiments, we first verified that the crossed group II inhibition was present with the spinal cord intact with intracellular recordings from a sample of motoneurons. The spinal cord was then transected at a low thoracic level (T11–12). Local anesthetic (2% lidocaine) was applied topically with small balls of cotton–wool to minimize trauma during this procedure. A second sample of motoneurons was then taken to verify that the crossed IPSPs had been lost. The selective 5-hydroxytryptamine $(5\text{-HT})_{1A}$ agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT; Research Biochemicals, Natick, MA) was then given intravenously in physiological saline. Doses between 0.1 and 1.3 mg/kg were used. A third sample of motoneurons was taken after administration. Finally, the selective 5-HT_{1A} receptor antagonist WAY-100135 (Fletcher et al., 1993) (Wyeth Research, Boston, MA) was given, and a final group of motoneurons was sampled. WAY-100135 was solubilized in dimethyl formamide and given in physiological saline (doses 0.7–3.7 mg/kg, i.v.). In other experiments, drugs solubilized in dimethyl formamide were given but did not reverse the crossed IPSPs, indicating that there are no effects of the vehicle in this system (our unpublished observations). Both 8-OH-DPAT and WAY-100135 act centrally to exert profound effects on the cardiovascular system (hypotension). In these experiments, these actions were countered as much as possible with an intravenous infusion of adrenaline (0.025 mg/ml) given at a rate sufficient to maintain blood pressure at >90 mmHg. To preempt, rather than purely to respond to, changes in blood pressure in response to drug administration, we gave adrenaline simultaneously with giving drugs. As a control to ensure that any changes in crossed reflex inhibition were attributable to blood pressure fluctuation, we observed IPSPs evoked by ipsilateral group II afferents.

RESULTS

Anatomy of the descending pathway

To determine the location of the descending pathway responsible for control of the crossed inhibitory pathway, we performed six experiments in which we examined the effects of specific funicular lesions. In each experiment, we first recorded from a sample of motoneurons to verify that stimulation of contralateral group II afferents evoked IPSPs in motoneurons, as reported previously by Arya et al. (1991). In the six experiments, 31 of 32 GS motoneurons and 41 of 43 hamstring (SmAB and PBSt) motoneurons had crossed IPSPs in response to a single stimulus to the contralateral quadriceps nerve (cQ) at a strength sufficient to activate group II afferents [5 × threshold (5T)].

In the first experiment, the initial lesion included the dorsal funiculus (DF) and the most dorsal parts of both lateral funiculi [ipsilateral and contralateral to the motoneurons (iDLF and cDLF, respectively)]. After this lesion, crossed group II inhibition was much less frequent (3/14 vs 9/9 motoneurons recorded before the lesion in this experiment). Furthermore, in the three inhibited motoneurons the IPSPs were small. In subsequent experiments, lesions were made sequentially to localize the descending pathway further.

In two experiments, extensive lesions of the cDLF (in one experiment, with lesions of the ventral dorsolateral funiculus and the ventral funiculi as well) failed to abolish the crossed group II IPSPs. However, after subsequent lesion to the iDLF, the crossed IPSPs were abolished. Representative recordings from GS motoneurons from one of these experiments are shown in Figure 1. The frequencies of crossed IPSPs are given in Figure 1, and their mean sizes are given in the histograms of Figure 3*A*. There was no significant difference between the mean sizes of crossed IPSPs evoked from Q group II afferents with the cord intact or with lesions of only the cDLF in either GS or SmAB motoneurons (Student's *t* test). After the lesion of the iDLF, the incidence of crossed IPSPs was reduced substantially and many extensor motoneurons were excited rather than inhibited by contralateral group II afferents (Fig. 3*A*). In each of the motoneurons, we also tested the actions of ipsilateral group II afferents. After the second lesion (iDLF), which abolished the crossed IPSPs, the mean sizes of IPSPs evoked from ipsilateral Q group II afferents were slightly larger, but the difference was not statistically significant (Student's *t* test). The immediate implication of these results is that the descending pathway that enables crossed group II inhibition includes fibers in the DLF ipsilateral to the motoneurons (Burton and Edgley, 1993).

In three subsequent experiments, we again sampled motoneurons with the cord intact and then made our initial lesion in the iDLF. This initial lesion did not abolish the crossed IPSPs (Fig. 2*A*,*B*). The mean sizes of crossed IPSPs were smaller after this lesion in both GS and SmAB motoneurons (Fig. 3*B*), but the difference was not statistically significant (Student's *t* test). Subsequent lesions of the cDLF in these same experiments did reduce substantially or abolish the crossed group II IPSPs (Fig. 2*C*). After the cDLF lesion, only 1 of 22 GS motoneurons had crossed IPSPs, and EPSPs could be seen in 14 of 22 motoneurons. After the cDLF lesion, the incidence of crossed IPSPs in both GS and SmAB motoneurons was very low (Fig. 3*B*). Consistent with observations from the previous series of experiments, after the second lesion, which essentially abolished the crossed IPSPs, the mean size of the ipsilaterally evoked IPSPs in the GS motoneurons again was increased over the control values (from 3.1 ± 0.4 to 4.5 ± 0.5 mV). The increase was statistically significant ($p < 0.05$, Student's *t* test). These data imply that the crossed inhibition is enabled by a pathway descending in both dorsolateral funiculi, such that sectioning the DLF on either side alone does not affect the crossed group II IPSPs but that sectioning both abolishes them.

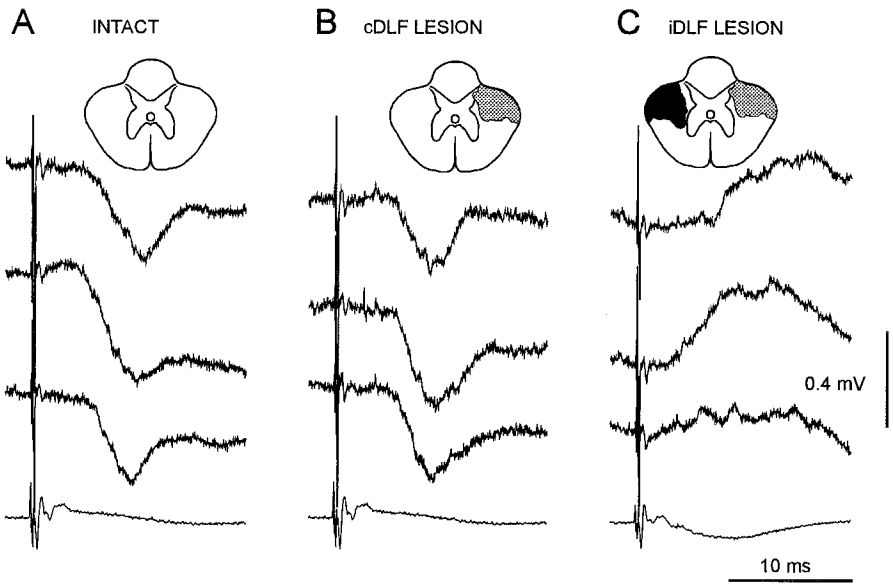

Figure 1. Effects of lesions of the dorsolateral funiculi on crossed group II inhibition. Each panel shows averaged (10 sweeps) intracellular responses from three different GS motoneurons to stimulation of the contralateral quadriceps nerve at 5T. The *bottom trace* in each panel is the volley recorded from the cord dorsum. At the *top* of each panel is indicated the greatest extent of the respective low thoracic cord lesion as reconstructed from histological sections for the animal from which the electrophysiological records shown here have been obtained. *A*, With the cord intact, almost all extensor motoneurons were inhibited on stimulation of contralateral group II afferents. *B*, Lesions at a low thoracic level of the dorsolateral funiculus contralateral to the motoneuron had little effect on the crossed IPSPs. *C*, After lesions of the dorsolateral funiculus ipsilateral to the motoneuron, contralateral group II afferents did not evoke IPSPs but, in many cases, evoked EPSPs. The frequencies of crossed IPSPs in the sample of gastrocnemius and hamstring motoneurons from both experiments of this type are indicated for each condition.

The pathway that was interrupted by the DLF lesions could act in a number of ways and at a number of sites to influence the pathway responsible for the crossed IPSPs. It is unlikely, however, that the effects were mediated directly via the motoneurons, because the IPSPs evoked in the same motoneurons from ipsilateral quadriceps group II afferents were not reduced—on the contrary, they were slightly increased. This suggests that the action of the descending pathway is not a postsynaptic action exerted on the motoneurons themselves, but at a site on the central pathway.

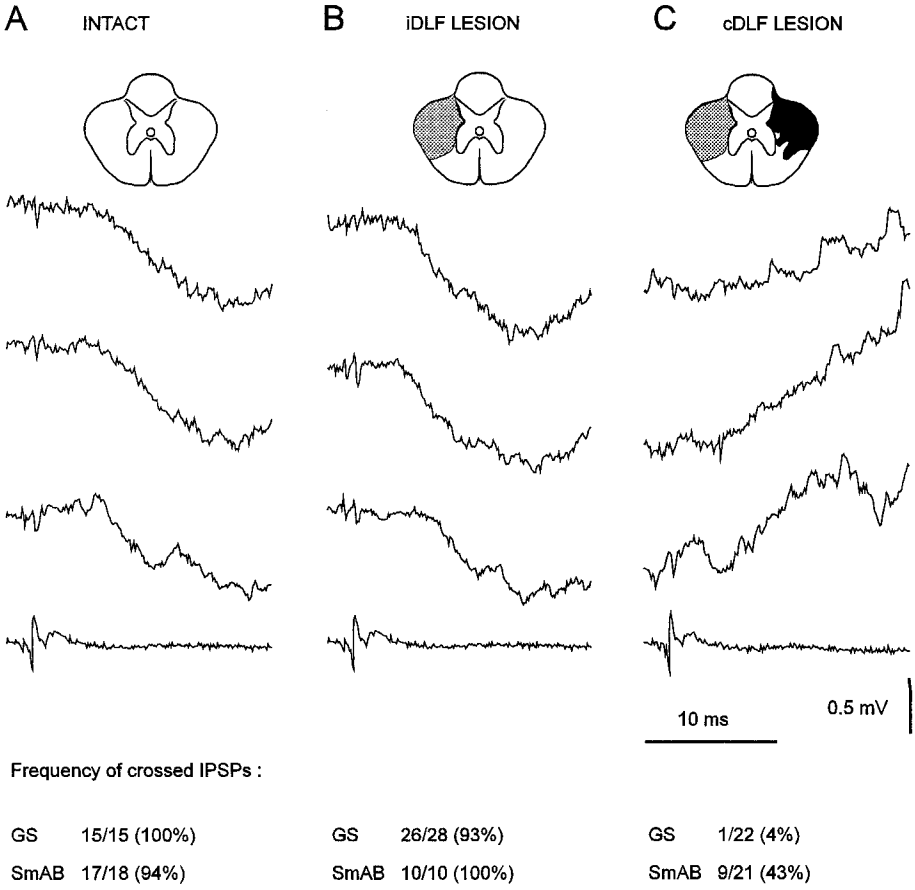

Figure 2. Effects of lesions of the dorsolateral funiculi on crossed group II inhibition. Each panel shows averaged (3–7 sweeps) intracellular responses from three different GS motoneurons to stimulation of the contralateral quadriceps nerve at 5T. The layout is similar to that of Figure 1 with the exception that in these experiments iDLF rather than cDLF was interrupted first. *A*, With the cord intact, almost all extensor motoneurons were inhibited on stimulation of contralateral group II afferents. *B*, Lesions of the dorsolateral funiculus ipsilateral to the motoneuron at a low thoracic level had little effect on the crossed IPSPs. *C*, After lesions of the dorsolateral funiculus contralateral to the motoneuron, contralateral group II afferents did not evoke IPSPs but, in many cases, evoked EPSPs. The frequencies of crossed IPSPs in the sample of gastrocnemius and hamstring motoneurons from the three experiments of this type are indicated for each condition.

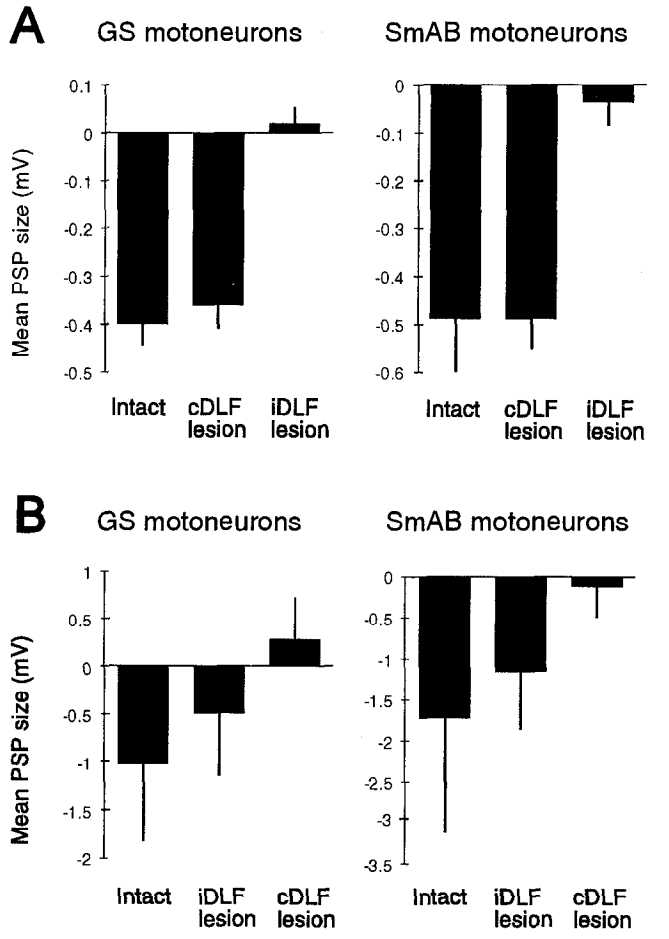

Figure 3. Summary histograms of the mean sizes of postsynaptic potentials from the two sets of experiments shown in Figures 1 and 2. *A*, Results from the two experiments in which the cDLF was interrupted before the iDLF. Only after bilateral lesion was the size of the crossed IPSPs significantly reduced—indeed, in the case of GS motoneurons, it was reduced to net crossed excitation. *B*, Results from the three experiments in which the iDLF was interrupted before the cDLF. Again, only after bilateral lesion was the size of the crossed IPSPs significantly reduced. In the case of the GS motoneurons, net crossed excitation ensued. *Bars*, ±SEM.

Observations relating to the chemical identity of the descending pathway

Tonic descending actions on spinal reflex pathways often are associated with monoaminergic descending pathways. Accordingly, we investigated the possibility that the descending control of crossed group II inhibition is mediated via such a pathway. Given its location in the DLF, it is possible that fibers containing 5-HT are involved, because serotonergic fibers pass through this funiculus (Hylden et al., 1985). We investigated this possibility in four experiments using pharmacological agents selective for 5-HT_{1A} receptors. Figure 4 shows representative recordings from one of these experiments, and Figure 5 summarizes population data from GS and SmAB motoneurons from four experiments.

As a first step, in each of these experiments we recorded from a sample of motoneurons (>10) with the spinal cord intact to verify that stimulation of group II afferents from contralateral quadriceps evoked IPSPs. All of the GS and SmAB motoneurons tested had crossed IPSPs in response to stimulation of group II afferents (15 and 31 motoneurons, respectively). Crossed extension was not seen with the cord intact in response to single stimuli in either GS or SmAB motoneurons. Subsequently, the spinal cord was transected at a low thoracic level (T11–12). Beginning 15 min later, a second sample of extensor motoneurons was tested to verify that the crossed inhibition had been abolished (Fig. 4). Crossed quadriceps group II IPSPs no longer were detectable in any of the SmAB motoneurons recorded (n = 15), whereas small IPSPs were present in a few of the GS motoneurons (3/26, 11%; Fig. 5*A*). In this sample, the mean size of the IPSPs evoked from the ipsilateral quadriceps was not significantly altered (Student's t test). As has been reported previously (Arya et al., 1991), spinal transection was accompanied by the appearance of later crossed EPSPs (i.e., a crossed extension reflex) in response to activation of group II afferents in 18 of 26 (69%) GS motoneurons and in 9 of 13 (69%) SmAB motoneurons (see Fig. 5*A*).

To test the possible involvement of 5-HT_{1A} receptors, 8-OH-DPAT was administered intravenously. In an initial experiment, a large dose (1.3 mg/kg) restored the crossed IPSPs. In subsequent experiments, smaller doses (0.1–0.3 mg/kg) also successfully restored the crossed IPSPs (Fig. 4). After 8-OH-DPAT administration, crossed IPSPs were seen in a high proportion of motoneurons: 26 of 29 GS motoneurons and 18 of 20 SmAB motoneurons (90% in both cases; see Fig. 5*A*). The mean size of the crossed IPSPs after spinal section and 8-OH-DPAT administration was not as large as the size of the IPSPs before spinal section (Fig. 5*A*), but the difference is not statistically significant (Student's t test). A similar decrease in the mean size of the crossed IPSPs in SmAB motoneurons, from -1.09 ± 0.27 to -0.63 ± 0.15 mV, also is not statistically significant. After 8-OH-DPAT administration, the late EPSPs that had appeared after spinal section were not seen. These changes in crossed IPSPs were not accompanied by any significant changes in the mean size of the IPSPs evoked from the ipsilateral quadriceps group II afferents (Student's t test).

In sampling from different motoneurons, the time course of action of 8-OH-DPAT cannot be determined precisely, but IPSPs were seen within 5–10 min of administration of the drug in most experiments and within 20 min in nearly all motoneurons. In a few instances (n = 4), we were able to maintain intracellular recordings from a single motoneuron during delivery and for a few minutes after 8-OH-DPAT was given. The time course for restoration of the crossed IPSPs in one of these instances is illustrated in Figure 6*A*.

Finally, to provide further evidence that the actions of 8-OH-DPAT were exerted at 5-HT_{1A} receptors, we administered the selective 5-HT_{1A} receptor antagonist WAY-100135. In all four experiments in which it was given, WAY-100135 (0.7–3.7 mg/kg, i.v.) antagonized the effects of 8-OH-DPAT and thus reduced the incidence of the crossed group II IPSPs (see Fig. 5*B*). As after spinal section, abolition of crossed IPSPs was accompanied by the appearance of later crossed EPSPs, in line with the crossed extension reflex. This was particularly dramatic in one experiment from which the records of Figure 4 were taken. This effect of WAY-100135 was not attributable to some global change in the membrane properties of motoneurons, because the IPSPs evoked in the same GS and SmAB motoneurons from ipsilateral quadriceps group II afferents were not altered significantly when compared with pre-experimental controls (Student's t test). The time course of action of WAY-100135 was estimated from the recovery of crossed IPSPs in four motoneurons in which the recordings were stable for 1–3 min after drug administration. An example is shown in Figure 6*B*.

Stimulation of the ipsilateral and contralateral sartorius nerve

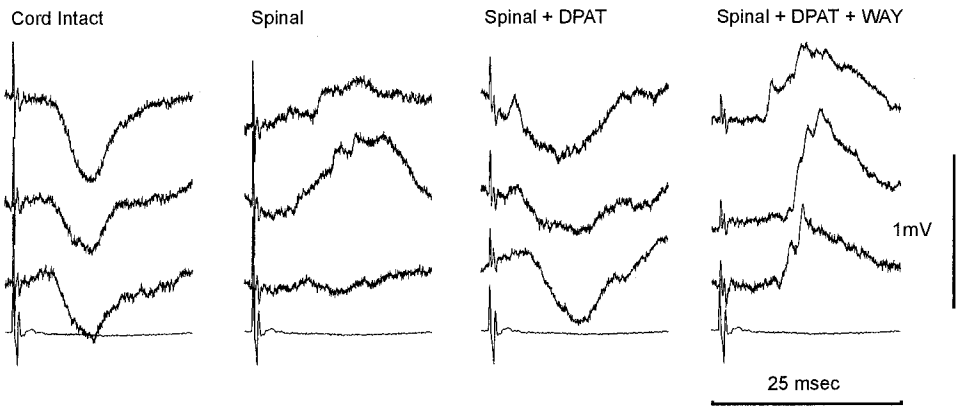

Figure 4. Effects on crossed IPSPs of spinal section, administration of 8-OH-DPAT, and WAY-100135. Averaged intracellular recordings from 12 GS motoneurons are shown, and in each case the response is stimulation of the contralateral quadriceps nerve at 5T. In each column, the *top three* recordings are from three different GS motoneurons, the *bottom trace* being the cord dorsum record. With the spinal cord intact, IPSPs are evoked. After spinal section, the IPSPs are abolished and in some cases EPSPs are evoked. Administration of 8-OH-DPAT restores the crossed IPSPs in the majority of extensor motoneurons. After subsequent administration of WAY-100135, no IPSPs were evoked, but in this experiment prominent EPSPs were evoked.

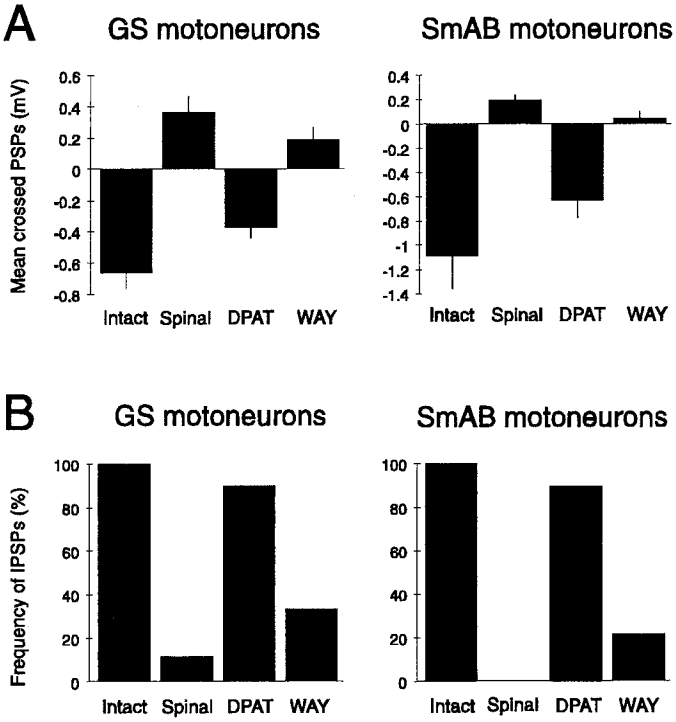

Figure 5. Histograms comparing the effects of 5-HT_{1A}-specific pharmacological agents on crossed inhibition. *A*, Sizes of EPSPs and IPSPs evoked from contralateral quadriceps group II afferents in GS and SmAB motoneurons under different conditions. After spinal section, the crossed IPSPs are abolished and crossed EPSPs are evoked. After 8-OH-DPAT administration, the IPSPs are restored, although they are smaller. In SmAB motoneurons, IPSPs after spinalization and 8-OH-DPAT administration were not significantly different than with the cord intact (Student's t test). In GS motoneurons, IPSPs after 8-OH-DPAT administration were significantly smaller than in the intact cord at $p < 0.05$ but not $p < 0.01$ (Student's t test). After WAY-100135 administration, EPSPs were evoked again. The IPSPs evoked by ipsilateral group II afferents were smaller after spinal section or drug treatment, but not significantly (Student's t test). *B*, Histograms showing the frequency of occurrence of crossed IPSPs in GS and SmAB motoneurons under different conditions in the same experiments as in *A*. Each histogram shows the percentage of neurons with crossed EPSPs with the spinal cord intact, after spinal section, after spinal section and 8-OH-DPAT administration, and after spinal section and 8-OH-DPAT and WAY-100135 administration. In a large proportion of neurons, 8-OH-DPAT restored the crossed IPSPs and WAY-100135 abolished them.

in the course of these experiments also resulted in similar patterns of IPSPs in extensor hindlimb motoneurons. Sartorius IPSPs were smaller in amplitude and less common than those evoked from quadriceps. The crossed IPSPs often were replaced by EPSPs after spinal transection, were restored with 8-OH-DPAT, and were abolished once again with WAY-100135, as in the IPSPs evoked from quadriceps.

DISCUSSION

These experiments provide clear evidence that a pathway descending in the dorsolateral funiculi of the spinal cord controls the operation of crossed group II IPSPs in extensor motoneurons. In addition, we provide evidence that activation of 5-HT_{1A} receptors mimics the activation of the descending pathway, and subsequent antagonism of these receptors mimics the action of spinal section. The simplest explanation of these findings is that a serotonergic pathway descending via the DLF enables the expression of crossed group II inhibition. Although there is no doubt that serotonin can influence spinal reflex pathways powerfully, monoamines are believed to exert a generally facilitatory or depressive action, often referred to as "gain setting" (for discussion, see Hultborn and Illert, 1992; Kuypers, 1982). This idea probably arises from observations of the behavioral consequences of administration of serotonin and the hope that all of the actions of serotonin might form part of a simple, coherent system (Jacobs and Formal, 1993).

The evidence from the current experiments is that, depending on the operation of the descending pathway, different spinal reflex pathways may be open. Thus, with the dorsolateral funiculi intact or after administration of 8-OH-DPAT, a fast crossed pathway from contralateral group II afferents inhibits motoneurons. With the descending pathway transected or with the actions of 8-OH-DPAT antagonized with WAY-100135, the same contralateral afferent input activates a pathway that evokes excitation in extensors (via a longer central pathway).

Our contention that the descending pathway is serotonergic depends on the selectivity of the drugs we have used. It is generally agreed that 8-OH-DPAT has good selectivity at 5-HT_{1A} receptors (Hoyer et al., 1994), but this view has become complicated by recent reports that 8-OH-DPAT has affinity for the recently described 5-HT_7 receptors (Ruat et al., 1993). WAY-100135 is established as a selective antagonist at 5-HT_{1A} receptors (Fletcher et al., 1993), but to our knowledge its actions at 5-HT_7 receptors have not been examined. Although we cannot differen-

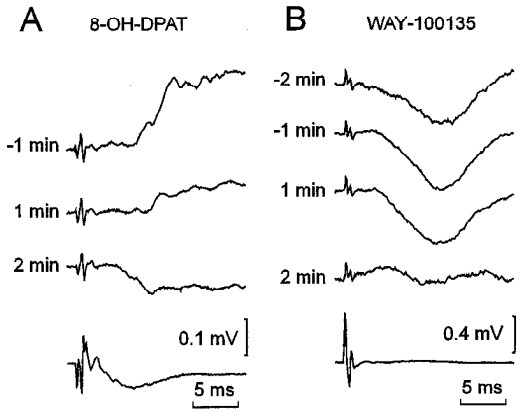

Figure 6. Time course of the actions of 8-OH-DPAT and WAY-100135 in individual motoneurons. *A*, Averaged intracellular responses of a GS motoneuron to stimulation of contralateral group II afferents. 8-OH-DPAT (0.2 mg/kg) was given at time 0. In this case, an EPSP was evoked with the spinal cord sectioned but before 8-OH-DPAT administration. One minute after administration the EPSP declined, and by 2 min an IPSP was beginning to appear. In this case, the recording quality declined after 3 min. *B*, Changes in the responses of a GS motoneuron after WAY-100135 (1.5 mg/kg) administration. Averaged intracellular responses show consistent IPSPs (before the recording, the spinal cord had been sectioned and 8-OH-DPAT had been given). Within 2 min after administration of WAY-100135, the crossed IPSPs had declined substantially; the recording conditions deteriorated after 3 min in this case.

tiate between actions at different receptor subtypes, the selectivity of these drugs for 5-HT receptors supports the involvement of a serotonergic system.

Where does 5-HT act?

The crossed IPSPs are evoked via a direct pathway, most likely disynaptically. The short central latency of the IPSPs and the fact that they are mediated via the midlumbar segments of the cord leave time only for a single intervening neuron (Arya et al., 1991; Aggelopoulos and Edgley, 1995). Candidate neurons that might mediate this effect are found in the deep parts of the intermediate zone (Jankowska and Noga, 1990). Given the simplicity of the central pathway with a single intervening neuron, there is a limited number of sites at which the control might be exerted: afferent terminals, interneurons, presynaptic terminals of the interneurons, or on motoneurons themselves. The control may not be exerted directly by serotonergic axons but, rather, might involve intermediary neurons.

Central pathways from group II afferents have been shown previously to be modulated by monoamines at the level of the afferent terminals. Focal monosynaptic potentials evoked from group II afferents in the dorsal horn of the midlumbar segments are effectively depressed by iontophoretic application of 8-OH-DPAT (Bras et al., 1991). Because these are monosynaptic potentials, the simplest explanation is that there is a serotonergic presynaptic control over the afferent terminals mediated by 5-HT_{1A} receptors. However, field potentials in the intermediate zone generated by collaterals of the same afferents are not affected by 8-OH-DPAT, but are reduced by application of adrenergic α_2 agonists (Bras et al., 1991). It is in this latter region in the intermediate zone that the neurons mediating the crossed IPSPs are likely to be located (Jankowska and Noga, 1990). It is unlikely that the effects seen in the current experiments were mediated directly via actions on the afferent terminals: our evidence suggests that the crossed IPSPs are expressed only when 5-HT_{1A} receptors in the cord are activated, but these receptors inhibit the actions of group II afferent terminals in the dorsal horn and have no detectable actions on the terminals of group II afferents in the intermediate zone. It is also unlikely that the motoneurons are the site of control, because the IPSPs evoked from ipsilateral group II afferents were not changed significantly by transection of the descending pathways or by manipulation of 5-HT_{1A} receptors.

We have no direct data on the potential actions of serotonin on the intervening neurons or at their terminals. However, there is evidence for a direct presynaptic action of serotonin on the synaptic terminals of spinal interneurons on motoneurons—in *Xenopus* embryos [on the terminals of Rohon–Beard cells (Sillar and Simmers, 1994)]. We have also found evidence recently for presynaptic depolarization at the terminals of midlumbar interneurons within the motoneuron pool, suggesting that presynaptic inhibitory processes operate at the terminals of midlumbar interneurons (Aggelopoulos et al., 1995). At present, we have not related this process to the actions of serotonin.

The depressive actions of 5-HT_{1A} receptors on group II-evoked field potentials in the dorsal horn of the midlumbar segments may be related to the alterations in crossed reflex actions indirectly. One action of the dorsal horn neurons is to generate presynaptic inhibition at group II afferent terminals (Jankowska and Riddell, 1995). If this were generated at the terminals of group II afferents on the interneurons that mediate the crossed IPSPs in motoneurons, or at the terminals of the interneurons, it may explain our observations. We might speculate that, with the cord intact or with 5-HT_{1A} receptors activated, strong presynaptic inhibition allows activity in the pathway mediating crossed IPSPs but not in the pathway mediating crossed extension. Removal of this inhibition (spinal transection or administration of WAY-100135) may reverse this situation.

Dorsal reticulospinal pathway

Obvious questions have been raised. What is the functional role of the crossed group II inhibition? What is the significance of the descending control? The present experiments demonstrate connectivity, rather than function. Nevertheless, we can speculate that the reflex pattern seen with the spinal cord intact is likely to promote bilaterally symmetrical actions in extensors, whereas with the descending control removed the pattern is more appropriate to reciprocal actions on the two sides. In this context, the descending pathway seems to have a role in selecting a specific reflex pattern. In parallel, but opposite, to the modifications of the pathway mediating crossed IPSPs are actions on a pathway mediating later crossed EPSPs in extensors (the EPSPs appeared during spinal section and when the actions of 8-OH-DPAT were antagonized). This latter observation is in keeping with the actions of a "dorsal reticulospinal system" that mediates tonic suppression of spinal reflexes by acting on interneurons, as described by Engberg et al. (1968). The existence of such a system descending in the dorsolateral funiculi was documented first by Holmquist and Lundberg (1959) (see also Baldissera et al., 1981; Lundberg, 1982). Subsequently, Engberg et al. (1968) demonstrated that this system could be activated by stimulation in the ventrolateral medulla.

At the time of the initial observations, the dorsal reticulospinal system was thought not to be serotonergic for two reasons: that the descending fibers were rapidly conducting (>20 m/sec), and that the effects were not mimicked by administration of the serotonin precursor 5-hydroxytryptophan (Engberg et al., 1968; Lundberg, 1982). It has been shown subsequently that some

serotonergic fibers run in the DLF and are myelinated (Westlund et al., 1992); thus, they can be relatively fast-conducting. Furthermore, a plethora of serotonin receptor subtypes exists (Hoyer et al., 1994). Administration of a nonspecific agonist such as 5-hydroxytryptophan will activate simultaneously several receptor subtypes and have unpredictable, sometimes opposing actions at different sites in a reflex pathway. These include potent actions directly on the motoneurons (Hounsgaard et al., 1988). Thus, it is a possibility that a system of fast-conducting serotonergic fibers acting via 5-HT_{1A}, or possibly 5-HT_7, receptors is responsible for the reflex suppression by the dorsal reticulospinal system. The same system, when active, not only would suppress certain reflexes (e.g., crossed extension) but would facilitate others (crossed group II inhibition of extensors).

The observations in this paper support the possibility that there exists a system of serotonergic fibers descending in the dorsolateral funiculus that operate via a specific set of 5-HT receptors that can select between two alternative crossed reflex pathways. The possibility that this system is rapidly conducting is intriguing, because it suggests that a degree of temporal specificity is also a requirement of its operation.

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