Disynaptic Pyramidal Excitation in Forelimb Motoneurons Mediated Via C₃–C₄ Propriospinal Neurons in the Macaca fuscata

B. Alstermark, T. Isa, Y. Ohki, and Y. Saito. Disynaptic pyramidal excitation in forelimb motoneurons mediated via C₃–C₄ propriospinal neurons in the Macaca fuscata. J. Neurophysiol. 82: 3580–3585, 1999. In contrast to findings in the cat, it recently has been shown that disynaptic pyramidal EPSPs only rarely are observed in forelimb motoneurons of the macaque monkey in the intact spinal cord or after a corticospinal transaction in C₃. This finding has been taken to indicate that the disynaptic pyramidal excitatory pathway via C₃–C₄ propriospinal neurons (PNs) is weakened through phylogeny when the monosynaptic cortico-motoneuronal connection has been strengthened. We reinvestigate this issue with special focus on the possibility that the inhibitory control of the C₃–C₄ PNs may be stronger in the macaque monkey than in the cat. The effect in forelimb motoneurons of electrical stimulation in the contralateral pyramid was investigated in anesthetized macaque monkeys (Macaca fuscata). We confirmed the low frequency of disynaptic pyramidal EPSPs in forelimb motoneurons. However, after intravenous injection of strychnine, disynaptic EPSPs could be evoked in 39 of 41 forelimb motoneurons recorded after lesion of the corticospinal fibers in C₅. After a corresponding lesion in C₂, disynaptic pyramidal EPSPs were observed in 2 of 25 motoneurons. In contrast to previous reports, we conclude that C₃–C₄ PNs can mediate disynaptic pyramidal excitation in high frequency of occurrence to forelimb motoneurons in the C₆–C₈ segments and that this transmission is under a stronger inhibitory control than in the cat. Thus, the hypothesis that the disynaptic excitatory cortico-motoneuronal pathway via the C₃–C₄ PNs is weakened in parallel with the strengthened monosynaptic connection through phylogeny is not supported by the present findings.

I N T R O D U C T I O N

In the cat, Illert et al. (1977) first demonstrated that disynaptic pyramidal excitation in forelimb motoneurons can be mediated via spinal interneurons with cell bodies located in the C₃–C₄ segments (cf. also Alstermark and Sasaki 1985; Illert and Wiedemann 1984). This premotoneuronal system was denoted the C₃–C₄ propriospinal system and it has since been analyzed in detail (cf. Alstermark and Lundberg 1992). In behavioral studies using either selective spinal cord lesions or transneuronal labeling, it was shown that C₃–C₄ propriospinal neurons (PNs) can mediate the descending command for forelimb target reaching (Alstermark and Kümmel 1990; Alstermark et al. 1981). So far the C₃–C₄ propriospinal system is unique because it is the only example of a command mediating interneuronal system in the spinal cord for voluntary movements.

In primates, interest has focused on the monosynaptic corticospinal pathway to motoneurons, which is lacking in the cat (cf. Phillips and Porter 1977). Therefore it was of considerable interest when non-monsynaptic excitation was first demonstrated in man (Baldissera and Pierrot-Deseilligny 1989; Malmgren and Pierrot-Deseilligny 1988). However, the interpretation by Gracies et al. (1994) that disynaptic pyramidal excitation can be mediated by PNs located rostral to the forelimb segments has been criticized strongly by Maier et al. (1998). In the macaque monkey, Maier et al. (1998) found that electrical stimulation in the contralateral pyramid evoked disynaptic excitatory postsynaptic potentials (pyramidal EPSPs) only rarely compared with the cat. They concluded that their “results provide little evidence for significant corticospinal excitation of motoneurons via a system of C₃–C₄ propriospinal neurons in the monkey.”

It is interesting that Bortoff and Strick (1993), who studied the corticospinal termination in the Cebus monkey (which is phylogenetically more advanced than the macaque monkey) found a heavy termination laterally in Rexed laminae VI–VII in the C₃–C₄ segments exactly where the C₃–C₄ PNs are located in the cat (Illert et al. 1978). Bortoff and Strick (1993) carefully stated that “Although as much as 30% of the corticospinal input terminates in the ventral horn of the Cebus monkey, it is clear that the majority of corticospinal terminations are located within the intermediate zone of the spinal cord. . . . Thus, the importance of corticospinal projections to interneurons should not be underemphasized.”

We have reinvestigated whether disynaptic pyramidal excitation in forelimb motoneurons can be mediated via C₃–C₄ PNs in the macaque monkey. It will be shown that this interneuronal system also exists in primates and that the inhibitory control of the C₃–C₄ PNs is stronger than in the cat.

M E T H O D S

Preparation

Experiments were performed on four macaque monkeys (Macaca fuscata) with body weights 3–5 kg. The animals were sedated with 0.1 ml/kg Xylazine followed by initial anesthesia with 0.2 ml/kg Ketamine, 0.5–1.0% halothane during surgery, and later up to 100 mg/kg α-chloralose. The anesthesia was regularly supplemented with small doses of ~0.5 mg/kg pentobarbital sodium (Nembutal) to maintain a stable anesthetic depth (regular respiration and blood pressure ~80 mmHg). During recording, pancuronium bromide (Myoblock) was

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introduced and artificial respiration started. Respiratory rate was adjusted to keep pCO₂ ~3.8%. In addition, i.v. injections with Ringer-glucose including lactate, atropine, and prednisolon were given to maintain a good general condition of the animals. Rectal temperature was maintained at ~37°C. The deep radial (DR), median (Med), and ulnar (Uln) nerves were dissected and mounted on silver electrodes or burried electrodes for stimulation. Laminitectomy was performed to expose the spinal cord segments C₂–T₁. Craniotomy was performed to place the pyramidal electrode. The experiments were approved by the ethical committee of the National Institute for the Physiological Sciences, Japan and were performed in accordance with the NIH guideline for the Care and Use of Laboratory Animals.

Recording and stimulation

In this study we focused on the pyramidal excitation in forelimb motoneurons in the C₆–C₈ segments. Of the 85 motoneurons recorded, 35 were DR, 8 Med, 1 Uln, and 41 unidentified. The antidromically identified motoneurons had spike amplitudes 60–100 mV. Unidentified motoneurons with low input resistance <2 MΩ and spike heights of ≥60 mV during depolarization were found in the vicinity of the DR, Med, and Uln motor nuclei defined by the antidromic field potentials. Furthermore, some unidentified motoneurons received heteronymous Ia EPSPs from the DR, Med, or Uln nerves.

The effect of electrical stimulation of the corticospinal fibers in the contralateral pyramid was investigated by intracellular recordings from 19 forelimb motoneurons (5 DR and 14 unidentified) in the intact spinal cord, 41 (21 DR, 2 Med, and 18 unidentified) after a C₅ lesion (cf. below), and 25 motoneurons (9 DR, 6 Med, 1 Uln, and 9 unidentified) after a C₂ lesion (cf. below). Intracellular recordings were made with sharp glass microelectrodes with tip diameters of 1–2 μm filled with 2 M K-citrate with impedances 3–5 MΩ. Cord dorsum recordings were made with a silver ball electrode at the dorsal root entry zone in the same segment as the intracellular recordings. The corticospinal axons were stimulated electrically in the contralateral pyramid, at the medullary level, using cathodal pulses (0.2 ms duration; 100–200 μA strength).

Strychnine injection

Intravenous injections of strychnine (1 mg/ml) were regularly administered during intracellular recordings; the standard dose was 0.1 mg/kg. Approximately twenty injections of strychnine were made during one experiment that lasted ~18–20 h. The general condition of the animal remained good although the blood pressure increased transiently for ~2 min after a single injection.

Spinal cord lesions

The corticospinal lesion was evaluated by recording the descending pyramidal volley with a cord dorsum electrode (cf. Illert et al. 1977). The direct pyramidal volley can be seen in Fig. 2B1. After the C₅ lesion of the dorsal part of the lateral funiculus, the major part of the negativity of the pyramidal volley was abolished (Fig. 2B2). The histological control is shown in Fig. 2A. We intentionally spared the ventral part of the lateral funiculus because the axons of the C₃–C₄ PNs are known to be located in this region in the cat (Illert et al. 1978). Some corticospinal fibers with direct projection to motoneurons are located in this region (Bortoff and Strick 1993). Note that part of the synaptic volley (Fig. 2B1, arrow) remained after the C₅ lesion (B2). It is likely that part of this synaptic volley is due to activation of the C₃–C₄ PNs (cf. discussion). The C₅ lesion was made in the same manner as the C₃ lesion and is illustrated in Fig. 3A. The direct pyramidal volley (Fig. 3B1) was completely abolished (Fig. 3B2). This lesion was made in the monkeys that had the C₅ lesion and after repeated strychnine injections. Measurements of segmental latencies were made from the direct pyramidal volley recorded before lesions.

RESULTS

Intact spinal cord before and after strychnine injections

Figure 1A shows that stimulation in the contralateral pyramidal evoked monosynaptic EPSPs that were followed by IPSPs. Monosynaptic EPSPs were found in 16 (84%) of 19 motoneurons (Fig. 1H, open bars). These observations confirm the results from previous investigations of the monkey and baboon by Landgren et al. (1962), Shapovalov (1975), Fritz et al. (1985), Jankowska et al. (1976) and Maier et al. (1998).

In order to test whether the low frequency of disynaptic and oligosynaptic pyramidal EPSPs was caused by inhibition at a premotoneuronal level, we systematically gave intravenous injections of strychnine (cf. METHODS). Figure 1B shows the effect 40 s after the strychnine injection. Note that the monosynaptic pyramidal EPSPs appeared without the addition of di- and oligosynaptic EPSPs. Thus, removing glycinergic inhibition at the motoneuronal level did not reveal di- or oligosynaptic EPSPs. This finding shows that lack of di- and oligosynaptic pyramidal EPSPs was not caused by an underlying inhibition in forelimb motoneurons. However, 120 s after the strychnine injection, di- and oligosynaptic EPSPs appeared along with the monosynaptic EPSPs (Fig. 1C). The result of averaging the recordings in Fig. 1, B and C is illustrated in Fig. 1D1 and the subtracted average Fig. 1, C minus B is shown in Fig. 1D2. Note that the segmental latency was in the disynaptic range (1.6 ms; D₂, arrow). Temporal facilitation was necessary to elicit di- and oligosynaptic pyramidal EPSPs as shown in the sequential recordings (Fig. 1, E–G).

Recordings from another forelimb motoneuron before and after strychnine injection were illustrated in Fig. 1, I–L. Mainly monosynaptic pyramidal EPSPs were found before the strychnine injection (Fig. 1J). However, 60 s after the injection oligosynaptic EPSPs components were evoked (Fig. 1J). The quite pronounced oligosynaptic pyramidal EPSPs compared with the monosynaptic EPSPs are shown with a slower sweep speed (Fig. 1K). We also observed a recovery from the effect of strychnine (Fig. 1L). In this case, the pyramidal EPSPs were reduced after 3 min. However, such clear recovery was only apparent after the first few injections, thereafter there was an accumulating effect of strychnine.

No oligosynaptic pyramidal EPSPs were found in the seven forelimb motoneurons that were recorded before the strychnine injection. This result is in agreement with that of Maier et al. (1998) who found oligosynaptic EPSPs only in a small percentage of the motoneurons. However, after strychnine injections oligosynaptic pyramidal EPSPs were observed in all 15 tested cells. In nine of these motoneurons the EPSPs were within a disynaptic range (1.2–1.8 ms) and the remaining six EPSPs were either tri- or polysynaptic in nature (Fig. 1H, closed bars).

Note the synaptic volley (marked by arrows) that appeared after direct corticospinal volley (after 3rd and 4th stimuli; Fig. 1, A–C and I–L). This synaptic volley was enhanced after strychnine injection (Fig. 1, C and J) and declined after recovery (Fig. 1L).

Segmental location of interneurons mediating di- and oligosynaptic pyramidal EPSPs

In order to test whether the disynaptic pyramidal EPSPs can be mediated via C₃–C₄ PNs as in the cat (Illert et al. 1977), we
first transected the corticospinal tract at the C₄/C₅ segmental border, denoted as C₅ lesion (cf. METHODS).

**Figure 2**, C–F illustrates a motoneuron recorded after the C₅ lesion. Eleven strychnine injections were given before recording this motoneuron. As seen in Fig. 2C, already a single pyramidal stimulus evoked small EPSPs which were markedly facilitated by the second (Fig. 2D) and third (Fig. 2E) pyramidal stimuli. The segmental latency of this pyramidal EPSP was 1.3–1.4 ms as observed in the expanded records taken with higher sweep speed in Fig. 2F. The result suggests a strong disynaptic linkage from the corticospinal tract.

Figure 2G shows that the effects from the contralateral pyramid were not due to current spread. The stimulating electrode was positioned somewhat laterally in the pyramid to avoid spread to the ipsilateral pyramid (cf. Illert et al. 1977). Disynaptic pyramidal EPSPs were evoked when the stimulating electrode was in the contralateral pyramid (Fig. 2, G1 and G2) but disappeared just above it (Fig. 2, G3 and G4). Thus the disynaptic and oligosynaptic pyramidal EPSPs found in the forelimb motoneurons were the result of stimulation of corticospinal axons in the contralateral pyramid. However, more dorsally in the most ventral part of the reticular formation, another oligosynaptic EPSP appeared in records (Fig. 2, G5 and G6). These EPSPs may be mediated via bulbospinal neurons and/or spinal interneurons.

Our findings with pyramidal stimulation after a C₅ lesion are in agreement with the findings by Maier et al. (1998) that disynaptic and oligosynaptic pyramidal EPSPs found in the forelimb motoneurons were the result of stimulation of corticospinal axons in the contralateral pyramid. However, more dorsally in the most ventral part of the reticular formation, another oligosynaptic EPSP appeared in records (Fig. 2, G5 and G6). These EPSPs may be mediated via bulbospinal neurons and/or spinal interneurons.
tonurons, the pyramidal EPSPs were within a disynaptic range (Fig. 2H). We found three motoneurons with remaining monosynaptic pyramidal EPSPs in one experiment (Fig. 2H, open bars) when the lesion did not extend into the ventrolateral part of the lateral funiculus (Fig. 2A). In the experiments with more ventrolateral extension of the C5 lesion, we found no monosynaptic pyramidal EPSPs, but di- and oligosynaptic EPSPs could still be evoked.

The second step in localizing the intercalated neurons mediating the di- and oligosynaptic pyramidal EPSPs was to transect the corticospinal axons at the C3/C4 segmental border (cf. METHODS) as was done in the cat (Illert et al. 1977). After complete transection of the corticospinal axons in C2 as shown for the pyramidal volley in Fig. 3B and the histology in Fig. 3A, we observed disynaptic pyramidal EPSPs in 2 (8%) of 25 motoneurons and EPSPs with segmental latencies longer than 2.5 ms in 11 (44%) cells as shown in Fig. 3G. All motoneurons after the C2 corticospinal lesion were recorded after repeated strychnine injections. The results from one of the recorded forelimb motoneurons, after the C2 lesion, is illustrated in Fig. 3, C–F taken at fast (Fig. 3, C1–F1) and slow (Fig. 3, C2–F2) sweep speeds. Note the absence of short latency pyramidal EPSPs and the presence of oligosynaptic EPSPs with latencies about 4–5 ms (Fig. 3F1, arrow). These pyramidal EPSPs were followed by a mixture of late EPSPs and IPSPs, which probably reflects a high excitability after repeated strychnine injections.

From these results we conclude that disynaptic pyramidal EPSPs in forelimb motoneurons can be effectively mediated via C3–C4 PNs in the Macaca fuscata provided that inhibition of this premotoneuronal system is reduced. However, we tentatively propose that disynaptic pyramidal EPSPs can sometimes be mediated via bulbospinal neurons projecting directly to forelimb motoneurons.

**DISCUSSION**

Our results leave no doubt that disynaptic pyramidal EPSPs in forelimb motoneurons can be mediated via C3–C4 PNs after the C5 corticospinal lesion and after strychnine injection. We
cannot exclude the possibility that strychnine removes tonic inhibition of the C3–C4 PNs, but it seems more likely that the appearance of EPSPs via the C3–C4 PNs is the result of the reduction of pyramidal IPSPs in them. In cats, disynaptic IPSPs are evoked in the C3–C4 PNs via two different pathways: 1) the feed-forward pathway which is excited from the same descending pathways as the C3–C4 PNs (Alstermark et al. 1984a) and 2) the feed-back pathway which is activated from forelimb afferents and the corticospinal tract (Alstermark et al. 1984b). The effect of strychnine may be due to removal of IPSPs from either or both of these pathways. It is tempting to suggest that the stronger inhibition in primates compared with that in cats should be ascribed to the feedforward pathway and that it reflects the need for a more focused excitatory control of the C3–C4 PNs in the primates. However, we can by no means exclude that the feedback inhibitory pathway is also involved.

The axonal location of the C3–C4 PNs in the macaque monkey is not known. We have assumed that they at least partly have a similar location in the ventral part of the lateral funiculus as has been shown in the cat (Illert et al. 1978). However, because we observed a reduction of the synaptic pyramidal volley (Fig. 2B2), presumably mediated via the C3–C4 PNs, it cannot be excluded that some of the propriospinal axons in the macaque monkey have a somewhat more dorsal location than the cat. Our findings are in contradiction with those of Maier et al. (1998) who did not observe a propriospinal volley after a C3 corticospinal transection.

One possible difference is that the C3–C4 PNs were strongly inhibited in the study by Maier et al. (1998) and thus could not mediate the synaptic pyramidal volley. Further investigation is necessary to find the detailed axonal location of the C3–C4 PNs with projection to forelimb motoneurons.

Let us consider the general hypothesis regarding a phylogenetical replacement of the C3–C4 propriospinal system by monosynaptic cortico-motoneuronal excitation (Lemon 1999; Maier et al. 1996 and 1998). Their common theme is that as the monosynaptic cortico-motoneuronal excitation gradually becomes stronger through phylogenesis because of increased need for control of dextrous finger movements, the phylogenetically older C3–C4 propriospinal system should become weakened and vanish in higher monkeys and man. The evidence is not only the findings by Maier et al. (1998), which we now have explained, but also that of Maier et al. (1997) and Nakajima et al. (1999) who found that disynaptic pyramidal EPSPs are more frequent in the squirrel monkey, which has less advanced finger movements compared with the macaque monkey. Maier et al. (1998) conclude that these EPSPs are mediated via C3–C4 PNs even if they have not made the necessary C2 lesion.

Furthermore, Lemon (1999) suggested that “the positive correlation across species between more advanced hand function and the strength of the cortico-motoneuronal system is accompanied by a negative correlation between this function and the strength of the PN system.” This is a surprising hypothesis because the behavioral work on the C3–C4 propriospinal system has shown that it can mediate the command for target reaching and is not conveying the command for food-taking which involves digit movements, wrist flexion, and supination. Therefore it is not logical that this particular system would need to be replaced with the monosynaptic cortico-motoneuronal connections, which, as is generally accepted, are of special importance for dextrous finger movements.

Recently, Lemon (1999) suggested that the studies by Pierrot-Deseilligny (1996) on a propriospinal system in man “may need to be reevaluated to obtain a better understanding of corticospinal control in man.” Because our results demonstrate the existence of C3–C4 PNs in primates, the objective by Lemon (1999) regarding Pierrot-Deseilligny’s conclusion is
removed. Thus, it now seems that the need for such a reevaluation is not so urgent.

We thank Prof. Anders Lundberg and Dr. Lars-Gunnar Pettersson for constructive criticism on the first version of this paper. Furthermore, we thank Dr. H. Aizawa for valuable discussion and M. Seo, C. Suzuki, Y. Takeshima, and J. Yamamoto for technical assistance.

This work was supported by Grants 08458266, 08279207, and 09268238 from the Ministry of Education, Science, Sports and Culture of Japan, as well as grants from CREST of the Japan Science and Technology Corporation, the Daiko Foundation, the Naito Memorial Foundation, and the Mitsubishi Foundation to T. Isa.

Address reprint requests to B. Alstermark.

Received 13 July 1999; accepted in final form 7 September 1999.

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