Properties of Propriospinal Neurons in the C3–C4 Segments Mediating Disynaptic Pyramidal Excitation to Forelimb Motoneurons in the Macaque Monkey

Tadashi Isa,1,4,* Yukari Ohki,2 Kazuhiko Seki,1 and Bror Alstermark3,*

1Department of Developmental Physiology, National Institute for Physiological Sciences, Okazaki, Japan; 2Department of Physiology, Kyorin University Medical School, Tokyo, Japan; 3Department of Integrative Medical Biology, Section of Physiology, Umeå University, Umeå, Sweden; and 4Core Research for Evolutionary Science and Technology of Japan Science and Technology Agency, Kawaguchi, Japan

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Isa, Tadashi, Yukari Ohki, Kazuhiko Seki, and Bror Alstermark. Properties of propriospinal neurons in the C3–C4 segments mediating disynaptic pyramidal excitation to forelimb motoneurons in the macaque monkey. J Neurophysiol 95: 3674–3685, 2006. First published February 22, 2006; doi:10.1152/jn.00103.2005. Candidate propriospinal neurons (PNs) that mediate disynaptic pyramidal excitation to forelimb motoneurons were studied in the C3–C4 segments in anesthetized macaque monkeys (n = 10). A total of 177 neurons were recorded (145 extracellularly, 14 intracellularly, and 1 both) in laminae VI–VII. Among these, 86 neurons (73 extracellularly, 14 intracellularly, and 1 both) were antidromically activated from the forelimb motor nucleus or from the ventrolateral funiculus just lateral to the motor nucleus in the C3/C4 segments and thus are identified as PNs. Among the 73 extracellularly recorded PNs, 60 cells were fired from a train of four stimuli to the contralateral pyramidal segment with segmental latencies of 0.8–2.2 ms, with most of them (n = 52) in a monosynaptic range (<1.4 ms including one synaptic delay and time to firing). The firing probability was only 21% from the third pyramidal volley but increased to 83% after intravenous injection of strychnine. In most of the intracellularly recorded PNs, stimulation of the contralateral pyramidal evoked monosynaptic excitatory postsynaptic potentials (EPSPs, 12/14) and disynaptic inhibitory postsynaptic potentials (14/14), which were found to be glycinergic. In contrast, cells that did not project to the C6–Th1 segments where forelimb motoneurons are located were classified as segmental interneurons. These were fired from the third pyramidal volley with a probability of 71% before injection of strychnine. It is proposed that some of these interneurons mediate feed-forward inhibition to the PNs. These results suggest that the C3–C4 PNs receive feedback inhibition from the pyramid in addition to monosynaptic excitation and that this inhibition is stronger in the macaque monkey than in the cat. Another difference with the cat was that only 26 of the 86 PNs (30%, as compared with 84% in the cat) with projection to the forelimb motor nuclei send ascending collaterals terminating in the lateral reticular nucleus (LRN) on the ipsilateral side of the medulla. Thus we identified C3–C4 PNs that could mediate disynaptic pyramidal excitation to forelimb motoneurons in the macaque monkey. The present findings explain why it was difficult in previous studies of the macaque monkey to evoke disynaptic pyramidal excitation via C3–C4 PNs in forelimb motoneurons and why—as compared with the cat—the monosynaptic EPSPs evoked from the LRN via C3–C4 PNs were smaller in amplitude.

INTRODUCTION

It has been established that the corticospinal tract in primates exerts monosynaptic excitation and disynaptic inhibition on spinal motoneurons (MNs) (Fritz et al. 1985; Jankowska et al. 1976; Landgren et al. 1962; Maier et al. 1998; Shapovalov 1975). Anatomical studies have shown that a large number of terminal boutons of corticospinal fibers are actually distributed outside the motor nuclei, mainly in the intermediate layers, laminae VI–VIII (Bortoff and Strick 1993; Ralston and Ralston 1985), where segmental interneurons and propriospinal neurons (PNs) are located. However, the functional significance of corticospinal connections to many of these spinal cord interneuronal systems has not been clarified in the monkey. In the cat, the shortest corticomotoneuronal pathway is disynaptic (Ilbert et al. 1976). It has been shown that a substantial portion of the disynaptic pyramidal effect on forelimb MNs is mediated by PNs with cell bodies located in the C3–C4 segments which are denoted as “C3–C4 PNs” (Alstermark and Sasaki 1985; Ilbert and Wiedemann 1984; Illert et al. 1977, 1978) as well as segmental interneurons located in the same segments as the MNs (C6–Th1) (Alstermark and Sasaki 1985; Illert and Wiedemann 1984; Kitazawa et al. 1993). The cell bodies of the C3–C4 PNs are located mainly in laminae VI–VII of the C3–C4 segments and mediate convergent inputs from several descending tracts such as the corticospinal, rubrospinal, tectospinal, and reticulospinal tracts to forelimb MNs via axons passing through the ventral portion of the lateral funiculus (Illert et al. 1978). In addition, most of the C3–C4 PNs have ascending collaterals to the lateral reticular nucleus (LRN) in the medulla, which may send internal feedback signals to the cerebellum via mossy fibers (Alstermark et al. 1981a; Illert and Lundberg 1978). Based on behavioral studies using either selective spinal cord lesions or activity-dependent retrograde transneuronal labeling techniques, it has been shown that the C3–C4 PNs can mediate descending commands for forelimb reaching movements in cats (Alstermark and Kümmer 1990; Alstermark et al. 1981b). In man, the existence of disynaptic excitatory corticomotoneuronal pathway mediated via the propriospinal neurons has been suggested (Pierrot-Deseilligny 2002). This is based on the demonstration of nonmonosynaptic Ia excitation of wrist flexor motoneurons by facilitation of H reflexes, suggesting that the facilitation was mediated by PNs located rostral to the segments containing the wrist flexor motoneurons (Malmgren and Pierrot-Deseilligny 1988). The facilitation was enhanced at...
the onset of voluntary movements (Baldissera and Pierrot-Deseilligny 1989). Furthermore, apparent disynaptic pyramidal excitation can be mediated by PNs by using transcranial magnetic stimulation (Pauvert et al. 1998). Finally, results from a patient with a lesion of the ventrolateral funiculus in C6/C7 suggested interruption of rostrally located PNs in the cervical cord (Marchand-Pauvert 2001). Earlier studies in the monkey showed that, stimulation of the corticospinal fibers evoked monosynaptic excitatory postsynaptic potentials (EPSPs) and disynaptic inhibitory postsynaptic potentials (IPSPs) in spinal MNs, but no clear evidence for di- or oligosynaptic EPSPs was described (Fritz et al. 1985; Jankowska et al. 1976; Langgren et al. 1962; Shapovalov 1975; cf. Phillips and Porter 1977). Efforts were made to find C3–C4 PNs in the macaque monkey by Lemon and colleagues, but in their studies, disynaptic corticomotoneuronal EPSPs could be found in only a small proportion of MNs (3% in the intact spinal cord). Based on these results, they concluded that the C3–C4 PNs are not playing an important role in primates as in cats (Maier et al. 1998) or have been replaced by monosynaptic corticomotoneuronal connections (Nakajima et al. 2000). Lemon and colleagues also showed the absence of disynaptic corticospinal excitation of forelimb motoneurons by using single motor-unit recordings and transcranial magnetic stimulation (Olivier et al. 2001). To clarify this issue, we performed similar motor-unit recordings and transcranial magnetic stimulation to determine the existence of an ascending projection to the LRN and the axonal location of the descending axons. We present evidence showing that strong feed-forward glycineric inhibition reduces the firing probability of the C3–C4 PNs in response to pyramidal stimulation and that only a minority of the cells have ascending collaterals to the LRN.

METHODS

Experiments were performed in 10 macaque monkeys (8 Macaca fascata and 2 M. mulatta) with body weight ranging from 3.3 to 7.0 kg. The experimental procedures followed the National Institutes of Health Guideline for the Care and Use of Laboratory Animals and were approved by the Committee of Animal Experimentation of the Okazaki National Institutes.

Surgical preparation

The animals were sedated with 1 mg/kg xylazine followed by initial anesthesia with 5–10 mg/kg ketamine. After insertion of tracheal cannula, anesthesia was maintained by 1.0–1.5% isoflurane during surgery. After surgery, anesthesia was switched to intravenous injection of α-chloralose (50–100 mg/kg). During recording, intravenous pancuronium bromide (Mibloc, Organon) and artificial respiration were used. Respiration rate was adapted to keep pCO2 within 3.3–4.2%. The anesthesia was regularly supplemented by doses of 1 mg/kg pentobarbital sodium (Nembutal, Abbott Laboratories) to maintain a stable depth of anesthesia. The adequacy of anesthesia was assessed by reference to blood pressure (stable at 80–100 mmHg), heart rate, and pupillary reflex. In addition, intravenous injections of Ringer glucose including lactate, atropine, and decadrone were given to maintain good general condition of the animals. Rectal temperature was maintained at 36–37°C. The deep radial (DR), median (Med), and ulnar (Ul) nerves were dissected and mounted on silver electrodes or buried electrodes for stimulation. A laminectomy was performed to expose the spinal cord segments C2–Th1, and a caudal craniotomy was performed to place a stimulating electrode in the medullary pyramid and LRN.

Recording and stimulation

In this study, we focused on the effect of pyramidal stimulation in the PNs with the cell bodies in the C3–C4 segments. The experimental arrangement is summarized in Fig. 1F based on the neural circuit that we propose. A tungsten needle electrode for stimulation of the medullary pyramid was placed 1–3 mm rostral to the obex and 1.2 mm lateral to the midline by monitoring cord dorsum potentials evoked by the stimulation through the electrode. The appropriate depth of the electrode was determined when the threshold of the pyramidal volley became <10 μA (cathodal square wave pulse with 0.1-ms duration). For identification of the PNs, a tungsten needle intraspinal stimulating electrode was placed either in the ventral part of the lateral funiculus or in the DR motor nucleus at the C6/C7 segments. All sampled PNs were antidromically activated with current intensity <200 μA. Criteria for identifying antidromic spikes included constant latency, the ability to follow high-frequency stimulation (>100 Hz) and collision with preceding orthodromic or spontaneous spike (Lipski 1981). The location of DR motor nucleus was determined by observing an antidromic field potential after stimulation of the DR nerve. The lateral funiculus stimulating electrode was placed 200 μm lateral to the most lateral track where motoneurons were found. The depth of the electrode was 4.5–5.0 mm from the dorsal surface of the spinal cord. To exclude the long PNs with projections to lumbar segments, bipolar silver ball electrodes were placed on the surface of the left and right lateral sides of the Th3 segment. Neurons that could not be antidromically activated with 5-mA current stimulus at this location were judged as lacking projection to the lumbar segments. In this article, C3–C4 neurons that were antidromically activated from the C6/C7 segments but not from the Th3 will be denoted as “C3–C4 PNs.” Cells in the C3–C4 segments that were not antidromically activated from the C6/C7 segments were classified as segmental interneurons (C3–C4 sINs).

To determine whether C3–C4 PNs possessed ascending collateral projections to the ipsilateral lateral reticular nucleus (LRN), a tungsten needle electrode was placed at 1 mm caudal to the obex, 3.5 mm lateral to the midline, and depth about 5.5 mm, corresponding to the mediodorsal edge of the LRN. The precise position of the LRN electrode was determined so as to induce the largest field potential in the motor nuclei (Alstermark et al. 1981a; Maier et al. 1998).

For recordings from PNs, glass micropipettes containing 2 M potassium citrate (tip diameters: 0.5–1.5 μm, impedance: 3–10 MΩ) were used. In the present study, we also recorded from DR MNs that were identified by antidromic activation from the muscle nerve. We also recorded from unidentified MNs. The unidentified MNs had low input resistance (<2 MΩ) and were found in the vicinity of DR, Med, or Ul motor nuclei defined by the antidromic field potentials. For motoneuron recording, glass micropipettes with the same solution but larger diameters (1.0–2.0 μm, impedance: 2.5–5.0 MΩ) were used. Cord dorsum potentials were recorded with a silver ball electrode placed at the dorsal root entry zone in the same segment as the intracellular or extracellular recording.

Strychnine injection

Intravenous injection of strychnine (1 mg/ml) was regularly administered during intracellular or extracellular recordings. The standard
dose was 0.1 mg/kg. Ten to 20 injections were made during the experiment lasting for 18–20 h. The circulatory and respiratory conditions of the animal remained good, although blood pressure increased transiently for 2 min after strychnine injection. Effects of strychnine included increased blood pressure and high excitability of recorded neurons. The effects of strychnine disappeared within 10 min during the initial part of the experiments but near in the end of the experiment, relatively high excitability persisted in the spinal cord. Accordingly, we tested the effect of strychnine in a limited number of neurons that were recorded in earlier part of the experiments.

Histology

Electrolytic lesions were made by passing DC current (20 μA, 30 s) to mark several critical points of stimulation. Recording glass microelectrodes were left in some of the critical recording tracks. At the termination of the experiment, the animals were killed with Nembutal (75 mg/kg) and perfused with 10% formaline via the arterial line. The brain and spinal cord were processed for Nissl staining to confirm the location of the stimulating and recording electrodes.

RESULTS

A total of 177 neurons were recorded in the C3–C4 segments, of which 145 neurons were recorded extracellularly and 48 neurons intracellularly. Among these, 16 neurons were recorded both ways. Eighty-six neurons were antidromically activated from one of the electrodes placed in the C6 or in C7 segment and identified as C3–C4 PNs. In this study, we did not record from long PNs, which can be antidromically activated from the Th13 segment.

Extracellular recordings from C3-C4 PNs

Figure 1, A–D, shows the records obtained from a C3-C4 PN activated from the electrode placed in the ventrolateral funiculus of the C7 segment. The antidromic spikes are indicated by black arrows in B–D. This neuron could be fired (red trace) from the third pyramidal stimulus (Pyr) in only 1 of 10 trials as shown in Fig. 1A (red arrow). To investigate whether glycinergic feed-forward inhibition exists at the level of the PNs as observed in cats (Alstermark et al. 1984a,b), we tested the effect of intravenous injection of strychnine. To facilitate the
inspection of the traces, the time period after the fourth pyramidal stimulus and the C7 stimulus for antidromic activation in the left records, is expanded on the right in Fig. 1, B and C. It can be seen that no orthodromic spike was observed in B (2 min after injection), whereas the cell started to be orthodromically activated between 2 and 3 min after injection. Then in each case in which the cell fired orthodromically (red traces in C), the antidromic spike failed due to collision of the spikes and/or refractoriness in the soma. This is more clearly illustrated in single trace records in Fig. 1D. The horizontal lines and black dots indicate the synaptic and unitary field potential evoked by the fourth pyramidal stimulus. When the PN was not orthodromically activated from the fourth pyramidal stimulus, the antidromic spike was observed (left black). However, when the cell was orthodromically activated from the 4th pyramidal stimulus (red arrow on the right red) the antidromic spike was collided as shown in the right records in Fig. 1D (see black arrows with bracketed dash). Figure 1E shows a recording from an interneuron before strychnine injection, which could not be antidromically activated from C7 (not illustrated). Such a cell is defined as a C3–C4 sIN. The firing probability was calculated by counting the number of records with orthodromic activation by the third pyramidal stimulus among 10 sweeps and for this C3–C4 sIN, it was as high as 100% (average 71% for 10 sINs) before strychnine injection.

The firing probability of the C3–C4 PNs was measured in 17 neurons before injection of strychnine or when the effect of previous injection of strychnine had completely disappeared. The firing probability after strychnine was measured in 10 PNs that were recorded immediately after the injection, when glycinergic inhibition was blocked at a relatively constant level. The probability increased on average from 21% (before strychnine; 17 PNs) to 83% (after strychnine; 10 PNs). In both groups, only neurons are included which were not spontaneously active.

Among a total of 73 extracellularly recorded C3–C4 PNs, 60 neurons were orthodromically activated by one to four stimuli in a train of electrical pulses applied to the contralateral pyramid at 200 μA after administration of strychnine or when the effect of strychnine appeared to persist. We judged the cells to be activated when they fired at least in 30% of the trials. The segmental latencies of the orthodromic firing responses were measured from the initial positive peak of the pyramidal volley recorded simultaneously in the cord dorsum potential at the same segment. The C3–C4 PNs were fired from the pyramid with segmental latencies of 0.8–2.2 ms in 60 cells, and in 52 cells, the latencies were <1.4 ms (including 1 synaptic delay and time to firing; cf. Alstermark et al. 1990), suggesting a monosynaptic excitatory linkage (Fig. 1G).

These results show that ~71% (52/73) of the C3–C4 PNs recorded in this study were orthodromically activated via monosynaptic excitation from the contralateral pyramid. Further, the present results suggest that the C3–C4 PNs are under glycinergic inhibition, which prevented these neurons from responding vigorously to the pyramidal stimulation under the same anesthetic condition (Alstermark et al. 1999; Maier et al. 1998). Thus strong pyramidal excitation of sINs even before strychnine injection suggests that the neurons mediate feed-forward inhibition, as is the case in cats (Fig. 1F).

Intracellular recordings from C3–C4 PNs and sINs

To investigate the role of glycinergic inhibition on the C3–C4 PNs, we obtained intracellular recordings from 14 PNs and 34 sINs in the C3–C4 segments.

Figure 2, A–C, shows an example of intracellular records from a C3–C4 PN. The blocked antidromic spike induced by stimulation of the C6 lateral funiculus at 100 μA is shown in Fig. 2C. In response to pyramidal stimulation, EPSPs were induced with fixed segmental latency of 0.7 ms to each of the

FIG. 2. Intracellular recordings from a C3–C4 PN. Responses to a train of 3 pyramidal stimuli at 200 μA. A: effects of current injection. Ten sweeps are superimposed in each record. 1: +20 nA, 2: no current, and 3: −20 nA. Bottom traces indicate the cord dorsum potential recorded at the same segment as the PN. B: effect of intravenous injection of strychnine. Average of 10 sweeps (train of 3 pyramidal stimuli at 200 μA, with +20-nA current injection) recorded before and 4 min after strychnine injection are superimposed. C: antidromic spike (*) of the neuron induced by stimulation of the C6 lateral funiculus at 100 μA. D: segmental latencies of the EPSPs (above) and IPSPs (below). , records from PNs; , records from sINs.
three repetitive shocks at 200 µA, which suggested that the EPSPs were of monosynaptic origin. EPSPs were observed in 47 (14 PNs and 33 sINs) of the 48 intracellularly recorded cells. Latencies of pyramidal EPSPs in the 14/14 C3–C4 PNs (■) and 33/34 C3–C4 sINs (□) are shown in Fig. 2D (top columns). The latencies were shorter than 1.0 ms in 12 of the 14 identified C3–C4 PNs and in 28 of the 33 C3–C4 sINs, presumably in a monosynaptic range (Fig. 2C). Further, as shown in Fig. 2A2, individual EPSPs were followed by sharp hyperpolarizations (asterisk), which appeared to be enhanced in response to the third stimulus. The hyperpolarization also appeared to be enhanced when the cell was depolarized by constant current injection (+20 nA; Fig. 2A1) and disappeared when the cell was hyperpolarized by current injection (−10 nA; Fig. 2A3). These results suggest that the hyperpolarizations were caused by IPSPs. Figure 2B shows the effect of intravenous injection of strychnine during the intracellular recordings, tested in the same cell shown in A (n = 1). The IPSPs were reduced, which suggested that the IPSPs were glycergic. The IPSPs were observed in 40 (14 PNs and 26 sINs) of the 48 intracellularly recorded neurons (14 PNs and 34 sINs). IPSP latencies ranged from 1.1 to 2.7 ms (Fig. 2D, bottom columns), which suggested a disynaptic range. Taken together, the C3–C4 PNs and the sINs receive monosynaptic excitation and also disynaptic feed-forward inhibition from the pyramidal tract.

Figure 3 shows a summary of the location of 70 C3–C4 neurons recorded only extracellularly (circles, n = 59), intracellularly alone (diamonds, n = 5), or both extracellularly and intracellularly (asterisks, n = 6) recorded in three experiments in which location of the cells could be determined. C3–C4 PNs are shown in A (n = 37), whereas sINs are shown in B (n = 33). The neurons which received monosynaptic excitation from the pyramid are indicated by gray symbols. In the three experiments shown in the figure, we mainly tracked either in the lateral portion or medial portion of the gray matter. Because we did not record from this intermediate region, we cannot exclude that some C3–C4 PNs may be located in this region. In both groups of neurons, we found PNs with monosynaptic excitation from the pyramid, located mainly in the laminae VI and VII and at depths of 2.5–3.7 mm from the dorsal surface of the spinal cord.

**Frequency of bifurcating projection to the motor nuclei and LRN**

In cats, 84% of the C3–C4 PNs possess ascending projection to the LRN in the medulla (Alstermark et al. 1981a). In the present study, we investigated whether the PNs in the C3–C4 segments of the macaque monkeys project to the LRN. Figure 4A shows an example of a C4 PN, which showed antidromic spike responses to stimulation of C7 at a latency of 0.7 ms (Fig. 4A) and to stimulation of the LRN at a fixed latency of 1.5 ms (Fig. 4A). The spike responses to the C7 stimulation were collided by those evoked to the preceding LRN stimulation (Fig. 4A). These results show that this PN projected both to the LRN and C7. This neuron was orthodromically activated by the pyramidal stimulation with a monosynaptic latency (Fig. 4B; segmental latency was 1.1 ms; this recording was made after injection of strychnine). Note the collision of the antidromic spike by the preceding orthodromic spikes produced by the pyramidal stimulation.

Among a total of 177 neurons recorded from the C3–C4 segments in this study, 135 neurons were antidromically activated either from the LRN and/or C6/C7 but not from Th13. Among the 135 neurons that were antidromically activated from the LRN and/or C6/C7 segment, 26 neurons had bifurcating projections to the LRN and C6/C7. 49 neurons were activated only from the LRN and 60 neurons were activated only from the C6/C7. Thus only 30% (26/86) of the PNs projecting to C6/C7 segments possessed ascending branches to the LRN, which is a much smaller proportion than observed in cat (cf. discussion).

Figure 4C shows the spatial distribution of 70 neurons whose location could be reconstructed. The neurons with bifurcating axons and those projecting only to the LRN or C6 appeared to be intermingled with each other and were mainly distributed in the laminae VI and VII at depths of 2.4–3.7 mm.

Figure 4D shows the distribution of antidromic latencies of neurons with descending projection to the C6/C7. We always increased the stimulus intensity ≥ 200 µA, and when shortening of the antidromic latency was observed, we recorded the shortest latency within the above range of the stimulus intensity. Thus it is presumed that the latencies indicate the conduction time of the stem axons. Latencies ranged between 0.7 and 3.0 ms (Fig. 4D), and the conduction velocities of the descending axons ranged between 5.4 and 51.5 m/s (mean ± SD = 18.3 ± 8.8 m/s, Fig. 4F). Conduction velocities of bifurcating neurons (21.3 ± 11.9 m/s, Fig. 4F, ■) were slightly faster than those of neurons projecting only to the C6/C7 (17.1 ± 6.9 m/s, Fig. 4F, □; *p = 0.04, Student’s t-test). Figure

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**FIG. 3.** Distribution of the recorded PNs (n = 37, A) and sINs (n = 33, B) in the C3–C4 segments the location of which could be reconstructed (records from 3 experiments), respectively. In these experiments, recordings were performed either in medial tracks or in the lateral tracks. Separation between the 2 groups is simply due to lack of tracking in this region. Circles, extracellularly recorded neurons; diamonds, intracellularly recorded neurons; asterisks, neurons recorded both intra- and extracellularly. Gray symbols, neurons with monosynaptic pyramidal excitation; black symbols, those without monosynaptic pyramidal excitation.
Ascendng projection to the LRN

As the next step, we investigated whether the ascending branches projecting to the LRN region actually terminated in the nucleus. Figure 5, A–C shows an example of the antidromic threshold mapping in LRN of a PN in three different coronal planes at the caudal brain stem. The shortest latency foci, which would correspond to the location of the ascending stem axon, were distributed in the reticular formation medioventral to the LRN, while low-threshold foci with longer antidromic latencies extended into the LRN. In Fig. 5D, the low-threshold foci of five neurons (<100 μA) are superimposed. As shown in the figure, low-threshold foci were found inside the LRN in all five C3–C4 PNs. These results strongly suggest that ascending axons of the C3–C4 PNs pass through the reticular formation mediodorsal to the LRN and issue collaterals into the LRN. In addition, low-threshold foci were also distributed outside the LRN, dorsal to the nucleus in one cell.

Descending projection to the forelimb motor nuclei

We next investigated the location of descending axons and projection pattern of collaterals to the gray matter in the C6/C7 segments. Figure 6A shows an example of the axonal trajectory of a PN studied by threshold mapping. As shown in the figure, the presumed location of the stem axons was in the ventral portion of the lateral funiculus, just lateral to the edge of the gray matter and the low-threshold foci extended into the ventral horn. In Fig. 6B, the low-threshold foci of the seven tested neurons (<100 μA) are superimposed. These results show that the descending axons of the PNs pass through the ventral portion of the lateral funiculus and project to the motor nucleus in the C6/C7 segments.

LRN EPSPs in forelimb MNs

In cats, because of the high-frequency of bifurcating (84%) projection of C3–C4 PNs to the LRN and MNs, stimulation in the LRN induces large monosynaptic EPSPs in forelimb MNs by way of an “axon-reflex” (Alstermark et al. 1981a; Illert and Lundberg 1978). In the study by Nakajima et al. (2000), LRN EPSPs were smaller in the macaque monkey compared with the cat and squirrel monkey. On this basis, these authors proposed that C3–C4 PNs do not play a major role in mediating the pyramidal effects to MNs in the macaque monkey. However, given our new result that only ~30% of the C3–C4 PNs in the macaque monkey have dual projections to the LRN and the forelimb motor nuclei, it would be expected that the LRN EPSPs should be smaller in amplitude compared with those in the cat. We have now re-investigated the effects evoked by stimulation in the LRN.

We obtained intracellular recordings from 24 forelimb MNs (5 DR and 19 unidentified MNs located nearby the DR MNs). In all these MNs, LRN stimulation induced EPSPs with fixed latencies (at 1.4–2.1 ms from the stimulus artifact). One example of a LRN EPSP is illustrated in Fig. 7A. The EPSP was evoked at a fixed latency of 1.9 ms from the stimulus artifact. Recording was made after strychnine injection. The electrode track is shown in Fig. 7B. Dorsal to the LRN, at depth ~4.0 and ~5.0 mm (not shown), the threshold was between 100 and 200 μA, but decreased to around 50 μA in the LRN at depth ~6.0 mm. The amplitude of the EPSP increased more...
ventrally and reached a peak value of 7.8 mV with 500 μA stimulus. Because the shortest antidromic latencies of the bifurcating C3–C4 PNs from the LRN and C6/C7 were 0.7 and 0.6 ms, respectively (Fig. 4, D and E), the conduction times of the fastest bifurcating axons are ~1.3 ms. Based on this finding, LRN EPSPs recorded in the present study are likely of monosynaptic origin. The averaged amplitude of the EPSPs before strychnine injection was 0.95 ± 0.82 mV (n = 24), which is similar to the value reported by Nakajima et al. (2000) in the macaque monkey (1.2 ± 0.7 mV), but smaller than those found previously in the cat (5.2 ± 2.1 mV) (Alstermark and Sasaki 1986) and the squirrel monkey (1.7 ± 0.8 mV) (Nakajima et al. 2000). Our results and those of Nakajima et al. (2000) are compatible with the fact that a much smaller fraction of the C3–C4 PNs has ascending projections to the LRN in the macaque monkey compared with the cat. However, the amplitude of monosynaptic unitary LRN EPSPs in the macaque monkey has not been investigated, so we cannot exclude the possibility that there may be a species difference in this regard compared with the cat (Alstermark and Sasaki 1986). Thus it is too early to draw a conclusion on the number of PNs based on the amplitude of the monosynaptic LRN EPSPs.

On the other hand, the LRN EPSPs in the present study were sometimes mixed with IPSPs. As shown in Fig. 8A2, only a small LRN EPSP could be evoked. The early EPSP component was effectively truncated at a latency of 2.4 ms from the stimulus artifact. Depolarizing current injection of 6 nA (A1) revealed an underlying IPSP (onset is indicated by vertical gray line), which was partly reversed by hyperpolarizing current injection of 6 nA (A3). The latency of the EPSP was 1.7 and 2.4 ms for the IPSPs. The longer latency of the IPSPs suggests a disynaptic origin. Such mixture of LRN EPSPs and IPSPs were observed in 5 of the 11 MNs tested before injection of strychnine. The latencies of the LRN EPSPs and IPSPs ranged from 1.4 to 2.1 ms and 2.1 to 3.1 ms, respectively. Pyramidal stimulation also evoked mixed EPSPs (disynaptic EPSPs indicated with single asterisks in B, 1–3) and IPSPs (double asterisks in B, 1–2) in this cell. In this motoneuron there was no clear monosynaptic pyramidal EPSP. Maier et al. (1998) found that ~27% of their recorded motoneurons lacked monosynaptic pyramidal EPSPs. The latencies of the pyramidal IPSPs were in a disynaptic range (segmental latency: 1.4 ms) and the IPSPs could be mediated presumably via Ia inhibitory interneurons (INs in Fig. 8C). The latencies of the LRN IPSPs were slightly longer than the EPSPs, which suggest that the IPSPs were induced via disynaptic linkage. However, the latency of the shortest LRN IPSPs (2.1 ms) was close to the range of the monosynaptic LRN EPSPs. The possibility that the IPSPs were monosynaptic in some cases, mediated by the inhibitory PNs with dual projection to the LRN and MNs, as previously reported in cats (Alstermark et al. 1984b) will be discussed.

To study the LRN EPSPs without contaminating IPSPs, recording was made during intravenous injection of strychnine as shown in Fig. 8D. For comparison, a train of three pyramidal stimuli were applied in the same cell. As described in the preceding text, before strychnine injection only small LRN EPSPs could be evoked and pyramidal IPSPs predominated (D1). By 30 s after the strychnine injection, the LRN EPSPs became larger in amplitude and the pyramidal effect changed to predominating EPSPs (D2). Finally, after 2 min, the mono-
synaptic LRN EPSPs had grown (the onset is indicated by vertical gray line) and became larger than 4 mV in amplitude and clear disynaptic pyramidal EPSPs could be discerned (single asterisk in D3). After injection of strychnine, the effect of LRN stimulation was tested in 14 MNs and the averaged amplitude of the LRN EPSPs was 4.2/110061.8 mV (n/1100514), which was 4.4 times as large as before the strychnine injection. Disynaptic pyramidal EPSPs were observed in all the 14 MNs after strychnine injection.

These results suggest that concomitant IPSPs may be another reason why the LRN EPSPs were smaller in the macaque monkey than in cats.

DISCUSSION

Identification of C3–C4 PNs in non-human primates

In the present study, we recorded from C3–C4 PNs that were antidromically activated from the hand and arm motor nuclei or from the lateral funiculus just lateral to the motor nuclei of the C6/C7 segments. These neurons were located in laminae VI–VII of the C3–C4 segments, the axons descended through the ventral portion of the lateral funiculus and projected to the motor nuclei of forelimb muscles, but were not antidromically activated from Th13. These characteristics are similar to those of the C3–C4 PNs in cats (Illert et al. 1978). However, several different properties were found in the PNs of the macaque monkey. First, although they received monosynaptic excitation from the contralateral pyramid to a similar extent (71%, 52/73) as in the cat (74%) (Illert et al. 1978), the PNs were rarely excited orthodromically from the pyramid. Disynaptic feed-forward inhibition from the pyramid was first described in the C3–C4 PNs of the cat (Alstermark et al. 1984a,b), however, the strength of the inhibition appears to be weaker than in monkeys because repetitive stimulation of the pyramid induced orthodromic firing in most of the C3–C4 PNs with higher probabilities in cats. In contrast to the cat, the firing probabilities of C3–C4 PNs in the macaque monkey were lower, and intravenous injection of strychnine markedly increased the probabilities of orthodromic activation of the PNs. Second, the present study has shown that only about one-third of the PNs possess an ascending projection to the LRN. This ratio is considerably lower than in cats in which 84% of the C3–C4 PNs have ascending projection to the LRN. In previous experiments in cat (Alstermark et al. 1981a), a current intensity of 200 μA was used to sample the antidromically activated cells, whereas in the present study, we used 500 μA. The size of the brain stem at the level of the LRN is rather similar (<20% difference) in both species. Thus it is not likely that we underestimated the number of ascending neurons. On the other hand, it is possible that the value of 84% in the study of Alstermark et al. (1981a) could be an underestimate.

In this study, we did not record from long PNs that could be antidromically activated from the Th13 segment. Because the same method of stimulation as in the cat was used (Alstermark et al. 1991), and the size of the spinal cord at the level of stimulation is rather similar in both species of animals, it is not likely that the current intensity was too weak to activate the descending axons of the long PNs. The cell bodies of the long PNs are presumably located more ventromedially in lamina VII and in lamina VIII as is the case in the cat (Alstermark et al. 1987).

Feed-forward inhibition of C3–C4 PNs

Feed-forward inhibition of C3–C4 PNs was first described in the cat (Alstermark et al. 1984a,b). It was shown that the cortico-, rubro-, tecto-, and reticulospinal tracts, which all produce monosynaptic excitation (Illert et al. 1978, 1981), also evoked disynaptic inhibition in the C3–C4 PNs (Alstermark et al. 1984a,b). The feed-forward inhibition was mainly mediated via common sINs in the C3–C4 segments (Alstermark et al.
FIG. 8. Concomitant LRN IPSPs and the LRN EPSPs in a forelimb MN. A: synaptic responses to LRN stimulation at 500 μA. Effect of current injection; +6 nA (1), no current (2), and −6 nA (3). Vertical gray line indicates the onset of the IPSP and horizontal gray lines indicate the baseline level. B: synaptic responses to pyramidal stimulation at 200 μA. Effect of current injection; +6 nA (1), no current (2) and −6 nA (3). The single asterisks in 1–3 indicate the disynaptic EPSP and the double asterisks in 1–2 indicate the disynaptic IPSP. C: schematic drawing of the proposed circuit diagram. D: effect of intravenous injection of strychnine on the LRN and pyramidal stimulations. Records before injection (1), 30 s after injection (2), and 2 min after injection (3). Disynaptic EPSP is indicated with single asterisk in D1. Onsets of monosynaptic LRN EPSPs are indicated by vertical gray line and horizontal gray lines indicate the baseline level. A, B, and D: records from the same cell.

1984a,b). Despite a clear feed-forward inhibition in the cat, the probability of firing in single C3–C4 PNs by pyramidal stimulation (a train of 3 shocks and no strychnine) was on average 90% (B. Alstermark, J. Ogawa, S. Sasaki, unpublished results). In contrast, the present results in the monkey show that the firing probability of the C3–C4 PNs before strychnine injection was as low as 21%. After reduction of glycineric inhibition by strychnine injection, the firing probability increased to 83%. Thus the increased firing probability can to a large extent be attributed to a reduction of feed-forward inhibition because the candidate sINs in the C3–C4 segments showed a higher firing probability (71%) than the PNs before strychnine injection. These findings could explain why disynaptic pyramidal EPSPs are regularly evoked in forelimb MNs of the cat without previous injections of strychnine, whereas such EPSPs are only rarely evoked in forelimb MNs of the macaque monkey. In this regard, it was interesting that we found in our recent study that after functional recovery from a chronic lesion of the lateral corticospinal tract in C2/C3, disynaptic pyramidal EPSPs could be evoked in 50% of the forelimb MNs even without strychnine (Sasaki et al. 2004); consistent with a compensatory reduction of the feed-forward inhibition. In this study, we also observed feed-forward inhibition in the sINs in the C3–C4 segments like in the cat (Alstermark et al. 1984c). Our findings support the hypothesis proposed by these authors that the feed-forward inhibitory control of the sINs is used in parallel in the selection of subpopulations of PNs, which control different sets of motoneuronal pools.

From a functional point of view it is interesting to consider why a stronger inhibitory control of the C3–C4 PNs in the monkey compared with the cat might be important. One possibility is that feed-forward inhibition used for spatial control (Alstermark and Sasaki 1986; Alstermark et al. 1984b,c; Tantisira et al. 1996) might have evolved further during evolution to enable a broader repertoire of arm and hand movements in the monkey. Another aspect of feed-forward inhibition relates to the timing of firing of the C3–C4 PNs. The pattern of firing must be of critical importance in the control of the forelimb MNs especially during the final phase of reaching when grasping with the digit is to be accomplished. Many different muscles of the hand and arm are co-activated and fine tuning of the level of contraction is therefore needed. The amount of feed-forward inhibition could be used to finely control the firing duration and frequency of the C3–C4 PNs. Chronic recordings from identified C3–C4 PNs during ongoing reaching and grasping in the cat (Perfiliev et al. 2003) and monkey will be important to gain further understanding of how the descending feed-forward control is used and possible differences between the two species. In this regard, a recent study by Niwa et al. (2004) showed a possible contribution of the C3–C4 PNs in the control of wrist movements in intact animals by single-unit recordings from antidromically identified C3–C4 PNs in awake monkeys.

Our study has been focused on the feed-forward inhibition. However, in cat all C3–C4 PNs also receive feed-back inhibition evoked from forelimb nerves (Alstermark et al. 1984c). This remains to be investigated in the macaque monkey. In man it was recently shown that corticospinal excitation in forelimb MNs mediated via PNs, was suppressed by feed-back inhibition (Nicolas et al. 2001). Furthermore, our findings do not exclude a contribution of tonic inhibition as already discussed in our previous publications (Alstermark and Isa 2002; Alstermark et al. 1999).

Ascending projection of C3–C4 PNs to the LRN

It was first shown in the cat that C3–C4 PNs have bifurcating axons to forelimb MNs and to neurons in the LRN (Alstermark et al. 1981a; Ilbert and Lundberg 1978). Such a dual projection was found in the majority of the PNs (84%). In contrast, we have now observed a much lower frequency of ascending projection among the C3–C4 PNs with projection to forelimb MNs (30%) in the macaque monkey. The reason for this difference is not clear, but it cannot be excluded that the PNs in the macaque monkey have collaterals to other types of neurons that may send an efference copy signal to the cerebellum. In the cat it was found that some C3–C4 PNs have collateral connections to spinocerebellar neurons in the fore-
limb segments (Alstermark et al. 1990). This possibility can be investigated in future investigations. Also, in the present study, it was found that ascending collaterals of one PN projected dorsal to the LRN, but the final termination was not investigated (Fig. 5).

In the present study, we found that the stimulation of the LRN induced disynaptic IPSPs in some MNs. In the cat, it has been shown that the excitatory C3–C4 PNs do also have projection to Ia inhibitory INs in the forelimb segments (Illert and Tanaka 1978), and it was demonstrated that such excitatory C3–C4 PNs have ascending projection to the LRN as indicated in Fig. 8C (Alstermark et al. 1984b). Thus it is likely that the disynaptic LRN IPSPs were mediated by an excitatory C3–C4 PNs and Ia inhibitory interneurons. However, in some MNs, the latency of the LRN IPSPs was as short as 2.1 ms, which overlaps the latency of monosynaptic LRN EPSPs. The fluctuating onset of the IPSPs might be caused by concomitant monosynaptic EPSPs (Fig. 8A1). In cats, the existence of inhibitory C3–C4 PNs with dual projection to the LRN and forelimb MNs was demonstrated (Alstermark et al. 1984b), and we cannot exclude the possibility that such inhibitory C3–C4 PNs exist also in monkeys and mediate monosynaptic LRN IPSPs. It should be noted that we have observed disynaptic pyramidal IPSPs also after a chronic transection of this tract in C3/C5, which might be mediated via inhibitory C3–C4 PNs (S. Sasaki, T. Isa, L-G. Pettersson, B. Alstermark, K. Naito, K. Yoshimura, K. Seki, Y. Ohki. unpublished findings).

The current strength of 500 µA used for stimulation in the LRN is the same as was used to test the LRN EPSPs in MNs in the cat (Alstermark and Sasaki 1985). We do not claim that this stimulation was exclusive for the ascending axon collaterals of the C3–C4 PNs and would like to emphasize that part of the effects could be mediated by other descending or ascending systems. Future investigations are required to solve this problem in the monkey. It should be noted that despite the stimulation strength of 200 µA in the work by Nakajima et al. (2000), the amplitude of the LRN EPSPs were comparable (actually slightly larger) in size to those observed in the present study. In the cat, it was shown that 500 µA used for stimulation in the LRN, gave almost no spread to the medially located reticulospinal tract (Alstermark and Sasaki 1985). However, in the motoneuron shown in Fig. 7, tracks located 1–2 mm medially (not illustrated) showed that EPSPs of smaller amplitudes and with 0.5 ms longer latency could be evoked with 500 µA. Such EPSPs are not fully compatible with a monosynaptic linkage via the bifurcating C3–C4 PNs and are thus more likely mediated via another system.

One consequence of the lower occurrence of ascending projections to the LRN of the C3–C4 PNs with projection to forelimb MNs in the macaque monkey compared with the cat could be smaller amplitudes of the monosynaptic LRN EPSPs. The smaller amplitude of monosynaptic LRN EPSPs was confirmed in the monkey by recording from forelimb MNs in the present study. The peak amplitude of LRN EPSPs in the monkey was on average 0.95 mV, significantly smaller compared with 5.85 mV (DR MNs) in the cat (Alstermark and Sasaki 1986). Nakajima et al. (2000) also reported small amplitudes (1.2 mV, averaged value). However, the concomitant IPSPs might be another reason for small amplitudes of the monosynaptic LRN EPSPs. In the present study, after injection of strychnine, the average amplitude of LRN EPSPs became as large as 4.2 mV. Because the LRN EPSPs were not tested after injection of strychnine in the cat, we cannot directly compare the amplitude between the two species. The species difference in the amplitude of the unitary LRN EPSPs is not excluded. All together, the amplitude of LRN EPSPs is influenced by the number of bifurcating C3–C4 PNs (including the number of terminals on motoneurons), the amplitude of unitary LRN EPSPs and concomitant LRN IPSPs. These complications make it difficult to use the amplitude of the LRN EPSPs as a measure of the contribution of the C3–C4 PNs to mediating the disynaptic corticospinal excitation to MNs in the monkey.

We have previously shown that the prevalence of disynaptic pyramidal EPSPs mediated via C3–C4 PNs in forelimb MNs of the macaque monkey is the same as in the cat (Alstermark et al. 1999), when glycnergic inhibition of the C3–C4 PNs was reduced. In the article by Nakajima et al. (2000), the authors assumed that a similar proportion of the C3–C4 PNs in the macaque monkey would have ascending projection to the LRN as found in the cat (84%) (Alstermark et al. 1981a). Based on this assumption, Nakajima et al. (2000) concluded that the small LRN EPSPs in the macaque monkey supports their prediction that the transmission of corticospinal excitation mediated by the C3–C4 PN system is weaker compared with the cat. The authors also made a comparison with LRN EPSPs obtained in the squirrel monkey and found that they were larger (1.7 mV) than in the macaque monkey but smaller than in the cat. On basis of these findings, the fact that the monosynaptic CM connection is lacking in the cat but becomes gradually stronger in the squirrel monkey, macaque monkey, and man in parallel with increased dexterity of finger movements, Nakajima et al. (2000), proposed the “replacement hypothesis” as outlined in their Fig. 12. Their hypothesis suggests that the gradually weaker projection from the C3–C4 PNs has been replaced by the gradually stronger monosynaptic CM connection and that the propriospinal system is of less importance both with respect to reaching and grasping in the monkey and even more so in man. As described in the preceding text, however, the amplitude of the LRN EPSPs is not a useful measure of the contribution of C3–C4 PNs to mediating disynaptic pyramidal excitation to motoneurons in the macaque monkey. Our previous study (Alstermark et al. 1999) and the present data suggest that the transmission of corticospinal excitation mediated by C3–C4 PN system in the monkey is under strong feed-forward inhibition, but when the inhibition is reduced, the C3–C4 PN system can transmit substantial pyramidal excitation to forelimb MNs.

In behavioral experiments, we recently showed that the macaque monkey could still make the precision grip with the thumb and index finger within a few days after a complete transection of the lateral corticospinal tract in C3/C5, which completely interrupted the monosynaptic CM connection (Sasaki et al. 2004). Thus we suggest that indirect CM pathways mediated via more rostrally located (above C3/C5 including brain stem) intercalated neurons are also used in the control of the precision grip. Ongoing research aims at investigating if and to which extent the command for reaching and dexterous digit movements can be mediated via the C3–C4 PNs and/or bulbospinal systems in the macaque monkey by making a
lesion in C5 of the lateral corticospinal tract as previously made in the cat (Alstermark et al. 1981b).

We have previously proposed that the monosynaptic CM connection has evolved to add and not to replace the control already exerted by indirect CM systems like the C5–C4 pro-PN system (Alstermark and Isa 2002; Sasaki et al. 2004). The results of the present study strongly support this view.

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