The Flexor Reflex Mediated by Group II Afferent Fibers: Effects of Morphine-HCl and Mephenesin

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ABSTRACT—The effects of morphine-HCl and mephenesin on the flexor reflex mediated by group II afferent fibers were investigated. The flexor reflex was recorded by means of the electromyogram (EMG) evoked in the muscle tibialis anterior by stimulation of the ipsilateral tibial nerve in urethane-α-chloralose anesthetized rats. Afferent volleys corresponding to the phasic EMG component of the flexor reflex with 7.6-msec latency (flexor EMG: fEMG) were also recorded using the double volley technique. The threshold of the afferent volleys mediating the fEMG was approximately twice as high as that of the most excitable afferent volleys, which were considered the spikes of group I afferent fibers, and the conduction velocity of the afferent volleys was 39.9 ± 3.2 m/sec. Morphine-HCl (5 mg/kg, i.v.) did not change the amplitude of the fEMG, but mephenesin (40 and 80 mg/kg, i.v.) depressed it dose-dependently. These results suggest that the fEMG is a flexor reflex mediated by group II afferent fibers, which is not affected by morphine-HCl but depressed by mephenesin.

Keywords: Morphine, Mephenesin, Flexor reflex, Group II afferent fiber

The flexor reflex, a polysynaptic reflex (PSR) whose afferent fibers consist of groups II, III and IV afferent fibers, has been recorded by the withdrawal response of the hindlimb, the contraction of the flexor muscle or the electromyogram (EMG) evoked in the flexor muscles. These reflexes have been used to evaluate the effects of various drugs such as analgesics or centrally acting muscle relaxants; however, the effects of drugs are not always consistent. Morphine, a well-known analgesic, has been reported to depress the withdrawal response of the hindlimb (1–3), which is a flexor reflex induced by the nociceptive stimulation that is strong enough to activate group IV afferent fibers (4). On the other hand, the flexor reflex evoked by non-nociceptive stimulation is not affected by morphine (4). Mephenesin, a centrally acting muscle relaxant, has been reported to depress PSRs (5, 6) including the flexor reflex measured by the contractions of the flexor muscles (7, 8), while the withdrawal response of the hindlimb is not affected by mephenesin (9). These results indicate that the flexor reflex mediated by group IV afferent fibers is depressed by morphine and not affected by mephenesin. Although the flexor reflex mediated by non-nociceptive group II afferent fibers seems to be depressed by mephenesin and not affected by morphine, little work has been performed on the flexor reflex mediated by these fibers. So, the aim of the present study was to investigate the effects of morphine-HCl and mephenesin on the flexor reflex mediated by group II afferent fibers.

We attempted to use the phasic EMG component of the flexor reflex with a latency of 7.6 msec (flexor EMG: fEMG) as a flexor reflex mediated by group II afferent fibers because this component is thought to be evoked by non-nociceptive stimulation (10). As a first step, we recorded afferent volleys from the ipsilateral dorsal root simultaneously with the fEMG and confirmed that its conduction velocity (CV) fell into the group II afferent fibers range. Thereafter, the effects of morphine-HCl and mephenesin on the fEMG were investigated.

MATERIALS AND METHODS

Recording of the fEMG and afferent volleys

Eight-weeks-old male Wistar rats (295–320 g) were used. Animals were anesthetized with intraperitoneal injection of urethane (400 mg/kg) and α-chloralose (50
mg/kg), which were supplemented as required, and then they were placed and fixed in the belly position. The left sciatic nerve was exposed, and all branches were cut except for the common peroneal nerve. The tibial nerve was placed on bipolar electrodes for stimulation (0.1 Hz, 0.05 msec; Nihon Kohden MSE-3). A silver ball electrode was placed on the ipsilateral muscle tibialis anterior for recording the fEMG. Laminectomy was performed in the lumbo-sacral region, the left dorsal root of segment L5 was exposed, and the peripheral end was placed on a monopolar platinum electrode for recording afferent volleys. An indifferent needle electrode was inserted in the skin. The fEMG and afferent volleys were displayed on a memory oscilloscope (Nihon Kohden VC-10), averaged 6 times by an averaging computer (Nihon Kohden DAT-1100) and recorded on an X-Y recorder (Graphtec WX-4401). Skin pouches were formed, and the exposed tissues were covered with warm liquid paraffin maintained at 36 ± 1°C. Rectal temperature was monitored and maintained at 37 ± 1°C with a heat lamp.

The double volley technique, as described by Edgley and Jankowska (11), was used to record afferent volleys corresponding to the fEMG separately from those of the most excitable afferent fibers. Namely, the first stimulus, having a strength just below the threshold of the fEMG, was applied 0.9 msec prior to the second stimulus which generated the fEMG. Afferent volleys mediating the fEMG could be recorded separately by making the most excitable fibers refractory at the time of the second stimulus. These stimuli were given to two different points of the tibial nerve. CVs were calculated by dividing the distance between two stimulating points by the difference in the latencies of the corresponding peaks of afferent volleys.

**RESULTS**

**Recording of the fEMG and afferent volleys**

Afferent volleys were recorded from the dorsal root at segment L5 to exclude the antidromic volleys of afferent fibers. Since the conduction distance of afferent volleys in rats is too short, it is difficult to record the potentials of the group II afferent fibers separately from those of group I afferent fibers because the latter ones have lower threshold (12). Therefore we adopted the double volley technique (11) to record the distinct group II afferent volleys and measure their CVs correctly.

A phasic afferent spike (peak 1) was recorded from the L5 dorsal root by applying only the first stimulus to the ipsilateral tibial nerve (Fig. 1A). The second stimulus was gradually increased. When the stimulus was more than double the threshold value for peak 1, the late component of phasic afferent spike (peak 2) was observed, and the fEMG with a latency of 7.6 ± 0.4 msec (n = 4) appeared in the muscle tibialis anterior simultaneously (Fig. 1, B and C). When the amplitude of peak 2 reached its maximum, the fEMG increased no longer. Neither the EMG with a latency of more than 20 msec nor the afferent volleys with a longer latency than peak 2 were observed if the stimulus intensity was raised more than the maximum for peak 2. The CVs of peak 1 and peak 2 were 54.1 ± 8.1 and 39.9 ± 3.2 m/sec, respectively (n = 4).

**The effects of morphine-HCl and mephenesin on the fEMG**

Morphine-HCl at a dose of 5 mg/kg had no effect on the fEMG within 20 min and thereafter tended to reduce the fEMG; however, no significant change was observed (Fig. 2). Mephenesin at doses of 40 and 80 mg/kg depressed the fEMG dose-dependently and maximal depression was observed immediately after the administration. With 40 mg/kg, the inhibitory effect was observed within 5 min and with 80 mg/kg, within 40 min (Fig. 3).

**DISCUSSION**

The aim of this study was to investigate the effects of morphine-HCl and mephenesin on the flexor reflex mediated by group II afferent fibers. First, we recorded both the fEMG and the corresponding afferent volleys by stimulation of the ipsilateral tibial nerve to confirm the fEMG was mediated by group II afferent fibers.

Peak 1 evoked by subthreshold stimulation of the
Fig. 1. Typical records of the averaged fEMG (left hand traces) and afferent volleys (right hand traces) evoked by stimulation of the ipsilateral tibial nerve with two shocks (arrowheads). The responses to only the first stimulus (A). In the records from (B) to (D), the strength of the second stimulation was progressively increased.

Fig. 2. Effect of morphine-HCl on the fEMG. (A) Typical responses of the effect of morphine-HCl (5 mg/kg, i.v.) on the fEMG. (B) Time course of the effect of morphine-HCl on the fEMG. Ordinate: mean amplitudes of the fEMG, as percentages of the value just prior to the drug administration, with S.E.M. indicated (n = 4). Abscissa: time in min after the drug administration. ○: Saline. ●: Morphine, 5 mg/kg.
Fig. 3. Effect of mephenesin on the fEMG. (A) Typical responses of the effect of mephenesin (40 mg/kg, i.v.) on the fEMG. (B) Time course of the effect of mephenesin on the fEMG. Ordinate: mean amplitudes of the fEMG, as percentages of the value just prior to the drug administration, with S.E.M. indicated (n = 3-4). Abscissa: time in min after the drug administration. ○: Solvent; △: Mephenesin, 40 mg/kg; ■: Mephenesin, 80 mg/kg. *P < 0.05, **P < 0.01: statistically significant difference from the solvent (20% propylene glycol)-treated group (Dunnett’s test).

fEMG was considered to be the group I afferent spike. As the threshold of peak 2 was approximately twice as high as that of peak 1, peak 2 was regarded as the group II afferent spike (13). In rats, CVs for groups I and II afferent fibers were reported to be 32–52 and 16–36 m/sec, respectively, by Russell (14) and more than 30 and 14–30 m/sec, respectively, by Harper and Lawson (15). In our study, CVs for peak 1 and peak 2 were faster than those for group I and group II afferent fibers reported in the above-mentioned articles. The rats we used were older and/or heavier than those used in the studies of Russell and Harper and Lawson. Taken together with the fact that CVs of rats increase with age and weight gain up to 22–32 weeks of age (16, 17), the CVs of peak 1 and peak 2 were considered to fall into the ranges for group I and group II afferent fibers, respectively.

The amplitude of the fEMG changed synchronously with that of peak 2. Lloyd (18) reported that the latency of the group II reflex evoked in the common peroneal nerve by stimulating the ipsilateral tibial nerve was 5.8 msec in cats. The latency of the M-wave evoked in the muscle tibialis anterior by stimulation of the ipsilateral common peroneal nerve is 1.5 msec (K. Sakitama, unpublished data) and that of fEMG is 7.6 msec; therefore the difference between the latency of the fEMG and the M-wave is 6.1 msec, which is consistent with the latency of the group II reflex described by Lloyd. These results suggest that the fEMG is mediated by group II afferent fibers.

Next, we studied the effects of morphine-HCl and mephenesin on the fEMG. Morphine-HCl did not affect the fEMG at a dose of 5 mg/kg, which was an adequate dose to cause an apparent inhibition of the withdrawal response of the hindlimb (1–3). This reflex is thought to be induced by nociceptive stimulation that can activate group IV afferent fibers (4). Thus morphine-HCl is thought not to affect the flexor reflex mediated by group II afferent fibers, but to suppress that mediated by group IV afferent fibers. Our result is consistent with the results of Koll (4) who showed no effect of morphine, in the range of doses exhibiting analgesic effects, on the flexor reflex mediated by group II afferent fibers in man.

Mephenesin inhibited the fEMG dose-dependently. Satoh et al. (9) reported mephenesin had no effect on the withdrawal response of the hindlimb evoked by intraarterial administration of bradykinin which has been reported to excite group III and group IV afferent fibers without activating group II afferent fibers (19).
Taken together, mephenesin is thought to suppress the flexor reflex mediated by group II afferent fibers without affecting that mediated by group III and group IV afferent fibers.

Although the underlying mechanisms causing the difference in the effects of morphine-HCl and mephenesin on the flexor reflex according to the types of mediating afferent fibers are not understood, our results support the insistence of Duggan and North (20) that the investigations about PSRs should be performed after determining the probable fiber types mediating the reflex.

In summary, the fEMG, a phasic component of the EMG with a latency of 7.6 msec, evoked in the muscle tibialis anterior by stimulation of the ipsilateral tibial nerve was determined to be the flexor reflex mediated by group II afferent fibers, which is not affected by morphine-HCl but suppressed by mephenesin.

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REFERENCES


