Effects of Eperisone-HCl on the Stretch Reflex in Anesthetized Cats

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Abstract—The effect of eperisone-HCl on the spinal stretch reflex was investigated in cats anesthetized with urethane-chloralose. The reflex activity, elicited by muscle stretches, was recorded from the split ventral root and analyzed using a cross-correlation histogram between the motoneuronal spikes and muscle stretches. Eperisone-HCl was intravenously applied at a dose of 5 mg/kg. It was found that eperisone weakly inhibited the reflex activity of tonic motoneurons (approximately 30%), and that of the phasic ones was either moderately (approximately 70%) or completely (100%) inhibited by eperisone. The cross-correlation analysis suggested that the membrane potentials of both tonic and phasic motoneurons are equally lowered by eperisone or that monosynaptic transmission from primary afferents of muscle spindles to motoneurons is inhibited by eperisone.

Eperisone-HCl (4'-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride) is a new antispastic agent that exhibits strong antitremorin, antiphsostigmine and antinicotinic action (1). Tanaka et al. (2) have reported that this agent inhibits the mono- and polysynaptic reflex elicited by dorsal root stimulation but does not inhibit the patellar tendon reflex elicited by taps. It is known that the muscle spindle is sensitive to sinusoidal stretchings of a muscle at low amplitude (3). Homma (4) revealed that when a muscle is stretched with triangular waveforms, intracellular recording of an alpha-motoneuron innervating the muscle shows monosynaptic excitatory postsynaptic potential (EPSP) ripples corresponding to the triangular stretches, that continuous application of the stretches causes temporal summation of the EPSP ripples, and that when the summated membrane potential attains the firing threshold, the alpha-motoneuron fires. To further clarify the action of this agent on the spinal stretch reflex, the discharge pattern of motoneuronal spikes, elicited by muscle stretch and recorded from the ventral root filament, was investigated in anesthetized cats using cross-correlation analysis between the motoneuronal spikes and muscle stretches.

Experiments were performed in ten cats anesthetized with intraperitoneal injection of a mixture of 10% urethane and 1% alpha-chloralose (4–5 ml/kg). The cats were paralyzed with gallamine triethiodide and artificially ventilated during the experiment. Endtidal CO₂ concentration was maintained at approximately 4%. Rectal temperature was maintained at 37±1°C using a heating pad and an infra-red lamp. Mean blood pressure was maintained above 80 mmHg by infusion of Ringer’s solution when necessary.

Left hind limb nerves, except the medial and lateral gastrocnemius nerves, were cut. The left triceps surae muscle was carefully separated from the surrounding tissue, and divided into the medial, lateral gastrocnemius and soleus muscles and then covered with warm paraffin oil. A laminectomy was performed to expose the lumbar spinal segments from L5 to S1, which were then covered with warm paraffin oil.

An electromagnetic vibrator was attached to the tendon of the medial gastrocnemius muscle via a steel hook. To investigate the stretch reflex, the muscle was longitudinally stretched with a triangular waveform (rising and falling phase: 4 msec). Intervals between the stretches were randomly changed in a
uniform distribution from 15 to 55 msec. The amplitude of the muscle stretch was servo-controlled and adjusted at 50 μm. The initial muscle length was set at 10 mm released position from the maximum physiological length of the muscle determined at the fully dorsi-flexed position of the ankle.

The L7 ventral root was cut, and the central end of the ventral root was divided into fine filaments until functionally single ventral root activity was obtained in response to a manual, brief stretching of the muscle. Activity of the primary ending of muscle spindles, identified by the conduction velocity and discharge pause during the muscle contraction, was recorded from a filament of the dorsal root without cutting the fiber. The initial muscle length used in the present study was enough to increase muscle spindle activity as a background excitatory inflow to motoneurons but not enough to elicit motoneuronal spikes by itself.

Eperisone-HCI (supplied by Eisai, Tokyo, Japan) was dissolved in Ringer’s solution and intravenously injected at a dose of 5 mg/kg. The activities of the motoneuron and primary ending of muscle spindles, elicited by the muscle stretches, were recorded at 5 min before and at every 10 min up to 100 min after the eperisone injection. The neuronal activities and the stretch signals were stored on magnetic tape (MR-30, TEAC, Japan) and analyzed by use of a signal processor (7T17, NEC-Sanei, Japan) to calculate cross-correlation histograms between the motoneuronal spikes and electrical pulses which triggered the muscle stretches.

Figure 1A shows an example of multi-unit motoneuronal spikes elicited by random stretches of the medial gastrocnemius muscle.

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**Fig. 1.** Effect of eperisone on the stretch reflex activity of the spinal motoneurons. **A:** multi-unit recordings from a finely dissected ventral root filament (upper traces) elicited by random stretches of the medial gastrocnemius muscle (lower traces). Intravenous injection of eperisone-HCI (5 mg/kg) is indicated by the arrow. **B** and **C:** Cross-correlation histograms between phasic or tonic motoneuronal spikes and random muscle stretches, respectively. Ordinate: spike number for 10 sec, abscissa: latency from the onset of the muscle stretch. Bin width: 0.25 msec. A to C were recorded and calculated before and 10, 40 and 60 min after eperisone injection.
Before the intravenous injection of eperisone, several spikes are elicited. A motoneuronal spike with the lowest amplitude shows tonic firing, while motoneuronal spikes with the high amplitude show phasic firing in response to the stretches. Ten min after the injection, the phasic motoneuronal spikes completely disappear but the tonic spikes continue to fire, although the interspike intervals of the tonic spikes become longer than the control. The phasic motoneuronal spikes recovered to fire 40 min after the injection. One hour after the injection, firings of the tonic and phasic motoneuronal spikes return to the control level. No change was observed in the firing rate of the primary ending of muscle spindles after eperisone injection (not shown).

Rows B and C of Fig. 1 show the cross-correlation histograms between phasic or tonic motoneuronal spikes and the muscle stretches, respectively. After the amplitude discrimination, the histograms were obtained from motoneuronal spikes for 10 sec. Before the injection, phasic and tonic motoneuronal discharges are elicited with the minimum latency of 1.75 msec after the onset of the muscle stretch. Judging from the latency, the first peak in the histograms corresponds to the spikes elicited by monosynaptic transmission in the stretch reflex (5, 6). As seen in Fig. 1A, control, every muscle stretch does not always elicit a tonic or phasic motoneuronal spike. These facts indicate that the motoneuronal spike is elicited as a consequence of the summation of several monosynaptic EPSP evoked by muscle stretches. Row B of Fig. 1 shows that at 10 min after the injection, the phasic spikes are not elicited by the muscle stretches. At 40 min after the injection, the phasic spikes are gradually elicited by the muscle stretches with a longer latency than that of the control. At 100 min after the injection, the reflex almost recovers from the influence of eperisone, but the peak latency is still increased by 0.75–1.0 msec as compared to that of the control. Although the tonic motoneuron was not strongly affected by eperisone as seen in Row C, the monosynaptic activity was approximately one third of the control and the peak latency was slightly increased by 0.13 msec at 40 min after eperisone administration. The motoneurons that exhibit a phasic response to the homonymous muscle stretch are reported to correspond to the phasic motoneurons, while those showing a tonic response correspond to the tonic motoneurons (7). It was concluded from these results that the excitability of the motoneuronal monosynaptic reflex is lowered by intravenous injection of eperisone and that phasic motoneurons are more affected by eperisone than tonic ones.

The time courses of the effect of eperisone on the monosynaptic stretch reflex are shown in Fig. 2. The integrated area of the first peak in the cross-correlation histogram obtained at 10 min intervals after eperisone injection was normalized by that of the control and was expressed as plus and minus percent control. Approximately three groups of different time courses are noticeable in Fig. 2. The first group (indicated by filled circles) consists of the tonic motoneurons. The action of eperisone on tonic motoneurons was not always constant from 5 to 30 min after the injection. Weak inhibition of approximately 30% was seen at 40–50 min after the injection. The second and third groups (indicated by open circles) consist of phasic motoneurons. The second group shows moderate (approximately 70%) inhibition at 10 min after the injection which continued for 20 min. The third group shows strong (approximately 100%) inhibition at 10 min
after the injection which continued for more than 30 min with one exception which showed excitation at 40 min after the injection.

Since the ventral roots were cut in the present study, the possibility of an indirect effect of eperisone via gamma-motoneurons on the stretch reflex can be disregarded. Furthermore, the primary endings of muscle spindles were not affected by eperisone in the present study. Taken together, it was thought that the inhibitory effect of eperisone is due to either a direct action on alpha-motoneurons or an indirect action via interneurons in the spinal cord or via supraspinal centers. Although a local anesthetic effect of eperisone on ventricular neurons at a high dose is reported by Ohtsuka et al. (8), the local anesthetic effect on spinal motoneurons would be negligible in the present study, using eperisone at a dose of 5 mg/kg. Although the precise mechanism of the inhibitory action could not be clarified in the present study, the preferred action on phasic motoneurons and the prolongation of the latency of the monosynaptic reflex suggest that the membrane potentials of both tonic and phasic motoneurons are equally lowered by eperisone. Since the amplitude of monosynaptic EPSP elicited by a group Ia afferent volley is larger in tonic motoneurons than in phasic ones (9), it is reasonable to consider that EPSPs, which are summed at random sequence in phasic motoneurons, can not attain the firing threshold level, while it takes more time for EPSPs which are summed at random sequence in tonic motoneurons to attain the firing threshold level, since the membrane potentials are shifted toward a hyperpolarizing level by eperisone. This hyperpolarization induced by eperisone was recorded from motoneurons in the sliced spinal cord of young rats by Ohtsuka et al. (personal communication). However, the possibility that eperisone inhibits the monosynaptic transmission should be considered since a low amplitude and a prolonged rise time of EPSP are expected in this case.

The results obtained in the present study are in good agreement with the reports that the flexor reflex is inhibited by eperisone in experimental animals (2) and that an abnormal tendon reflex and clonus in spastic patients is effectively controlled by eperisone (10). However, the present results could not clarify the dissociative phenomena that eperisone inhibits the reflex elicited by dorsal root stimulation but does not inhibit the reflex elicited by tendon taps (2).

Since Fig. 2 was obtained from simultaneous recordings of multi-unit motoneuronal firings, it was supposed that the differing action of eperisone on tonic and phasic motoneurons is not due to a difference in the preparation or to the level of anesthesia.

In three out of ten cats tested, the blood pressure was decreased by approximately 20 mmHg from 120 mmHg after the eperisone injection. This hypotension may be due to the direct effect of eperisone on smooth muscles (11). However, since the basilar artery is dilated by eperisone (12), it is safe to consider that blood flow in the spinal cord is well-preserved in the present study and that the inhibition of the monosynaptic reflex induced by eperisone is not due to the systemic hypotension.

References
1 Kuroiwa, Y., Sobue, I., Tazaki, Y., Nakanishi, T., Ohtomo, E. and Itahara, K.: Effects of E-0646 on cases of spasticity — A double-blind comparison using tolperisone hydrochloride. Clin. Eval. 9, 391–419 (1981) (in Japanese)
2 Tanaka, K., Kaneko, T. and Yamatsu, K.: Effects of 4'-ethyl-2-methyl-3-piperidinopropiophenone on experimental rigidity and spinal cord activities. Folia Pharmacol. Japon. 77, 511–520 (1981) (Abs. in English)
3 Matthews, P.B.C.: Mammalian Muscle Receptors and Their Central Actions, Edward Arnold, London (1972)
4 Homma, S.: Frequency characteristics of the impulse decoding ratio between the spinal afferents and efferents in the stretch reflex. Prog. Brain Res. 44, 15–30 (1976)
5 Homma, S. and Nakajima, Y.: Input-output relationship in spinal motoneurons in the stretch reflex. Prog. Brain Res. 56, 37–43 (1979)
6 Nakajima, Y. and Homma, S.: Cross-correlation analysis of neuronal activities. Japan. J. Physiol. 37, 967–977 (1987)
7 Granit, R., Henatsch, H.-D. and Steg, G.: Tonic and phasic ventral horn cells differentiated by post-tetanic potentiation in cat extensors. Acta
Physiol. Scand. 37, 114–126 (1956)

8 Noma, S., Sasa, M., Ohno, Y., Matsuoka, I. and Takaori, S.: Effects of eperisone applied by microiontophoresis on neurons in the medial and lateral vestibular nuclei. Japan. J. Pharmacol. 40, 283–290 (1986)

9 Burke, R.E.: Group Ia synaptic input to fast and slow twitch motor units of cat triceps surae. J. Physiol. (Lond.) 196, 605–630 (1968)

10 Watanabe, S., Kitahara, H. and Nakagawa, T.: Clinical neurophysiological study on centrally acting muscle relaxant. Japan. J. Clin. Exp. Med. 58, 1610–1616 (1981) (in Japanese)

11 Suzuki, A., Yanagawa, T. and Fujii, Y.: On mechanisms of smooth muscle relaxation by E-0646. Med. Consultation and New Remedies 17, 2809–2813 (1980) (in Japanese)

12 Fujioka, M. and Kuriyama, H.: Eperisone, an antispastic agent, possesses vasodilating actions on the guinea-pig basilar artery. J. Pharmacol. Exp. Ther. 235, 757–763 (1985)