MECHANISMS OF DEPRESSANT ACTION OF MUSCLE RELAXANTS ON SPINAL REFLEXES: PARTICIPATION OF MEMBRANE STABILIZING ACTION

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The participation of local anesthetic action in spinal reflex inhibition produced by mephenesin-type muscle relaxants was examined by comparing the local anesthetic effects \((in\ vitre)\), the depressant effects on muscle afferent discharges \((in\ situ)\) and the depressant effects on spinal reflexes \((in\ situ)\) of the drugs in rats. At doses producing depression of spinal reflexes, mephenesin, tolperisone (mephenesin-type) and lidocaine (local anesthetic) reduced the frequency of afferent discharges from the muscle. The order of reducing afferent discharges by these drugs corresponded to that of their conduction blocking activities in the isolated sciatic nerve of rats. These results suggest the participation of a membrane stabilizing action in spinal reflex inhibition produced by mephenesin-type muscle relaxants. Baclofen (non-mephenesin-type) did not show any local anesthetic action.

**Keywords** --- muscle relaxant; mephenesin; tolperisone; baclofen; lidocaine; spinal reflex; muscle afferent; local anesthetic action; membrane stabilization

A centrally acting muscle relaxant, mephenesin, depresses mono- and polysynaptic reflexes in the spinal cord,\(^1\)\(^2\) and the synaptic transmission in the brain stem\(^3\) and superior cervical ganglion.\(^4\) Selectivity of action on the polysynaptic reflexes has been ascribed to the number of synapse which the drug affects.\(^5\)

However, the mechanisms of depressant action of the drug on the synaptic transmission have not been elucidated. We suggested that mephenesin stabilizes the motoneuronal membrane and resulted in inhibition of the monosynaptic spike generation.\(^6\)

It has been reported that mephenesin and procaine reduce afferent discharges from the muscle spindle.\(^7\) The depressant action of drugs on afferent discharges has been regarded as depending on the local anesthetic action of drugs \((in\ situ)\).\(^8\) Further, the local anesthetics depressed mono- and polysynaptic reflexes, and their activity on the spinal cord was roughly proportion-al to their local anesthetic potency.\(^9\)

In the present study, effects of mephenesin, tolperisone (mephenesin type),\(^10\) baclofen (non-mephenesin type)\(^11\) and lidocaine (local anesthetic) were examined on spinal reflexes, muscle afferent discharges \((in\ situ)\), and conduction of action potential \((in\ vitre)\) in rats, in an attempt to clarify the participation of membrane stabilizing action in the inhibition of the spinal reflex by mephenesin-type muscle relaxants. In addition, membrane stabilizing effects of tolperisone and lidocaine in motoneurone somata and primary afferent fibers were demonstrated using the excitability test.

MATERIALS AND METHODS

---Local Anesthetic Action---The sciatic nerves were isolated from anesthetized (urethane 1 g/kg and \(\alpha\)-chloralose 25 mg/kg, i.p.) male Wistar rats \((n = 12)\) and the desheathed tibial or peroneal nerve bundle was set at the nerve cham-
ber in vitro at about 30°C. One end of the nerve was stimulated using a pair of Ag-AgCl electrodes and the conducted compound action potentials were recorded from another end of the nerve using a pair of Ag-AgCl electrodes. Drugs were dissolved in Ringer’s solution, adjusted to pH 7.4 and applied to the nerve placed in a bath between the stimulating and recording electrodes. The concentration of drug in the bath was increased every 5 min by exchanging the drug solution until the drug finally abolishes the potentials. The action potentials just before the exchanging of solution were photographed.

Muscle Afferent Discharges — Male Wistar rats (n = 56) were anesthetized with urethane and α-chloralose (1 g/kg and 25 mg/kg, respectively). The left gastrocnemius-soleus muscle was isolated from the surrounding tissue, and then the Achilles tendon was severed distally and loaded with tension of 10 g. The left sciatic nerve was sectioned and the medial and lateral gastrocnemius nerve bundles were isolated from the tibial nerve and either of the bundles was placed on a pair of Ag-AgCl electrodes. Action potentials were displayed on an oscilloscope (Nihonkohden VC-6). Spikes higher than an arbitrary threshold were then transformed into square wave pulses and fed into an integrator (reset time 3 s), the output of which was recorded by an ink-writing recorder (San-Ei Instrument Rectigraph 8S). The drug effect as percentage of inhibition was calculated by taking the difference between the control value, and the experimental value, dividing by the control value, and multiplying by 100. The control and the experimental values represent the number of total discharges appearing for 1 min before, and from 2.5 to 3.5 min after administration, respectively. Since the arrangement in the present study did preferentially record higher amplitude impulses and a brief test stretch of the tendon produced increases of initially dynamic and subsequently static responses in their frequency, the recorded afferent impulses may be regarded as those from the muscle spinal which send the larger fiber to the spinal cord.

Spinal Reflexes — Male Wistar rats (n = 32) were anesthetized with urethane and α-chloralose. C1 spinal rats were made by transecting the cord after bilateral cervical vagotomy. Laminectomy was performed in the lumbo-sacral region. Ventral and dorsal roots below L4 were cut bilaterally and dorsal and ventral roots of segments L4 and L5 were isolated. A skin pouch was formed at the site of the dissection to cover the exposed tissues with liquid paraffin kept at 37 ± 0.5°C. Rectal temperature was maintained at 37 ± 0.5°C using a d.c. heating pad. The dorsal and ventral roots of the segment L5 were placed on a pair of Ag-AgCl electrodes for stimulation (0.2 Hz, 0.05 ms, supramaximal) and recording, respectively. Reflex potentials were amplified with an a.c. amplifier and displayed on an oscilloscope (Nihonkohden VC-6) and photographed.

Excitability Test — Excitability of the primary afferent fiber and motoneuron soma was measured in spinal rats (n = 6) according to the technique of Wall. A tungsten microelectrode insulated except its tip by cashew was inserted into the motoneuron pool, which was stimulated by negative pulses (0.2 Hz, 0.01 ms, 5 V submaximal). The antidromic action potentials which reflect excitability of the primary afferent fiber, were recorded from the dorsal root L5 using a bipolar Ag-AgCl electrode. The orthodromic action potentials which reflect excitability of the motoneuron soma, were recorded from the ventral root L5. The responses were displayed on an oscilloscope (Nihonkohden VC-7), and then photographed (four times superimposed) or averaged four times using an averaging computer (Nihonkohden ATAC-201).

Neuromuscular Junction — A pair of Ag-AgCl electrodes were placed on the severed end of the tibial nerve in the left hindlimb of urethane and α-chloralose anesthetized rats (n = 12) and used for stimulation (0.2 Hz, 0.05 ms, supramaximal). The contraction of the triceps surae muscle induced by the stimulation was recorded using an isometric transducer (Nihonkohden SB-1T).
Drugs — Drugs used were mephenesin (Myanol, Chugai), tolperisone-HCl (Nippon Kayaku), baclofen (Ciba-Geigy) and lidocaine-HCl (Iwaki). In the experiments except for local anesthesia, all were dissolved in 0.9% saline and injected into the cannulated femoral vein.

RESULTS
1. Effects on Compound Action Potentials (Local Anesthetic Action)

As shown in Fig. 1, lidocaine-HCl (1–16 $\times 10^{-3}$ g/ml), tolperisone-HCl (2.5–40 $\times 10^{-5}$ g/ml) and mephenesin (5–160 $\times 10^{-5}$ g/ml) dose-dependently reduced the amplitude of compound action potential in the isolated sciatic nerve. On the other hand, baclofen even in a concentration as high as 2 $\times 10^{-3}$ g/ml did not show any appreciable effect on nerve conduction.

2. Effects on Muscle Afferent Discharges

Lidocaine-HCl (2.5–20 mg/kg, i.v.), tolperisone-HCl (10–40 mg/kg, i.v.) and mephenesin (20–80 mg/kg, i.v.) dose-dependently reduced the afferent discharges from the deafferented triceps surae muscle. Ten mg/kg of baclofen (i.v.) did not show any appreciable effect on the afferent activity.

3. Effects on Spinal Reflexes

As shown in Fig. 3, mephenesin (50 mg/kg, i.v.), tolperisone-HCl (10 mg/kg, i.v.) and lidocaine-HCl (10 mg/kg, i.v.) depressed monosynaptic reflexes in intact and spinal rats. Although these drugs caused such depressant actions at a dose range effective for reducing afferent discharges from the muscle, the effect of tolperisone on spinal reflexes was somewhat stronger than that predicted from its depressant actions on the afferent discharges and nerve conduction. Baclofen, which had no local anesthetic action or inhibitory effect on the afferent discharges, in a dose of 2 mg/kg (i.v.) markedly depressed monosynaptic reflex.

4. Effects of Tolperisone and Lidocaine on Excitability

Effects of mephenesin and baclofen on excitability has already been reported.6) Upper and lower traces in Fig. 4 show the primary afferent fiber and motoneuron soma excitability, respectively. Second response in the lower traces is considered to be a monosynaptically evoked action potential. Tolperisone-HCl (10 mg/kg, i.v.) and

![Fig. 1. Dose-Response Relationships of Effects of Drugs on Conduction in Isolated Sciatic Nerve of the Rat](image1)

Abscissa: Concentration of drugs. Ordinate: means of relative amplitudes of compound action potentials in percentage of controls, with S.E.M. indicated (n=4).

![Fig. 2. Dose-Response Relationships of the Effects of Drugs on Resting Afferent Discharges from the Triceps Surae Muscle in Rats](image2)

The muscle was continuously loaded with 10 g tension. Abscissa: doses of drugs. Ordinate: mean of frequencies of afferent discharges in percentage of controls, with the S.E.M. indicated (n=4).
lidocaine-HCl (10 mg/kg, i.v.) reduced excitability of the primary afferent fiber (PAF) and motoneuron soma (MN) and more strongly the monosynaptic responses (MS). Thus, it seems likely that tolperisone and lidocaine produce a membrane stabilizing action at the primary afferent fiber and motoneuron soma.

5. Effects on Neuromuscular Junction

Mephenesin (80 mg/kg, i.v.), tolperisone-HCl (40 mg/kg, i.v.), lidocaine-HCl (20 mg/kg, i.v.) and baclofen (10 mg/kg, i.v.) did not reduce the twitch contraction of indirectly stimulated triceps surae muscles (n = 3, respectively).

DISCUSSION

The present study demonstrated that mephenesin, tolperisone and lidocaine reduced the afferent activity and the similar dose levels of the drugs depressed the mono- and polysynaptic reflexes (Figs. 2 and 3). Although the doses required for blocking the compound action potential in the sciatic nerve in vitro must be quite higher than those effective for reducing afferent discharge from the muscle in situ, the order of reducing afferent discharges by drugs in situ well corresponded to that of their conduction blocking activities in the isolated sciatic nerve of rats (Figs. 1 and 2). These results suggest that the membrane stabilizing actions participate in the inhibitory actions on the spinal reflex by mephenesin-type drugs. Tolperisone had somewhat stronger depressant effects on the spinal reflex than those predicted from the depressant actions on the afferent discharge and nerve conduction. Such property might depend on the preferential distribution or affinity of the drug on the central nervous system. We have no data to support the possibility, however. Baclofen did not show any membrane stabilizing action, instead showing strong and preferential depressant action on the monosynaptic reflex. This supports our earlier results that the effects of baclofen are not due to the membrane stabilization like local anesthesia on neuronal membranes. 

Depression of excitability of primary afferent fiber and motoneuron soma by tolperisone and lidocaine (Fig. 4) supported above suggestion. Both drugs may stabilize initial segment (IS) of

![Effects of Drugs (i.v.) on Spinal Reflexes in Intact (A) and Spinal (B) Rats](image-url)

Reflexes were recorded from the ventral roots (L5) in response to stimulations of the ipsilateral dorsal roots (L5). The recordings consist of 5 superimposed tracings. Calibrations: 1 mV, 3 ms.
motoneuron and inhibit the spike generation from the EPSP like mephenesin.\textsuperscript{63} Initiation of the antidromic action potential in the excitability test was inhibited by the drugs; however, once the action potential was initiated in the primary afferent fiber, it was hardly influenced by the drugs in a dose range effective in \textit{in situ} experiment and could conduct along the afferent fiber. These possibilities are supported by the finding of Curtis and Phillis that iontophoretically applied procaine prevented the initiation of neuronal spike without affecting EPSP.\textsuperscript{14} The membrane of motoneuron appeared to be more susceptible to the action of procaine than that of the terminal afferent fibers.\textsuperscript{14,15} The conduction of action potential along axon may be more resistant to local anesthetics than the generation of action potential from the EPSP at initial segment of motoneuron, because the conduction of action potential has a high safety factor. However, these drugs could not block the neuromuscular transmission. This may be due to the extremely higher amplitude of the end-plate potential (EPP) than that needed to trigger the action potential in skeletal muscle fibers.

The local anesthetics, when given intravenously in small doses, produce anticonvulsive or anesthetic effect.\textsuperscript{16} Convulsion produced by higher doses of local anesthetics has been ascribed to the depression of inhibitory transmission.\textsuperscript{16} Recently, therapeutic effects on the status epilepticus of lidocaine has been reported.\textsuperscript{17,18} These effects may be due to a block of spike generation in neurones in central nervous system as suggested in the present study.

Muscle spindles together with their afferent fibers and \( \gamma \)-motoneurons (the \( \gamma \)-motoneuron-muscle spindle loops) constitute an important complex within the system for controlling motor activity.\textsuperscript{19} Some drugs which pre-

\[ \text{FIG. 4. Effects of Tolperisone-HCl (10 mg/kg, i.v.) and Lidocaine-HCl (10 mg/kg, i.v.) on Excitability of Primary Afferent Fiber and Motoneuron Soma} \]

\[ \text{Abscissae: time in min after the drug injection. Ordinates: means of relative response amplitudes of evoked potentials in percentage of controls, with the S.E.M. indicated (n=3). Where the S.E.M. bars are not shown they lie within the dimensions of the symbols. PAF: excitability of an afferent fiber. MN: excitability of a motoneuron soma. MS: monosynaptic response produced in the ventral root.} \]
ferentially reduce afferent activity from muscle spindle have been reported to produce marked muscle relaxation.20-22 These facts together with the present results suggest that depressant effects of mephenesin-type muscle relaxants on afferent activity may provide supportive effects on the relief of spastic movements caused by exaggerated γ-loop.

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