Research on the H-reflex of Rabbits with Experimental Spinal Cord Injury ; Effects of a GABAB Agonist and a GABAB Receptor Agonist Positive Modulator on the H-reflex

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ABSTRACT. The H-reflex is sometimes used for evaluation of spasticity following spinal cord injury (SCI). Since it is difficult to perform any human experimental research related to changes in the H-reflex after the administration of new medicines or after invasive operations, I created an experimental SCI model using rabbits, and compared the waveforms of the H-reflex before and after the creation of SCI. The effects of intrathecal infusion of muscle relaxants on the H-reflex were also investigated. At first, appearance of the H-reflex before and 1 week after creating SCI was observed, and the amplitude ratio of the maximum H-reflex and the maximum M-wave (H/M ratio) was calculated. Then, the effects of baclofen (GABAB receptor agonist) and CGP7930 (GABAB receptor agonist positive modulator) on the H/M ratio of the SCI rabbits were investigated. The H-reflex was evoked by smaller amplitude stimulation after the creation of SCI than before. The H/M ratio after the creation of SCI was significantly higher than before the creation. The mean H/M ratio after infusion of baclofen (10 nmol) was significantly lower than before infusion. Single infusion of CGP7930 had no effect on the H/M ratio. The mean H/M ratio after combined infusion of baclofen (10 nmol) and CGP7930 (1 nmol) was significantly lower than before infusion, and the reduction was more significant than that after single infusion of baclofen. It was ascertained that the increase in the H/M ratio after the creation of SCI is caused by interception of central descending pathways projecting to Ia inhibitory interneurons and that baclofen activated the presynaptic GABAB receptors on Ia afferent terminals. If single infusion of baclofen is not effective in any patients, additional infusion of CGP7930 may be used for the management of severe spasticity in the future.

Key words : H-reflex — spinal cord injury — GABAB receptor agonist — GABAB receptor agonist positive modulator

Tendon reflexes become hyper and muscle tone increases gradually in many patients with upper motor neuron disorders, such as cerebrovascular accident (CVA) and spinal cord injury (SCI), in their subacute or chronic phases. This condition is called spasticity. When the degree of spasticity is mild, patients can walk and perform the activities of daily living (ADL)

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independently or with little assistance by utilizing spasticity practically after rehabilitation training. However, unexpected abnormal movements, secondary muscular spasms, or joint contractures that are caused concurrently in the patients become obstacles to ADL, if the spasticity is excessive.

The Hoffmann-reflex (H-reflex) first reported by Paul A. Hoffmann in 1910 is a name of an evoked potential that is used as one of the quantitative evaluation methods for spasticity. Generally, the tibial nerve is stimulated electrically at the popliteal region, and an evoked potential of the gastrocnemius is recorded from surface electrodes placed on the posterior part of the lower leg. The H-reflex, the wave of the evoked potential, cannot be recorded from every human muscle, so recordings are limited to some muscles, such as the triceps surae, quadriceps femoris, flexor hallucis brevis, and flexor carpi ulnaris for healthy persons.[1] Since recordings of the H-reflex are stable in the soleus, one of the triceps surae, the soleus is most often used in studies related to the H-reflex.[1-3] Ia afferent sensory impulses elicited by electrical stimulation of the tibial nerve pass through the posterior cord, and monosynaptically activate motoneurones. The reflex responses descend down the motor fibers of the same tibial nerve, and evoke action potentials in the innervated muscle fibers. The summation of these waves is H-reflex.[3] In upper motor neuron disorders such as CVA and SCI, the central descending pathways projecting to Ia inhibitory interneurons are intercepted. Since these interneurons act presynaptically on the gamma-aminobutyric acid (GABA) B receptors existing on Ia afferent terminals and decrease tendon reflexs, excitement of the lower motor neurons becomes excessive and, as a result, spasticity arises in patients with upper motor neuron disorders.

Hiersemenzel *et al*[4] reported serial changes in the H-reflex of human SCI, and Milanov[6] and Suzuki *et al*[5] reported ones in the H-reflex of CVA. Their reports indicated that the H-reflex is evoked easily with these conditions and that amplitude of the H-reflex increases.[4] Various forms of medical treatment including physical medicine, therapeutic exercise, and medication have been tried to help patients with spasticity.[7] Muscle relaxants are divided into two types based on their activate site ; that is, central and peripheral mechanism. Motor point blocks using phenol or botulinum toxin have sometimes been performed in patients whose severe spasticity could not be treated with oral medication.[7-10] Baclofen, a GABAB receptor agonist, and a central acting muscle relaxant, is usually used in clinics for the medical treatment of spasticity. It has been reported that baclofen decreases calcium ion influx into the Ia afferent terminals by changing the action of GABAB receptors in the terminals, and that glutamate release is inhibited. Consenquently, monosynaptic transmission is suppressed.[8,11] When oral administration of baclofen is not effective, enough continuous intrathecal infusion is tried for some patients. Since intrathecal infusion is effective in 1/50 of the dose given orally, this method is spreading gradually as a new therapy.[11,12] However, in a few patients, this therapy is ineffective. Urwyler *et al*[13] reported in their in vitro study that the action of baclofen on GABAB receptors was reinforced by combined administration of 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol

(CGP7930), a GABAB receptor function agonist positive modulator, to the GABAB receptors in cultured cortical neurons of the rat.

In the research work on experimental SCI models, rats, cats and dogs are often used. There have been few reports, however, on the appearance of spinal reflex in normal and SCI models using rabbits.[14] Although rats, cats, and dogs can eventually walk even after their spinal cords are transected completely, SCI rabbits cannot acquire the ability to walk, which is also the case for SCI.[15] Therefore, I examined the appearance of the spinal reflex electrophysiologically in detail before and after creating SCI using rabbits, and investigated the effects of CGP7930 on the reflex.

MATERIALS AND METHODS

1) Materials

The materials I used in the experiments were 94 male Japanese white rabbits (mean weight, 2.9 ± 0.4 kg). The animals were allowed free access to solid rabbita feed and water. All animals were reared in animal quarters where the temperature was kept at 25°C. Halothane or propofol (Diprivan®) was used for anesthesia in the experiments (1) and (2), while the other experiments were performed only by propofol anesthesia.

Concerning the handling of the animals and the experiments, approval of the Animal Experimentation Committee of Kawasaki Medical School was obtained (approval number : 02-071), and I performed them according to the ethical regulations that the Committee provided.

2) Methods for Recording H-reflex

The rabbits were placed in the prone position, and their extremities were fixed to an operating table with a rope. After the skin was incised extensively on the positerior part of a posterior extremity, the fascia between biceps femoris and semitendinosus were torn in the middle. The sciatic nerve, common peroneal nerve, tibial nerve, and sural nerve were surrounded by abundant fat. These fatty tissues were removed using a clamp carefully to avoid applying strain to the nerves. The common peroneal nerve was resected beforehand so that evoked potentials from other muscles innervated by the peroneal nerve would not be picked up during the recording of the H-reflex. The hip and knee joints were fixed in the extended position, and the ankle joint was manually fixed at 70° plantar flexion during measurements (Fig 1).

The stimulation electrode used consisted of a pair of hook-shaped electrodes whose width between the anode and the cathode was 3 mm. The stimulation electrode was attached to the clip of an instrument for fixation, and the tip of the electrode was placed on the tibial nerve. The duration of the electrical stimulation was 0.01 msec, and the stimulation frequency was 0.5 Hz. The M-wave and the H-reflex were recorded while the stimulation intensity increased 0.1 mA at a time gradually until the M-wave reached supramaximal amplitude. Wire electrodes were used as the recording electrode, reference electrode, and grounded electrode. The bared part of the electrode placed at the tip of the needle was 10 mm in length. The recording electrode was placed under the skin on the plantar side near

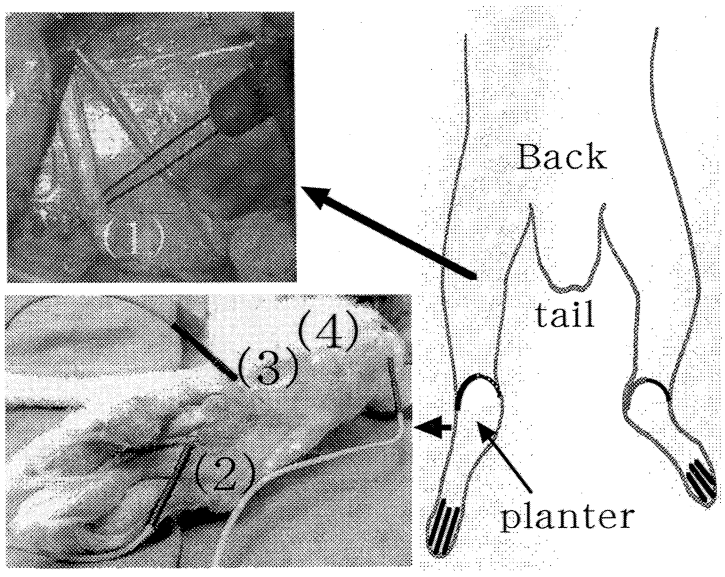

Fig 1. Positioning of extremities and recordings of the H-reflex
The experiments were performed in prone posture. The hip and knee joints were extended, and ankle joint was maintained at 70°
(1) : simulation electrode ; (2) : reference electrode ; (3) : recording electrode ; (4) : grounded electrode.

the second metatarsus, while the reference electrode was placed under the skin on the plantar side of the second metatarso phalangeal joint.[16-18] The grounded electrode was placed between the stimulation electrode and the recording electrode. The low and high frequency filters were used to record the waves ranged from 20 Hz-3 kHz. The Neuropack-Σ (Nihon Kohden Co.) was used for electrical stimulation and recording in this study. Each waveform was stored on floppy discs, and drawn on recording paper. Differences in the waveforms between Group 1 and Group 2 were compared. The peak-to-peak amplitude of the M-wave and the H-reflex was analyzed.

The following factors were examined : (1) the effects of anesthesia on the H-reflex ; (2) the stable method for creating SCI and identification of the H-reflex ; (3) the comparison of the H-reflex in SCI and normal rabbits ; (4) the relationship between muscle relaxants and the H-reflex ; (5) the effects of CGP7930 on the H-reflex.

3) Effects of anesthesia on the H-reflex

Twenty rabbits were anesthetized with halothane (Group 1) and 10 rabbits were anesthetized with propofol (Group 2). The amplitudes of the H-reflex were compared between 10 rabbits in Group 1 and 5 rabbits in Group 2. The other 15 rabbits were used for the establishment of anesthesia or for experimental maneuvers.

After 0.5 ml/kg of pentobarbital sodium (Nembutal®) was injected into the auricular vein, 2% of halothane was used continuously for inhalation anesthesia in Group 1. The dose of anesthesia was suitably changed in the

range without pain to investigate the effect of the depth of anesthesia on the waveforms of the H-reflex. If the rabbits would move their bodies, additional halothane would have been inhaled to minimize their pain. As the induction of anesthesia in Group 2, 10 mg/kg of propofol was injected through a 22-gauge indwelling injection needle that was placed in the auricular vein. Induction of an adequate depth of anesthesia was confirmed with painful stimuli to the tip of earlobe. The depth of anesthesia was maintained by infusing 60 mg/kg/hr of propofol using a microinjection pump. If the rabbits moved their bodies during the experiment, 3 mg/kg of propofol was injected additionally to minimize their pain. All rabbits in both groups inhaled 1 l/min of oxygen continuously during the experiment.

4) Stable method for creating SCI and identification of the H-reflex

Following the experiment described above, I tried to create an experimental SCI model using 10 rabbits in Group 1 and 5 rabbits in Group 2. The anesthesia was the same as in the first experiment. The skin was incised along the midline of the back between the twelfth thoracic spine (Th11) and the first lumbar spine (L1), and the thoracolumbar fascia was exposed. The thoracolumbar fascia was incised on the midline and the interspinous ligaments between the Th11 and the L1 (Th11/Th12 & Th12/L1) was removed. After the muscles attached to the transverse process, spinous process, and vertebral arch of Th12 were exfoliated, a laminectomy of Th12 was performed to expose the spinal cord. Following additional volus infusion of 5 mg/kg of propofol to maintain the condition of deep anesthesia, the SCI model was created by complete transection of the spinal cord using a surgical knife. Immediately after creating the SCI, hemostasis was accomplished with bone wax, and the skin and subcutaneous tissues incised in the back were sutured completely.

The rabbits were maintained in animal quarters where the temperature was kept at 25°C for 1 week. Clean intermittent catheterization for the management of dysfunctional voiding using 6Fr catheters, frequent changes in position to prevent pressure ulcers, and range of motion exercises to prevent contractures were carried out on the rabbits every day. One week after creation of the SCI, the contralateral tibial nerve was exposed, and the H-reflex was evoked.

Although it is reported that the tibial nerve was innervated by 2 dorsal roots (7th lumbar and 1st sacral roots), it takes a lot of time to resect both of the roots. Then, elimination of the H-reflex was attempted by resection of one of the dorsal roots connected with the tibial nerve in 4 rabbits in Group 1 and 5 rabbits in Group 2. The skin was incised along the midline of the back between L6 and the third sacral spine (S3), and the interspinous ligament between S1/S2 was removed. After the muscles attached to the mastoid processes, spinous processes, and vertebral arches were exfoliated, interlaminar fenestration between S1 and S2 was performed using a microscope and a dental drill. The first sacral dorsal root was resected microscopically.[15,19]

5) Comparison of the H-reflex in SCI and normal rabbits

The H-reflex and the M-wave in the tibial nerve were recorded using 44

normal rabbits different from the previous experiments as control data. After the recordings, I attempted to create an SCI in all the rabbits. Since 15 rabbits died during the first week, electrophysiological measurements were performed on the contralateral tibial nerve of the surviving 29 SCI rabbits 1 week after the operation.

After measuring the amplitude of the H-reflex and the M-wave, the amplitude ratio of the maximum H-reflex and the maximum M-wave (H/M ratio) was calculated. The mean H/M ratio in the normal rabbits was compared to that in the SCI rabbits using the Student t-test. In addition, the H/M ratio before and 1 week after the creation of SCI was compared using paired t-test for the 29 rabbits that survived after the creation of SCI.

6) Relationship between muscle relaxants and the H-reflex

To examine the effects of central acting muscle relaxant on the H-reflex, baclofen was administered to 4 SCI rabbits. The vehicle was administered to 5 other SCI rabbits as a control. Baclofen or the vehicle was injected intrathecally. The same dose of propofol was used for anesthesia during both the operation and intrathecal infusion, as mentioned before. The skin was incised along the midline of the back between L4 and L7, and the interspinous ligaments between L5/L6 and between L6/L7 were removed. After the muscles attached to the transverse processes, spinous processes, and vertebral arches were exfoliated, a partial laminectomy of L5 and L6 was performed microscopically. A polyethylene catheter (SP-8 : 0.2 mm in its inner diameter and 0.5 mm in its outer diameter) filled beforehand with lactated Ringer's solution (Vein F, pH 7.0) was inserted from the laminectomized portion of L6 into the epidural space, and the tip of the catheter was put under L7. Dura mater near L7 was incised with a 20-gauge injection needle for drip infusion, and the tip of the catheter was advanced about 10 mm into the subarachnoidal space. One drop of quick-drying glue was dripped onto the dura mater to fix the catheter and to close up the space between the dura mater and the catheter. The skin was incised on the posterior part of the neck. The proximal end of the catheter was led to outside from the posterior part of the neck through the subcutaneous tissue of the back. The catheter was looped at two places (L4, Th12) subcutaneously, and was fixed with quick-drying glue.

After dissolving the baclofen powder with 1N HCI, it was diluted with lactated Ringer's solution 10 times, and the concentration was adjusted to 4 nmol/μl (3 rabbits) and 10 nmol/μl (1 rabbit). Then 1 μl of baclofen solution was applied intrathecally into the four rabbits at a speed of 4 μl/min, while 1 μl of the vehicle was administered intrathecally to the 5 control rabbits. The H-reflex was measured before infusion, and 30, 60, 90, and 120 min after infusion.

7) Effects of CGP7930 on the H-reflex

The effects of baclofen and CGP7930 on the H-reflex of the SCI rabbits were investigated. Nineteen rabbits were newly operated on to create an SCI model. From the 4 to 7 days after the creation of SCI, the H-reflex was measured in all rabbits.

After the measurements, 1 μl of the solution of baclofen, the concentration of which was 10 nmol/μl, was infused intrathecally in 4 rabbits (Group A). The solution of baclofen was made using the same method as that used in the previous experiments.

After dissolving the CGP7930 powder with 99.5% of ethanol, it was diluted with lactated Ringer's solution 100 times, and the concentration was adjusted to 1 nmol/μl. Then 1 μl of the solution of CGP7930 was infused intrathecally in 5 rabbits (Group B). In addition, 1 μl of a solution of both baclofen and CGP7930 was infused into another 5 rabbits (Group C). As a control, 1 μl of the vehicle was infused into another 5 rabbits (Group D). The H-reflex was measured before infusion of the agents, and 30, 60, 90, and 120 minutes after infusion.

The H/M ratio before infusion was compared with that after infusion of the agents using the paired t-test. If there were significant differences in the H/M ratio before and after infusion of the agents, the decreasing rate (%) in the H/M ratio was calculated by the following formula [H/M ratio after infusion/H/M ratio before infusion] $\times$ 100. The mean decreasing rate in amplitude of the H/M ratio after infusion of each agent was compared with each other using the Student t-test.

RESULTS

1) Effects of anesthesia on the H-reflex

In Group 1 halothane was used for anesthesia. The first appeared wave was the M-wave, as the intensity of the stimulation increased. The latency was about 5.0 msec. After the stimulation gradually increased more, a waveform with low amplitude appeared in a latency of about 10 msec, later than the M-wave. Although this waveform was considered to be the H-reflex, there was no proof that it was the H reflex at this time. Then, I named the waveform long latency wave (LL) temporarily here. When the stimulation increased more and more, the amplitude of the LL reached the maximum, and gradually decreased after that. The amplitude ratio of the

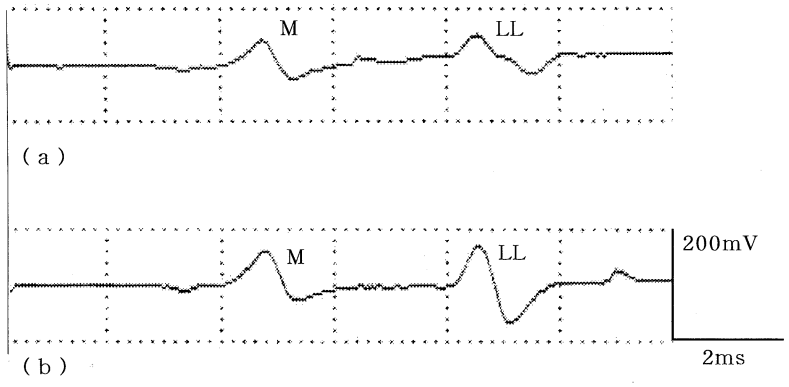

Fig 2. Effects of anesthesia on the H-reflex
(a) The H-reflex of the normal rabbit anesthetized with enough dose of halothane.
(b) The amplitude of LL increased when the depth of anesthesia using halothane became shallow. M : M-wave ; LL : long latency wave.

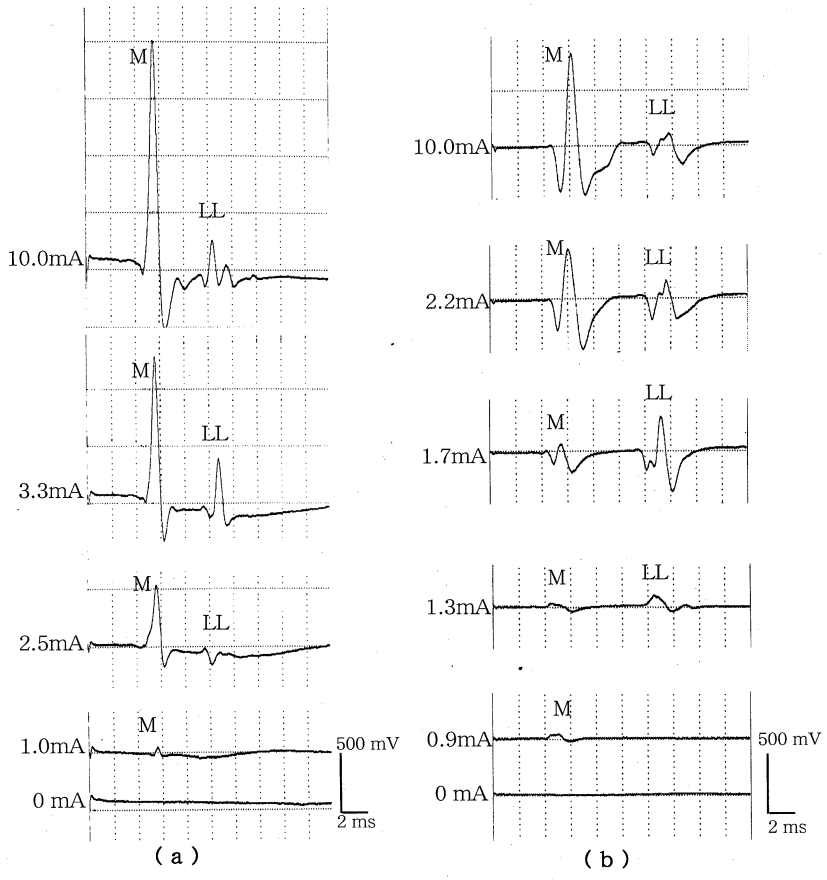

Fig 3. Waveform of the H-reflex of normal and SCI rabbit anesthetized with propofol (a) Normal rabbit. (b) SCI rabbit. The amplitude of LL changes in accordance with the intensity of stimulation. M : M-wave ; LL : long latency wave.

maximum LL and the maximum M-wave (LL/M ratio) was 0.23 ± 0.05.

The amplitude of the LL increased when the depth of anesthesia using halothane became shallow (Fig 2). On the other hand, the amplitude of the LL decreased when the condition of anesthesia was returned. Since the rabbits did not move their bodies during the experiment, I judged that the rabbits did not feel pain.

In Group 2 propofol was used for anesthesia. The LL was evoked in 2 of 5 rabbits by small amplitude stimulation that the M-wave was not evoked. Although the order of the appearance of the LL was the same as that in animals with halothane anesthesia in 3 other rabbits (Fig 3a), the amplitude of the LL seemed to be higher than in Group 1.

2) Stable method for the creation of SCI and identification of the H-reflex

Although the number of the rabbits in which I attempted to create SCI was 15 (Group 1 : 10 rabbits, Group 2 : 5 rabbits), 2 rabbits died of massive leakage of cerebrospinal fluid into the epidural space, and 1 rabbit died of a urethral injury. The rabbits anesthetized with propofol were

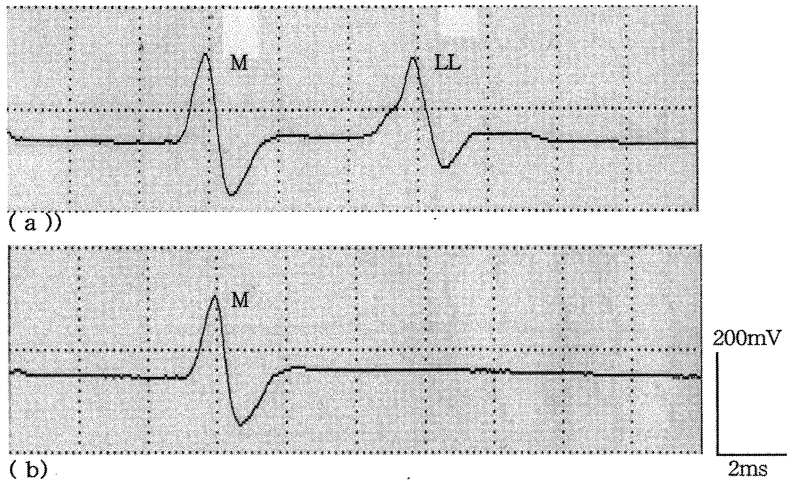

Fig 4. Effect of dorsal root resection on the H-reflex
(a) Recording before resection. (b) Recording after resection. M : M-wave ; LL : long latency wave.

awakened earlier than those anesthetized with halothane.

The LL was evoked by stimulation of smaller amplitude after the creation of SCI than before in Group 2 (Fig 3b). In addition, the LL/M ratio after the creation of SCI seemed to be higher than before they were made. The amplitude of the LL became considerably lower immediately after the S1 dorsal root was resected (Fig 4). Therefore, the LL was identified as the H-reflex. The conduction velocity of the H-reflex (HCV) could be calculated from the latency of the H-reflex (HL : msec), the latency of the M-wave (ML : msec), and the distance between the stimulation point and the transverse process of the L7 (L : mm). It is considered that there is a minimal delay of 1.0 msec at the synaptic cleft. When the HCV was calculated using the equation [HCV=2L/(HL-ML-1)], the HCV was estimated to be 80 ± 10 m/sec.

3) Comparison of the H-reflex in SCI and normal rabbits

The mean H/M ratio was 0.28 ± 0.13 before the creation of SCI (n=44), and 0.43 ± 0.28 after their creation of SCI (n=29). The H/M ratio after the creation of SCI was shown to be statistically higher than before their creation (p=0.022) (Fig 5).

Concerning only the 29 rabbits who survived after the creation of SCI, the mean H/M ratio before the creation of SCI was 0.29 ± 0.13, and that after the creation of SCI was 0.43 ± 0.28. There is a significant difference between the two mean values when analyzed by the paired t-test (p=0.002) (Fig 5).

4) Relationship between muscle relaxants and the H-reflex

In 3 of 4 rabbits that 4 nmol/μl of baclofen was administered, the mean H/M ratio was 0.35 before infusion and 0.28 after infusion of baclofen. In the remaining 1 rabbit, the H/M ratio also decreased from 0.40 to 0.29 following the administration of 10 nmol/μl of baclofen. The

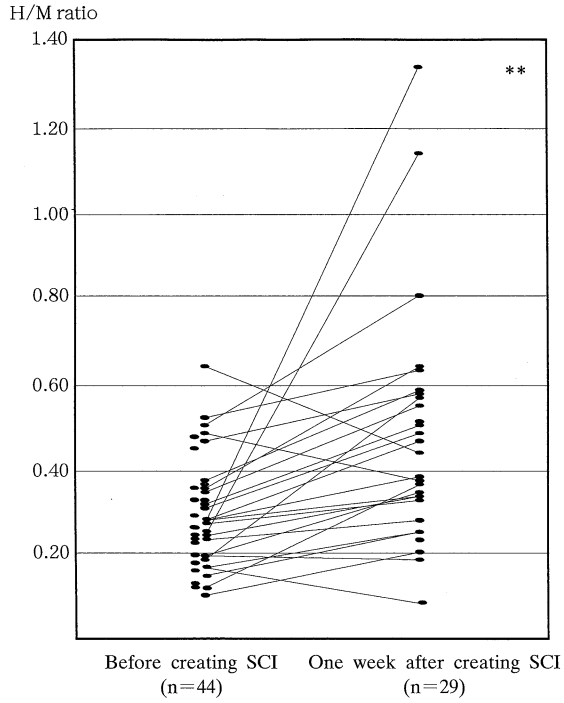

Fig 5. Comparison of the H/M ratio between before and after creating SCI
H/M ratio : amplitude ratio of the maximum H-reflex and the maximum M-wave. Double asterisk (**) means $p<0.01$.

lowest values of the H/M ratio were obtained 90 min after infusion in all 4 rabbits. So the H/M ratio 90 min after infusion of each drug was compared to the control. The mean H/M ratio was 0.45 before infusion, and 0.43 after infusion of the vehicle. There were no significant differences before and after infusion.

5) Effects of CGP7930 on the H-reflex

The mean H/M ratio was 0.29 ± 0.07 before infusion of baclofen, and 0.17 ± 0.08 after infusion in Group A ($n=4$). The H/M ratio after infusion of baclofen was significantly lower than before infusion ($p=0.02$). The lowest values of the H/M ratio were obtained 90 min after infusion in 3 rabbits, and 120 min after infusion in 1 rabbits. The mean H/M ratio was 0.35 ± 0.06 before infusion of CGP7930, and 0.34 ± 0.07 after infusion in Group B ($n=5$). There were no significant changes before and after infusion of CGP7930 alone ($p=0.46$). The mean H/M ratio was 0.38 ± 0.06 before combined infusion of baclofen and CGP7930, and 0.14 ± 0.01 after infusion in Group C ($n=5$). The H/M ratio after combined infusion was significantly lower than before infusion ($p=0.001$). The lowest values of the H/M ratio were obtained 90 min after infusion in 2 rabbits, and 120 min after infusion in 3 rabbits. The mean H/M ratio was 0.37 ± 0.09 before infusion of the vehicle, and 0.36 ± 0.08 after infusion in Group D ($n=5$). There were no significant changes before and after infusion of the vehicle

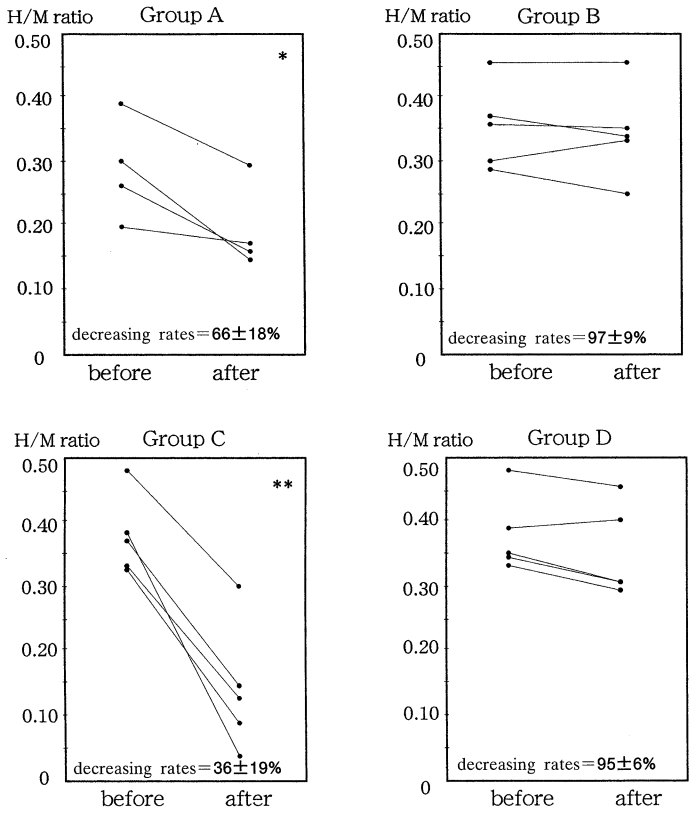

Fig 6. Effects of baclofen and CGP7930 on the H-reflex
Group A : baclofen ; Group B : CGP7930 ; Group C : combined baclofen and CGP7930 ; Group D : vehicle. Double asterisk (* *) means $p < 0.01$, and single asterisk (*) means $p < 0.05$.

($p=0.13$) (Fig 6).

The decreasing rates (%) in the H/M ratio was calculated for Group A and Group C. The mean decreasing rate in Group C ($36 \pm 19\%$) was more remarkable than in Group A ($66 \pm 18\%$) ($p=0.043$).

DISCUSSION

1) The H-reflex in normal and SCI

The H-reflex is used as one of the quantitative methods for evaluation of spasticity following upper motor neuron disorders, and it involves a type of compound muscle action potentials (CMAP) elicited by electrical stimulation of the tibial nerves. Ia afferent impulses passing through the posterior cord are said to monosynaptically activate motoneurones.[3] The reflex responses are conducted through the motor fibers of the same tibial nerve and evoke the CMAP in the innervated muscle. Recording of the H-reflex in humans is not easy, and the electrical stimulation should be small to obtain a clear waveform, because the reflex responses through the alpha-motor fibers may collide with the antidromic motor impulses that the

electrical stimulation activates simultaneously. Additionally, the amplitude of the H-reflex is small under normal conditions, because this monosynaptic reflex is inhibited presynaptically by the upper neurons.

In the present study, the H-reflex was evoked by smaller amplitude stimulation and the H/M ratio was significantly higher in SCI rabbits than in normal ones. The reason why the H-reflex was evoked easily after the creation of SCI is based on interception of the central descending pathways projecting to Ia inhibitory interneurons. This result was in good agreement with past reports using rats, cats, and dogs.[20,21] SCI humans and SCI rabbits cannot walk, while SCI rats and SCI cats are said to be able to walk independently. Hiersemenzel *et al*[4] reported that the H-reflex already appeared during spinal shock, whereas the H/M ratio was lower in SCI humans than normal for long period. It is said that the H/M ratio does not change until about eight weeks after injury and that it increases gradually after that. It increases earlier in SCI animals than in SCI humans, because the period of spinal shock is shorter in animals. It has been reported that the period of spinal shock is several weeks in humans, several days in monkeys, and several hours in dogs and cats.[22] To the best of our knowledge, there have been no reports about the period of spinal shock in rabbits, but the period may be short if judged by the present results.

2) Factors affected to the wave of the H-reflex

Since the waves of the H-reflex are affected by many factors such as the position of the extremities, posture, muscle contraction condition, and emotional changes, recordings of the H-reflex in humans are considered to be unstable.[1,19,21,23] The recordings of the H-reflex in this study were kept relatively stable by keeping the positions of the extremities and posture constant. However, it became clear that the waves tend to be affected by anesthesia, that is, halothane seems to inhibit appearance of the H-reflex. Because halothane has an action like that of a GABAB receptor agonist, it is not desirable to use halothane for H-reflex related research. As Jewett *et al*[24] reported that propofol did not affect monosynaptic reflexes, it was proved that propofol anesthesia was useful to the recording of the H-reflex in this study.

The style of apearance of the H-reflex in rabbits differed somewhat from that of the H-reflex in humans. Unlike in humans, the H-reflex in rabbits was hardly recordable with a small stimulus that M-wave could be barely recorded. Moreover, the H/M ratio was a little lower in the rabbits than in humans. It was reported that the mean H/M ratio was about 0.5 in healthy persons.[4] These results were similar to those in reports about rats.[25] Although it is considered that this is due to the higher susceptibility of Ia afferent fibers to electrical stimulation or to the wide distribution of conduction velocities, more research is needed in the future.

3) Effects of baclofen and CGP7930 on the H-reflex

It is well known that GABAergic interneurons participate in presynaptic inhibition of the H-reflex, and that GABAB receptors exist on Ia afferent terminals. Baclofen, a GABAB receptor agonist, has been reported to act

on the GABAB receptors, and to inhibit the appearance of the H-reflex. In the present study, I confirmed these results using rabbits. It have been reported that baclofen changes the action of GABAB receptors by decreasing calcium ion currents in the Ia afferent terminals. Urwyler *et al*[13] reported in their in vitro study using cultured cortical neurons of the rat that influx of calcium ions from the calcium channels decreased more after combined administration of baclofen and CGP7930 than after single administration of baclofen. CGP7930 is said to act positively only in the presence of a GABAB receptor agonist. Since there have been no in vivo studies about the action of CGP7930 on the spinal cord, our study appears to be the first one. Because the reduction in the H/M ratio after combined infusion of baclofen and CGP7930 was more remarkable than the reduction in the H/M ratio after single infusion of baclofen, it is supposed that CGP7930 acts positively anywhere near the GABAB receptors on Ia afferent terminals. However, the single infusion of CGP7930 had no effect on the H/M ratio. So, CGP7930 itself is not a GABAB receptor agonist. Although CGP7930 seems to have some function in increasing the influx of calcium ion, the exact mechanism is unclear. Much is expected from future research.

Application of intrathecal infusion of baclofen for medical treatment is performed only in a restricted number of institutions now. The embedded type of continuous intrathecal infusion of baclofen will be introduced into Japan in 2004, and there is great hope for new medical treatment for spasticity with it. Until now, when several medicines prove ineffective as medical treatment for spasticity, rhyzotomy or myelotomy has sometimes been performed as surgical treatment. After further research has been performed on the side effects of CGP7930, additional infusion of CGP7930 may become a valuable form of treatment for the management of severe spasticity.

In conclusion, the H/M ratio was significantly higher in the SCI rabbits than in the normal rabbits. Baclofen was confirmed to act as a GABAB receptor agonist and to also inhibit the appearance of the H-reflex in rabbits. Furthermore, inhibition of the H-reflex was more remarkable after combined intrathecal infusion of baclofen and CGP7930 than after single infusion of baclofen.

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REFERENCES

1) Tanaka R : H-reflex of human : as a nearophysiological examination. J Physiol Soc

Jpn **48** : 719-734, 1986 (in Japanese)
2) Fisher MA : AAEM minimonograph #13 : H-reflexes and F-waves : Physiology clinical indication. Muscle Nerve **15** : 1223-1233, 1992
3) Hase K : Clinical application of electromyogram. J Clin Rehabil **10** : 614-622, 2001 (in Japanese)
4) Hiersemenzel LP, Curt A, Dietz V : From spinal shock to spasticity : neuronal adaptations to a spinal cord injury. Neurology **54** : 1574-1582, 2000
5) Suzuki T, Saitoh E, Tani M, Nabeta R, Daikuya S, Hirose H, Wakayama I, Fujiwara T : Characteristic appearances of the H-reflex and F-wave with increased stimulus intensity in patients with cerebrovascular disease. Electromyogr Clin Neurophysiol **42** : 67-70, 2002
6) Milanov I : Neurographic studies in hemiplegic patients. Funct Neurol **10** : 77-82, 1995
7) Asayama Y : Treatment of spasticity of cerebrovascular disease. Jpn J Rehabil Med **34** : 346-357, 1997 (in Japanese)
8) Kato J : Treatment of spasticity of the central acting muscle relaxants. Pain Clin **22** : 1693-1697, 2001 (in Japanese)
9) Sato F, Mano Y : Botulinum toxin and the management of spasticity. Jpn J Rehabil Med **37** : 465-482, 2000 (in Japanese)
10) On AY, Kirazli Y, Kismali B, Aksit R : Mechanisms of action of phenol block and botulinus toxin type A in relieving spasticity : electrophysiologic investigation and follow-up. Am J Phys Med Rehabil **78** : 344-349, 1999
11) Taira T, Hori T : Spinal intrathecal baclofen. J Clin Exp Med **189** : 833-837, 1999 (in Japanese)
12) Nance P, Meythaler J : Intrathecal drug therapy. Phys Med Rehabil Clin North Am **10** : 385-401, 1999
13) Urwyler S, Mosbacher J, Lingenhoehl K, Heid J, Hofstetter K, Froestl W, Bettler B, Kaupmann K : Positive allosteric modulation of native and recombinant gamma-aminobutyric acid (B) receptors by 2,6-Di-tert-butyl-4-(3-hydroxyl-2, 2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. Mol Pharmacol **60** : 963-971, 2001
14) Messina C, Cotrufo R : Different excitability of type 1 and type 2 alpha-motoneurons. The recruitment curve of H- and M-responses in slow and fast muscles of rabbits. J Neurol Sci **28** : 57-63, 1976
15) Greenaway JB, Partlow GD, Gonsholt NL, Fisher KR : Anatomy of the lumbosacral spinal cord in rabbits. J Am Anim Hosp Assoc **37** : 27-34, 2001
16) Cliffer KD, Tonra JR, Carson SR, Radley HE, Cavnor C, Lindsay RM, Bodine SC, Distefano PS : Consistent repeated M- and H-wave recording in the hind limb of rats. Muscle Nerve **21** : 1405-1413, 1998
17) Simpson RK, Gondo M, Robertson CS, Goodman JC : The influence of glycine and related compounds on spinal cord injury-induced spasticity. Neurochem Res **20** : 1203-1210, 1995
18) Simpson RK, Robertson CS, Goodman JC : The role of glycine in spinal shock. J Spinal Cord Med **19** : 215-24, 1996
19) Hagiya N : Modulation of spinal monosynaptic reflexes during rhythmical jaw movements and its central neural mechanisms. Kokubyo Gakkai Zasshi **61** : 21-38, 1994 (in Japanese)
20) Handa Y, Naito A, Watanabe S, Komatsu S, Shimizu Y : Functional recovery of locomotive behavior in the adult spinal dog. Tohoku J Exp Med **148** : 343-384, 1986
21) Chen XY, Wolpaw JR, Jakeman LB, Stokes BT : Operant conditioning of H-reflex increase in spinal cord injured rats. J Neurotrauma **16** : 175-186, 1999
22) Nakayama Y : A Textbook of Physiology. 2th ed, Tokyo, Chyugai Igaku. 1991, p55-84 (in Japanese)
23) Tanaka T : Depression of reciprocal Ia inhibition of crural motoneurons during rhythmical jaw movement in rabbit. Kokubyo Gakkai Zasshi **63** : 153-163, 1996 (in Japanese)
24) Jewett BA, Gibbs LM, Tarasiuk A, Kendig JJ : Propofol and barbiturate depression of spinal nociceptive neurotransmission. Anesthesiology **77** : 1148-1154, 1992
25) Gozariu M, Roth V, Keime F, Le Bars D, Willer JC : An electrophysiological investigation into the monosynaptic H-reflex in the rat. Brain Res **782** : 343-347, 1998