Functional Change in the Rat Spinal Cord by Chronic Spinal Transection and Possible Roles of Monoamine Neurons

Tsugutaka ITO, Kiyoshi FURUKAWA, Tadahiko KARASAWA, Toshiaki KADOKAWA and Masanao SHIMIZU
Research Laboratories, Dainippon Pharmaceutical Co., Ltd., 33-94 Enoki-cho, Suite, Osaka 564, Japan

Accepted March 26, 1985

Abstract—Two types of spinal reflex responses, extensor reflex and ventral root potential, were compared physiologically and pharmacologically in acute and chronic spinal cord transected rats. The recovery curve of the extensor reflex, recorded as evoked electromyogram, in chronic spinal rats was strikingly different from that in acute spinal rats. Namely, shortening of the reflex amplitude suppression period (stimulus interval: 20 msec) and appearance of the supernormal period (30–60 msec) were observed in chronic spinal rats. The recovery curves of ventral root potential (monosynaptic reflex) and M wave were almost the same in both preparations. In the frequency depression curve, the amplitude of the extensor reflex in chronic spinal rats was higher at high frequency stimulation than that in acute spinal rats. 5-Hydroxytryptophan, 5-methoxy-N,N-dimethyltryptamine and quipazine enhanced the extensor reflex in chronic spinal rats with a potency of 200–400, 8 and 4 times stronger than that in acute spinal rats, respectively. These drugs did not show consistent effects on the monosynaptic reflex of ventral root potential in chronic spinal rats. These results strongly suggest that the spinal interneurons where descending serotonergic fibers terminate become supersensitive and functionally modified in chronic spinal rats. It is speculated that the supersensitivity of these interneurons may play an important role in spasticity.

Spasticity, which results from a wide variety of the brain and spinal cord disorders, is characterized mainly by hypertonia of the antigravity muscles and hyperreflexia. The mechanisms underlying the phenomena remain obscure; however, these have been explained as mainly due to hypersensitivity of the spinal motoneurons in patients (1, 2) and in animals (3). Contrary to these lines, there are several reports suggesting the functional changes in spinal interneuron level as the cause of spasticity, i.e., reduction of presynaptic inhibition (4, 5) and of postsynaptic inhibition (6).

Animals with chronic spinal cord transection have been used in the research on the pathophysiology of spasticity. For example, Murray and Goldberger (7) reported that cutaneous flexor reflex as well as knee jerk become hyperactive in the lesion side of the chronic spinal hemisection in cats. Brenowits and Pubols (8) demonstrated that at least some of the primary afferent inputs to the dorsal horn increase their synaptic efficacy following chronic spinal hemisection in cats. In chronic spinal rats, facilitation by 5-hydroxytryptophan (5-HTP) of the flexor reflex has been observed (9). These evidence seemed to suggest an involvement of spinal interneurons in functional changes of the spinal cord after chronic spinal transection.

In the present study, in order to clarify an involvement of spinal interneurons in spasticity, extensor reflex (ER), a polysynaptic reflex (PSR) via two or more interneurons (10), and ventral root potential (VRP) in chronic spinal rats were physiologically and pharmacologically compared with those in acute spinal rats. First, recovery (R) curves and frequency depression (FD)
Curves of the reflexes were analyzed in both preparations to elucidate functional changes involved in neuronal excitability such as reduction of pre- and post-synaptic inhibition and shortening of the refractory period (11–14). Secondly, effects of monoaminergic drugs on both reflexes were examined. Preliminary reports of this work have been published (15, 16).

Materials and Methods

General procedure: Male Wistar rats weighing 250 g to 400 g were used. They were housed in temperature and humidity-controlled rooms with a 12/12 hr light-dark cycle, 5 in a cage, and fed food and water ad libitum. Animals were anesthetized with combined intraperitoneal injection of α-chloralose (50 mg/kg) and urethane (400 mg/kg). Thereafter, supplementary doses of the anesthetics were given during the course of the experiments. Under the anesthesia, several peripheral nerves were exposed at the popliteal fossa and the common peroneal nerve was dissected for the ER experiments. The animal was fixed in a stereotaxic apparatus (Todai Noken type) and clamped at the spine and tibial bone. For the VRP experiments, a dorsal laminectomy was performed at the lumbar region. The exposed neural tissues were covered with warm liquid paraffin, and body temperature was maintained constant at 36.5±0.5°C by an infrared lamp. A cannula was inserted into the femoral vein for the administration of drugs.

Recording of ER and VRP: The procedures have been described previously (10). For the ER experiments, a concentric needle electrode was inserted into the ipsilateral gastrocnemius muscle, and a stimulating electrode placed on the central end of the common peroneal nerve. Square wave pulses of 0.1 msec duration supramaximal in strength via an isolation unit were applied once every 10 sec for eliciting the evoked electromyogram (EMG). For the VRP experiments, a recording electrode was placed on the L5 ventral root, and stimuli were applied to the L5 dorsal root via an isolation unit. Stimulus conditions were the same as those in the evoked EMG experiments. The M wave was also recorded.

The tibial nerve was exposed and the peripheral end of the transected tibial nerve was stimulated under the same stimulus conditions as those in the evoked EMG experiments. These electrical activities, amplified with an AC amplifier (Nihon Kohden, RB2), were displayed on a cathode ray oscilloscope (Nihon Kohden, VC-9). The amplitudes of ER, VRP and M wave were measured on photographs of five superimposed oscilloscope tracings.

Preparations of acute and chronic spinal rats: Under additional anesthesia with diethylether, the rat was spinalized at the cervical region (C1) using a spatula and artificially ventilated with room air at a rate of 60 strokes/min. The recordings were started 60 min after discontinuance of diethylether. In some experiments, acute spinal rats transected at thoracic region (T8) were used. Chronic spinal rats were prepared as follows: Under anesthesia with diethylether, a dorsal laminectomy was performed at the thoracic region. The spinal cord (T8) was completely transected with a self-made spatula under visual control. The wound was closed and antibiotic drug applied locally. Thereafter, the rats were housed in individual cages, and their bladders were emptied twice a day, and the skin cleaned with sterile alcohol solution. These animals, showing athetoid movements by light touch stimuli 10–20 days after the transection of the spinal cord, were used for the experiments.

Drugs: All drugs were injected intravenously into a cannulated femoral vein over a 30-sec period after the responses had remained stable over 15–30 min. 5-HTP (Nakarai) and L-DOPA (Nakarai) were dissolved in 0.9% saline containing a minimal quantity of HCl, and 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT, Sigma), quipazine maleate (synthesized in our Lab.), cyproheptadine HCl (Merck Sharp & Dohme) and apomorphine HCl (Macfarlan Smith Ltd.) were dissolved in 0.9% saline before the injection. These were injected in a volume of 0.8 ml/kg.

Statistics: For the statistical evaluation of the response, the amplitude obtained in each rat was expressed as a percentage of the non-conditioned value for the physiological
study and of the preinjection value for the pharmacological study. The mean values were calculated from 5–8 separate experiments. Statistical analyses were carried out by the Student’s t-test.

**Results**

R curves of ER, VRP and M wave: R curves were determined by plotting the test response amplitude as a percentage of the response to a conditioning stimulus at various intervals. The R curve of ER observed in chronic spinal rats were strikingly different from that in acute spinal rats (Fig. 1). Namely, marked suppression of amplitude was observed at the stimulus intervals of 5 and 10 msec in chronic spinal rats. Following the increase of stimulus intervals, the suppression was more rapidly released, and changed to the enhancing direction, as compared with that in acute spinal rats. At the stimulus intervals of 30–60 msec, a supernormal period over 100% of the control level was observed in 7 out of 8 animals. On the other hand, acute spinal rats transected at C1 showed no supernormal period. In addition, the R curve in acute spinal rats transected at T8 was similar to that in C1 transected rats, and no supernormal period was observed (amplitude of test response: 6.9%, 36.0%, 59.0% and 52.9% of the conditioning response at the stimulus interval of 20, 30, 40 and 60 msec, respectively). The R curve of the monosynaptic reflex (MSR) of VRP did not show any supernormal period in chronic spinal rats (Fig. 2). Furthermore, there was no difference between the curves in chronic and acute spinal rats, except that the amplitudes at stimulus intervals of 2–10 msec were markedly suppressed in chronic spinal rats. The R curves of M wave were almost the same in both preparations (Fig. 2).
FD curve of ER: ER amplitude was declined when the stimulus frequency was increased. The decline in chronic spinal rats was less pronounced as compared with that in acute spinal rats (Fig. 3). Namely, in acute spinal rats, ER amplitude was decreased to 57.6%, 22.0% and 1.3% of the control level at the stimulus frequency of 1, 5 and 20 Hz, respectively. On the other hand, ER amplitude in chronic spinal rats was yet 36.1% of the control level even at 20 Hz. The M wave showed only a 20% decrease even at 30 Hz in both preparations.

Effects of drugs on ER: 5-HP (50 mg/kg, i.v.) increased ER amplitude to 119.0% of the control level in acute spinal rats (Fig. 4). In chronic spinal rats, 5-HP increased it markedly with far lower doses. 5-HP at a dose of 0.25 mg/kg increased it to 141.0% of the control level. A potency of 5-HP in chronic spinal rats was 200–400 times stronger than that in acute spinal rats (Fig. 5). Figure 6 shows the antagonism of 5-HP-induced ER facilitation by the 30-min pretreatment of cyproheptadine (1 mg/kg) in chronic spinal rats. 5-MeO-DMT and quipazine, serotonin agonists, also facilitated the ER. Potencies of these two drugs in chronic spinal rats were 4–8 times stronger than those in acute spinal rats. L-DOPA (25 mg/kg) increased it to 183.9% of the control level in chronic spinal rats. The potency in chronic spinal rats was 4–8 times stronger than that in acute spinal rats. On the other hand, apomorphine, a dopamine agonist, decreased it in both preparations, and the potency in chronic spinal rats was 4 times stronger than that in acute spinal rats.
Fig. 3. Frequency depression curves of the extensor reflex response in chronic and acute spinal rats. Ordinate: height of the sustained extensor reflex (evoked EMG) response during the repetitive stimulation. Abscissa: frequency of stimulation (Hz). - - - - : chronic spinal rats, - - - - - - : acute spinal rats. Each point represents the mean in seven separate experiments with the S.E.M. indicated. Differences from acute spinal rats that are statistically significant: *P<0.05 and **P<0.01 (Student's t-test). Inserted figures show typical responses at 0.1 and 10 Hz in chronic and acute spinal rats.

Fig. 4. Effects of 5-HTP on the extensor reflex (evoked EMG) in acute and chronic spinal rats. - - - - : 5-HTP, 25 mg/kg, i.v.; - - - - : 5-HTP, 50 mg/kg, i.v.; - - - - : 5-HTP, 0.25 mg/kg, i.v.; - - - - : 5-HTP, 0.5 mg/kg, i.v.; - - - - : saline, 0.8 ml/kg, i.v. Ordinate: height of extensor reflex, expressed as a percentage of preinjection value. Abscissa: time after injection (min). Each point represents the mean in five separate experiments with the S.E.M. indicated. Differences from the control that are statistically significant: *P<0.05 and **P<0.01 (Student's t-test). Inserted figures show typical responses observed on the oscilloscope.
Fig. 5. Effects of monoaminergic drugs on the extensor reflex (evoked EMG) in chronic and acute spinal rats. Column and bars: maximal effect (mean of five separate experiments) of the drugs within 30 min after the injection, expressed as a percentage of preinjection value, and their S.E.M., respectively. Differences from the control that are statistically significant: *P<0.05 and **P<0.01 (Student's t-test).

Fig. 6. Effects of cyproheptadine on 5-HTP-induced enhancement of extensor reflex (evoked EMG) in chronic spinal rats. Cyproheptadine HCl (1.0 mg/kg, i.v.) was pretreated 30 min before the injection of 5-HTP (1 mg/kg). Ordinate: height of the extensor reflex, expressed as a percentage of preinjection value. Abscissa: time after injection (min). Each point represents the mean in five separate experiments with the S.E.M. indicated. Differences from the control that are statistically significant: *P<0.05 and **P<0.01 (Student's t-test).
Effects of drugs on VRP: Figure 7 shows the maximal effects of the drugs on MSR and PSR of the VRP in acute and chronic spinal rats. 5-HTP (1 mg/kg, i.v.) decreased MSR and increased PSR in chronic spinal rats, while being without effect in acute spinal rats. Quipazine (0.1 mg/kg) increased both MSR and PSR in both preparations. 5-MeO-DMT (20 μg/kg) pronouncedly decreased MSR and increased PSR in both preparations. L-DOPA (25 mg/kg) increased MSR to 176.2% of the control level in acute spinal rats. In chronic spinal rats, however, MSR was only slightly increased by L-DOPA. Apomorphine (1 mg/kg) decreased MSR in both preparations.

**Discussion**

In the present study, the R curve of ER in chronic spinal rats was found to be strikingly different from that in acute spinal rats. Namely, the amplitude suppression period was shortened and the supernormal period was induced in chronic spinal rats. Furthermore, the amplitude of the ER induced by a high frequency stimulation in chronic spinal rats was found to be higher than that in acute spinal rats. On the other hand, R and FD curves of the M wave were almost the same in both preparations, indicating little involvement of the efferent system to muscles in these functional differences between chronic and acute spinal rats. Therefore, these results strongly suggest that some functional changes occur in the spinal polysynaptic pathway after chronic spinal transection in rats.

Numerous descending fibers from cortical and subcortical areas are known to terminate exclusively on interneurons in laminae V–VII (17, 18). Therefore, chronic spinal cord transection causes degeneration of the descending fibers to lead to hyperexcitability on spinal neurons. For example, Murray and Goldberger (7) have demonstrated that in spinal cord partially hemisected cats, the regions of increased and expanded

| Drug              | Dose | Decrease (%) | Ventral root potential | Increase (%) |
|-------------------|------|--------------|------------------------|--------------|
| Saline            | i.v. 0.8 ml/kg | M 50          | P 0                    | M 50         |
| 5-HTP             | 1 mg/kg | M **          | P **                   | M **         |
| 5-MeO-DMT         | 20 μg/kg | M **          | P **                   | M **         |
| Quipazine maleate | 100 μg/kg | M **          | P **                   | M **         |
| L-DOPA            | 25 mg/kg | M **          | P **                   | M 76.0%      |
| Apomorphine       | 1 mg/kg | M **          | P **                   | M **         |

**Fig. 7.** Effects of monoaminergic drugs on the ventral root potential in chronic and acute spinal rats. Abbreviations: M=monosynaptic reflex, P=polysynaptic reflex. Column and bars: maximal effect (mean of five separate experiments) of the drugs within 30 min after the injection, expressed as a percentage of preinjection value, and their S.E.M., respectively. Differences from the control that are statistically significant: *P<0.05 and **P<0.01 (Student's t-test).
dorsal root input, showing signs of collateral sprouting in response to degeneration of descending tracts, can be correlated with electrophysiologically determined location of interneurons in the path of cutaneous reflexes and of stretch reflex facilitation, and suggested that the increased dorsal root projection to these interneurons mediates the exaggerated cutaneous flexor reflex. There is also a development of denervation supersensitivity following destruction of the descending fibers (19). Matsushita and Smith (11) reported in post-ischemic rigid rats that shortening of the amplitude suppression period in the R curve of MSR may be due to reduction of presynaptic inhibition based on destruction of the interneurons. In this study, PSR of VRP was observed in chronic spinal rats, and the R curve of MSR in chronic spinal rats was almost the same as that in acute spinal rats, indicating that there is little functional change in the interneurons primarily related to motoneuronal excitability in chronic spinal rats. Accordingly, it is likely that following the chronic spinal transection, spinal interneurons where descending fibers terminate become supersensitive and functionally modified due to anatomical and functional changes such as collateral sprouting, denervation supersensitivity, and reduction of inhibitory mechanisms.

The exact mechanisms underlying these functional changes in the interneuron levels are not fully elucidated. There are, however, several findings on this aspect. Monoamine contents are depleted in the spinal cord below the chronic spinal cord transection (10, 20, 21). Denervation supersensitivity to serotonin occurs in chronic spinal rats (19). To determine if there is an involvement of monoaminergic receptors in the functional changes, effects of the drugs involved in monoaminergic transmission were examined on ER in both preparations. 5-HTP enhanced the ER, and its potency in chronic spinal rats was 200–400 times stronger than that in acute spinal rats. In addition, such facilitation by 5-HTP in chronic spinal rats was antagonized with the pretreatment of cyproheptadine, a serotonin antagonist. The results support the findings of Barbeau et al. (22) that 5-HTP-induced enhancement of the EMG discharges in chronic spinal rats is antagonized by cyproheptadine. Furthermore, two serotoninergic agonists, 5-MeO-DMT (23) and quipazine (24), were found to enhance the ER in chronic spinal rats with potencies that were 8 and 4 times stronger than those in acute spinal rats, respectively. On the other hand, 5-HTP and 5-MeO-DMT depressed the MSR of VRP in chronic spinal rats, while quipazine enhanced it in both preparations. The results indicate that the effects of these three drugs on MSR were inconsistent. From these results and the physiological findings obtained in this study, it is suggested that supersensitivity to serotonin receptors on the interneurons occurs following the chronic spinal cord transection.

L-DOPA enhanced the ER in chronic spinal rats with a potency that was 4 times stronger than that in acute spinal rats. Accordingly, supersensitivity to catecholamine may occur as in the case of serotonin. However, apomorphine, a dopamine agonist, was found to depress the ER in chronic spinal rats. The enhancing effect of L-DOPA is expected to be due to noradrenaline formed from L-DOPA or L-DOPA itself.

In conclusion, spinal interneurons in the dorsal horn where descending serotoninergic fibers terminate are considered to become supersensitive and functionally modified as the results of degeneration of the descending fibers in chronic spinal rats. The function of the spinal cord is modified in the patients with brain and spinal cord trauma, as described previously (1, 2, 4–6). Accordingly, abnormal excitability on spinal interneurons may be strongly involved in spasticity in man.

References
1 Angel, R.W. and Hofmann, W.W.: The H reflex in normal, spastic and rigid subjects. Arch. Neurol. 8, 591–596 (1963)
2 Olsen, P.Z. and Diamantopoulos, E.: Excitability of spinal motoneurones in normal subjects and patients with spasticity, Parkinsonian rigidity, and cerebellar hypotonia. J. Neurol. Neurosurg. Psychiatry 30, 325–331 (1967)
3 Aoki, M., Mori, S. and Fujimori, B.: Exaggeration of knee- jerk following spinal hemisection in monkey. Brain Res. 107, 471–485 (1976)
4 Burke, D. and Ashby, P.: Are spinal "presynaptic" inhibitory mechanisms suppressed in spasticity?
5. Delwaide, P.J.: Human monosynaptic reflexes and presynaptic inhibition. In New Developments in Electromyography and Clinical Neurophysiology, Edited by Desmedt, J.E., Vol. 3, p. 508-522, Karger, Basel (1973)

6. Herman, R. and Mecomber, S.A.: Vibration-elicited reflexes in normal and spastic muscle in man. Am. J. Phys. Med. 50, 169-183 (1971)

7. Murray, M. and Goldberger, M.E.: Restitution of function and collateral sprouting in the cat spinal cord: The partially hemisected animal. J. Comp. Neurol. 158, 19-36 (1974)

8. Brenowits, G.L. and Pubols, L.M.: Increased receptive field size of dorsal horn neurons following chronic spinal cord hemisections in cats. Brain Res. 216, 45-69 (1981)

9. Nozaki, M., Bell, J.A., Vanpel, D.B. and Martin, W.R.: Responses of the flexor reflex to LSD, tryptamine, 5-hydroxytryptophan, methoxamine, and d-amphetamine in acute and chronic spinal rats. Psychopharmacology (Berlin) 55, 13-18 (1977)

10. Ito, T., Furukawa, K., Karasawa, T., Kadokawa, T. and Shimizu, M.: Functional changes in the rat spinal cord by chronic spinal transection and a possible role of monoamine neurons. Eighth International Congress of Pharmacology IUPHAR, Tokyo, Abstracts p. 592 (1981)

11. Ito, T., Furukawa, K., Karasawa, T., Kadokawa, T. and Shimizu, M.: Effect of serotonergic drugs on polysynaptic reflex in chronic spinal rats. Japan. J. Pharmacol. 32 Supp. 69P (1982)

12. Nyberg-Hansen, R.: Functional organization of descending supraspinal fiber systems to the spinal cord. Anatomical observations and physiological correlations. Rev. Anat. Embryol. Cell Biol. 39, 6-48 (1966)

13. Clemente, C.D.: Neurophysiologic mechanisms and neuroanatomic substrates related to spasticity. Neurology 28, 40-51 (1978)

14. Decandia, M., Provini, L. and Táboriková, H.: Mechanisms of the reflex discharge depression in the spinal motoneurone during repetitive orthodromic stimulation. Brain Res. 4, 284-291 (1967)

15. Anden, N.-E., Haggendal, J., Magnusson, T. and Rosengren, E.: The time course of disappearance of noradrenaline and 5-hydroxytryptamine in the spinal cord after transection. Acta Physiol. Scand. 62, 115-118 (1964)

16. Carlsson, A., Magnusson, T. and Rosengren, E.: Monoamines of the spinal cord normally and after transection. Experientia 17, 359 (1963)

17. Fuxe, K., Holmstedt, B. and Jonsson, G.: Effects of 5-methoxy-N,N-dimethyltryptamine on central monoamine neurons. Eur. J. Pharmacol. 19, 25-34 (1972)

18. Jacoby, J.H., Howd, R.A., Levin, R.S. and Wurtman, R.J.: Mechanisms by which quipazine, a putative serotonin receptor agonist, alters brain 5-hydroxyindole metabolism. Neuropharmacology 15, 529-534 (1976)