The Japanese Journal of Physiology
19, pp. 1-10, 1969

GAMMA ACTIVITY OF RIGID CAT CAUSED BY TETANUS TOXIN

Masaakira KANO* AND Kohsi TAKANO

Department of Physiology, School of Medicine, Chiba University, Chiba, Japan

SHERRINGTON (1905) first observed that tetanus toxin converts inhibition into excitation in the spinal reflex pathway. He suggested that the action of this toxin was similar to that of strychnine which has a convulsive effect on the central nervous system. BROOKS *et al.* (1957) showed that tetanus toxin diminished or abolished five forms of spinal inhibition on alpha-motoneurons in the lightly anesthetized spinal cat. WILSON *et al.* (1960) later showed that tetanus toxin depressed or abolished both recurrent inhibition and facilitation of these motoneurons. That tetanus toxin may similarly affect gamma-motoneurons has been suggested by ERZINA (1961). Others (ANDREW and BARR, 1958), however, have considered that no gamma-motoneuron activation is involved in local tetanus. The present work therefore was performed to study the effect of tetanus toxin on the gamma-loop and to explore the role of gamma-motoneuron on the rigidity of local tetanus in cat. The preliminary study was already published (KANO *et al.*, 1967).

METHODS

A total of 15 adult cats with rigidity produced by tetanus toxin were anesthetized by mixture of urethane (300 mg/kg) and chloralose (30 mg/kg). In some experiments a laminectomy was performed to expose the spinal cord, which was then covered with parafin oil. In these cats the dorsal root of L_7 and S_1 were stimulated or cut during the experiment. The triceps surae muscle of both sides was dissected free from the surrounding tissue. The legs were firmly fixed to the table and the Achilles tendon was connected to a strain gauge myograph with steel wire.

Tension and electrical activity of the muscles were recorded, after suitable amplification, by a pen recorder. In some preparations, the internal capsule (CI) was electrically stimulated with bipolar steel electrodes inserted stereotaxically. The brain stimulus consisted of trains of square wave pulses (50 c/sec) with a pulse duration of 0.1 msec and an amplitude less than 5 volts.

The dose of tetanus toxin used was 12.5×10^4 mouse minimum lethal doses (m.l.d)

Received for publication November 29, 1967

* Present adress: Brain Research Institute, School of Medicine, Chiba University.
加濃正明，高野光司

per ml, which was obtained from a culture of A-47 (Harvard) strain of Clostridium tetani. 150-15000 m.l.d of toxin was injected intramuscularly, into the left triceps surae muscle. 18 to 24 hr after the injection, the typical symptoms of local tetanus were observed in the hind leg on the injected side. No significant difference in the degree of rigidity was found despite different doses of the toxin or different incubation periods.

RESULTS

Elicitation of the pinna reflex (SHERRINGTON, 1905, 1917) has been shown by GRANIT *et al.* (1953) to evoke augmented discharge first of gamma motoneurons and then of muscle spindles. When a pinna reflex was elicited in cat with local tetanus the EMG and tension measured were markedly augmented within a few seconds on the rigid side. The augmentation lasted for several dozens of seconds and then decayed slowly. The slow decay of the electrical and mechanical activity of the muscle was similar to that of the discharge of gamma motor fibres and muscle spindles occurring after pinna stimulation (GRANIT *et al.*, 1953; SCHOMBURG, 1965). FIG. 1 shows electrical (each upper trace) and mechanical (each lower trace) activity recorded from a rigid muscle (*l*) and from the control side (*r*) during and after a pinna reflex. On the control side there was no effect of pinna activation.

When the dorsal roots of L_7 and S_1 on each side were stimulated by rectangular pulses (50 c/sec) the triceps surae muscles were reflexly activated bilaterally. The response was pronounced on the side with tetanus and rather feeble on the control side. The rigid muscle was activated when the ipsilateral side (FIG. 2*a-l*) and also when the contralateral side (FIG. 2*b-l*) was stimulated. The time course of the decay of activity in the rigid muscle was similar to that seen with activation of the muscle by the pinna reflex. On the control side the muscle activity increased slightly when the ipsilateral dorsal roots was stimulated (FIG. 2*b-r*), but little when the contralateral dorsal roots were stimulated (FIG. 2*a-r*).

Electrical stimulation of the internal capsule (CI) contralateral to the rigid limb resulted in muscular contraction. At a moderate stimulus strength, the activity of the rigid muscle increased slowly and persisted for a long time (FIG. 3*a-l*). On the ipsilateral (control) side the augmentation of muscle activity was small and little tension change was observed (FIG. 3*a-r*). When the stimulus intensity was strong, muscle activation was observed on both sides (FIG. 3*b*). On the control side, the activation was observed only during the stimulation and this suggested activation of alpha-motoneurons only. On the rigid (contralateral) side, however, the activation was prolonged, possibly reflecting the time course of gamma-motoneuron activation.

Since the gamma-motoneurons have a lower threshold than alpha-motoneurons to electrical stimulation of brain (GRANIT and KAADA, 1953), it is likely

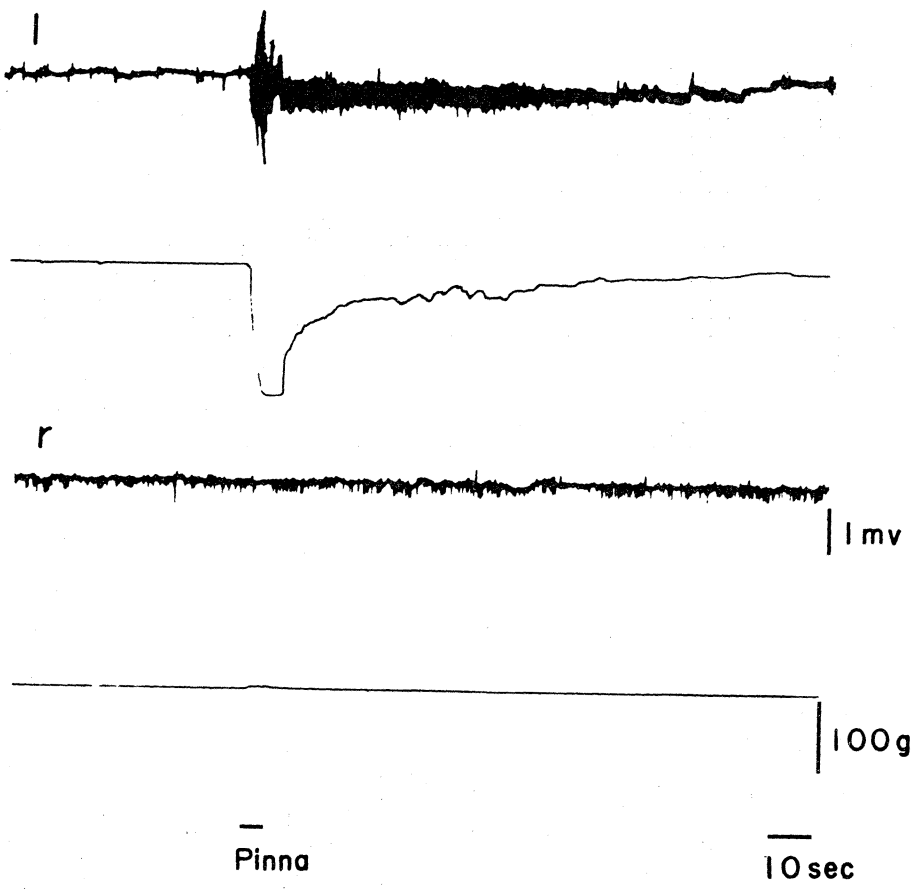

Fig. 1. Lightly anesthetized cat. Electromyogram (each upper trace) and tension record (each lower trace) of the left (rigid) muscle (*l*) and in the right (control) muscle (*r*). Both the EMG and tension records of the rigid muscle show a strong response to pinna-twist (indicated by the horizontal line).

with moderate stimulation that only gamma-motoneurons were excited. Thus the gamma system of the rigid side, in a state of increased excitability, is capable of being activated by moderate CI stimulation. When the stimulus was stronger, alpha-motoneurons on both sides were activated, thereby producing a large increase in tension in the muscles bilaterally. On the control side, however, the tension faded away instantaneously after cessation of stimulation, probably because of a lack of a support by coincident gamma-motoneuron activity. By analogy with the blockade of the inhibition converging onto alpha-motoneurons by tetanus toxin (BROOKS *et al.*, 1957) it is possible that the inhibition of gamma-motoneurons is also depressed.

The CI was also stimulated after bilateral deafferentation by sectioning the dorsal roots of L_5 to S_3. In FIG. 4, the CI, both contralateral and ipsilateral

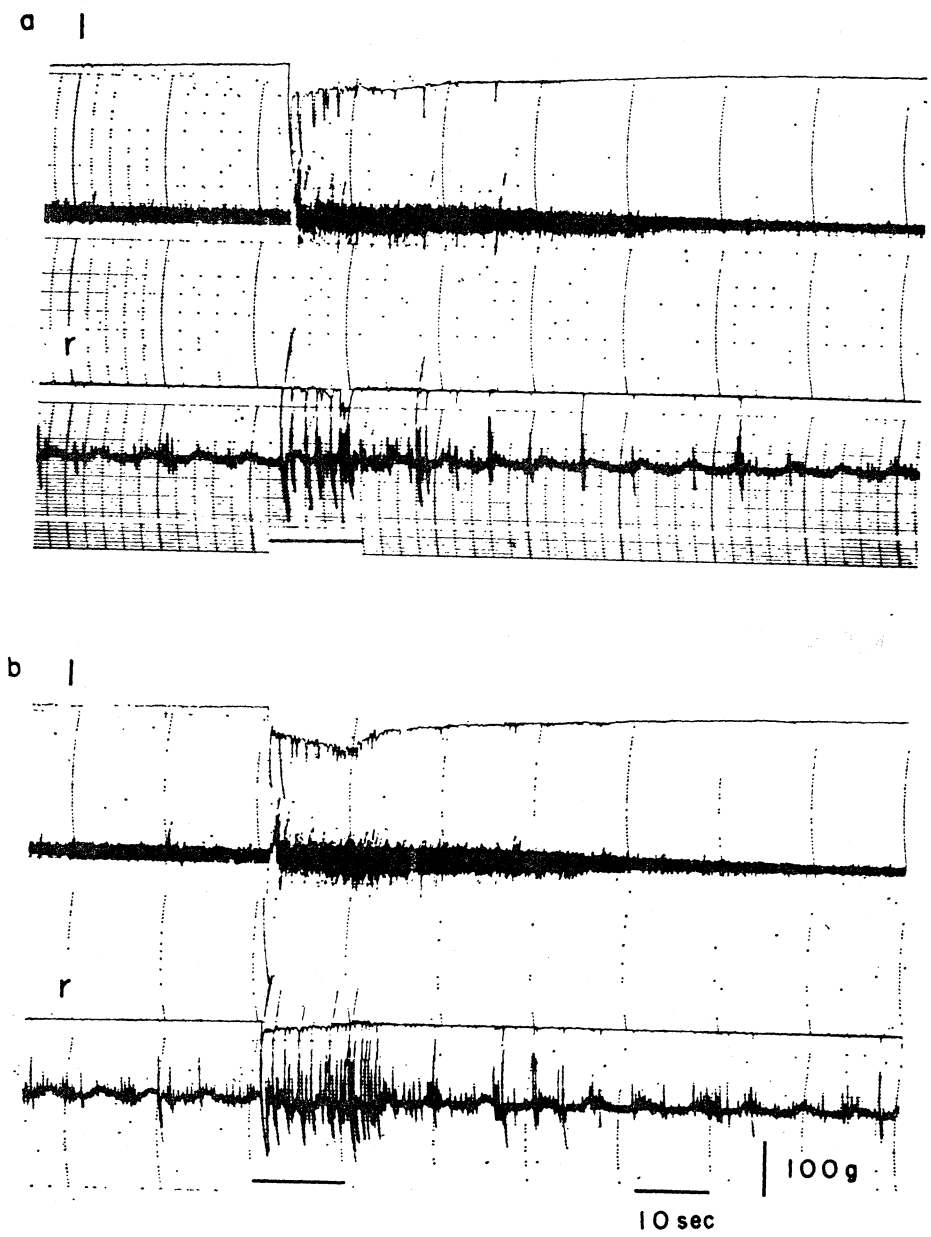

Fig. 2. Lightly anesthetized cat. Tension record (each upper trace) and electromyogram (each lower trace) in the left (rigid) muscle (*l*) and in the right (control) muscle (r). Dorsal roots of L_7 and S_1 of left side (*a*) and of right side (*b*) were stimulated at 50 c/sec. Note that the rigid muscle is activated by both ipsi- and contralateral stimulation while the control muscle is activated only by stimulation of the ipsilateral dorsal roots. Stimulation indicated by the horizontal line.

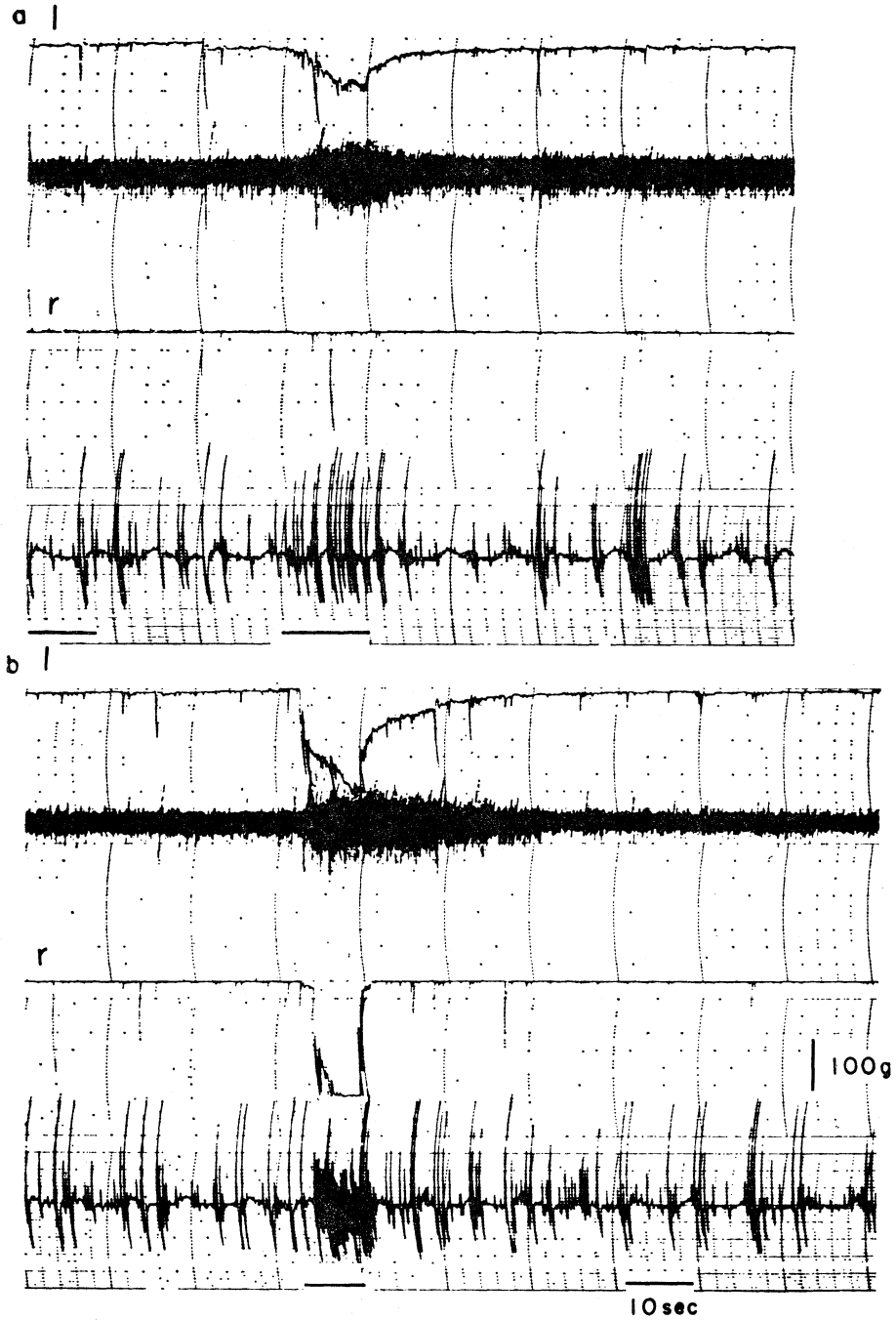

Fig. 3. Lightly anesthetized cat. Tension record (each upper trace) and electromyogram (each lower trace) in the left (rigid) muscle (*l*) and in the right (control) muscle (*r*). Moderate (*a*) and strong (*b*) tetanic (50 c/sec) stimulation of the right internal capsule. With moderate stimulation only the rigid muscle (contralateral) is tonically activated (*a–l*). With strong stimulation the rigid muscle shows both phasic and tonic activation (*b–l*), while the control (ipsilateral) muscle shows only a phasic type of activation (*b–r*). Stimulation indicated by the horizontal line.

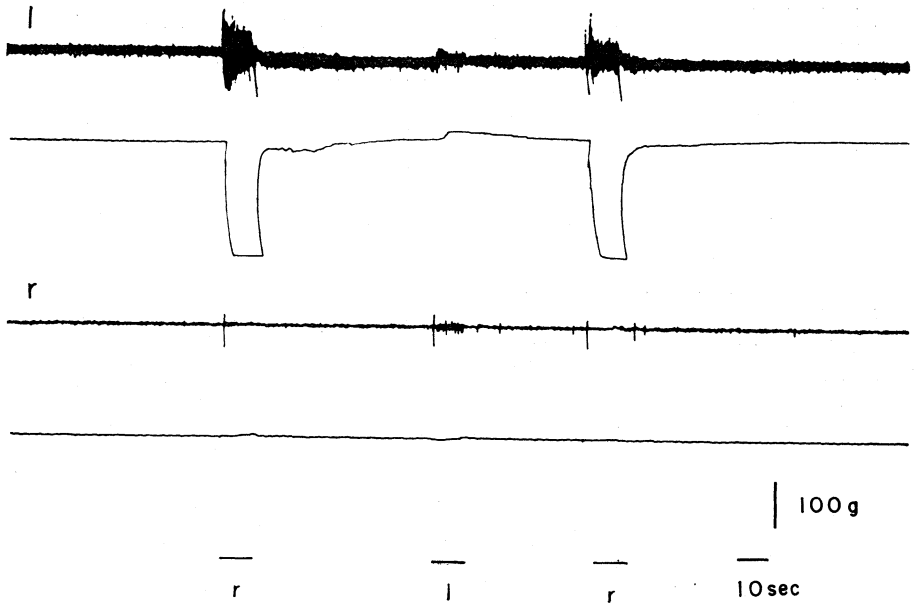

Fig. 4. Lightly anesthetized cat. Dorsal roots of L_5–S_3 cut bilaterally. Right (*r* under bar) or left (*l* under bar) internal capsule was stimulated at 50 c/sec. Electromyogram (each upper trace) and tension record (each lower trace) in the left (rigid) muscle (*l*) and in the right (control) muscle (*r*). Only when the internal capsule contralaterary to the rigid muscle is stimulated phasic activation occurs during the stimulation without after-discharge. Stimulation indicated by the horizontal bar.

to a rigid muscle was stimulated. With contralateral stimulation, the electrical activity in the rigid muscle appeared only during stimulation (l–r underbar, l–$\bar{r}$). There was no persistense of tonic activity in the muscle after the phasic activity. With ipsilateral stimulation there was little change of the rigid muscle in the electrical or mechanical activity (l–$\bar{l}$). Stimulation of CI contralateral to the control muscle resulted in a small change in the electrical activity but not the tension of the muscle (r–$\bar{l}$). Thus when the gamma loop was intact, the response to CI stimulation was found in both the contralateral rigid and the ipsilateral control muscle (FIG. 3*b*). However, by interrupting feed-back through the gamma loop by deafferentation the effect of CI stimulation on the ipsilateral rigid side is lost (FIG. 4).

In FIG. 5, CI stimulation of moderate strength ipsilateral to the rigid muscle resulted in gamma-activation in the rigid muscle (l–$\bar{l}$). With stimulation of the contralateral side there was both alpha- and gamma-activation (l–$\bar{r}$). In the control muscle there was only alpha-activation when stimulation was of the contralateral CI (r–$\bar{l}$).

When a muscle nerve is bathed in 0.25% procaine-Ringer solution the gamma-fibers may be partially or completely blocked early in the course of

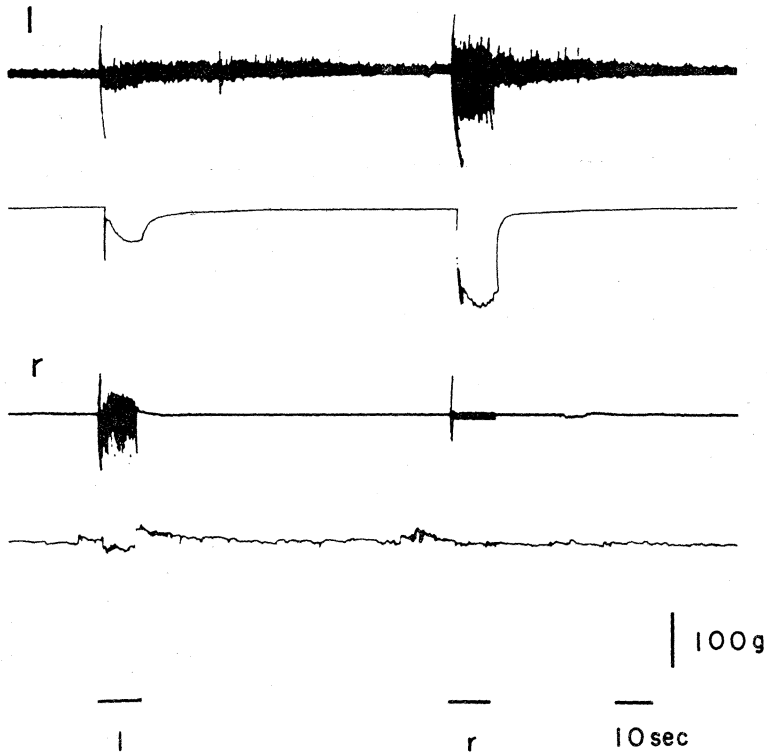

Fig. 5. Lightly anesthetized cat. The right (*r* under bar) or left (*l* under bar) internal capsule was stimulated at 50 c/sec. Electromyogram (each upper trace) and tension record (each lower trace) in the left (rigid) muscle (*l*) and in the right (control) muscle (*r*). Stimulation strength was moderate. The rigid muscle shows a tonic long lasting activation with ipsilateral stimulation (*l–l* under bar) and shows phasic and tonic activation with contralateral stimulation (*l–r* under bar). The control muscle shows only phasic activation with contralateral stimulation (*r–l* under bar) and no activity with ipsilateral stimulation. In *r–r* under bar only the stimulus artifact is seen.

anesthesia (MATTHEWS and RUSHWORTH, 1958) without affecting the alpha-fibers. To test the effect of pharmacological gamma blockade, the nerve branches to a rigid triceps surae muscle were immersed in the procaine-Ringer solution and the contralateral CI was stimulated. FIG. 6-0 is the control record of CI stimulation contralateral to the rigid muscle. FIG. 6-3, 6-6 and 6-9 are the records of 3 min, 6 min and 9 min after application of procaine-bath respectively. That at this stage of anesthesia only gamma-fibers were anesthetized leaving almost all alpha-fibers intact was shown in a preliminary study (TAKANO, unpublished). The electrical activity in the rigid muscle evoked by contralateral CI stimulation became smaller paralleling the progress of gamma-fiber blockade. In the experiment illustrated, only the fiber branches to the

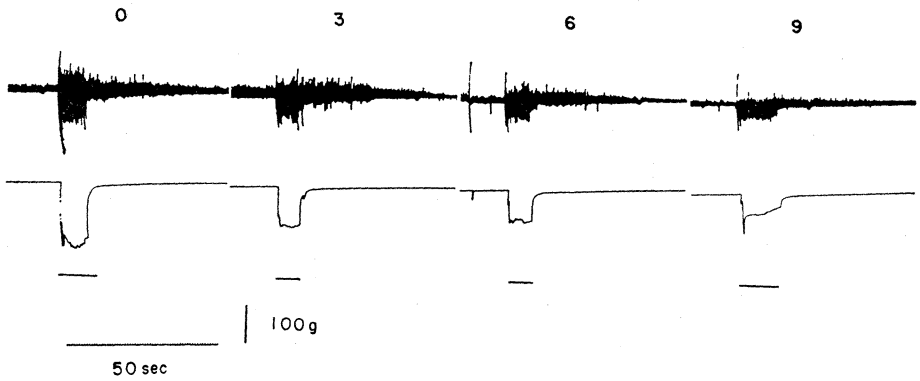

Fig. 6. Lightly anesthetized cat. The internal capsule contralateral to the rigid muscle was stimulated. Electromyogram (each upper trace) and tension record (each lower trace) of the rigid muscle. The nerve branches to the rigid muscle were immersed in 0.25% procaine-Ringer solution. 0 is the control record. 3, 6 and 9 are the records taken 3 min, 6 min and 9 min respectively after starting the bath. Note that the effect of internal capsule stimulation was depressed by selective blockade of gamma-fibers. Stimulation indicated by the horizontal bar.

triceps surae muscle on the rigid side were blocked. Since the feed-back pathways to the motoneurons of the triceps surae muscle from spindles in synergic muscles was left intact, the tonic activity of muscle seen after the CI stimulation was still observed in spite of gamma-fiber blockade.

DISCUSSION

In the physiological studies of the rigidity induced by tetanus toxin, the action of the toxin has only been shown to act on the spinal alpha-motor system (BROOKS *et al.*, 1957; WILSON *et al.*, 1960). Concerned with the role of gamma system in the tetanus rigidity, ANDREW and BARR (1958) observed that the activity of the gamma-motoneuron is unaltered in tetanus and KRYZHANOVSKYI (1967) suggested that the role of gamma-system in rigidity is merely supplementary.

In our experiments the electrical and mechanical responses of muscle were used as indicators of reflex activity. Reflex activation of the rigid muscle evokes both phasic and tonic responses. We have considered that the phasic response primarily represents activity of alpha-motoneurons while the tonic response results from co-activation of gamma-motoneurons. It is our assumption in this study that the tonic response represents gamma-activation for the following reasons: 1) The long lasting acceleration of spindle discharges is characteristic of the discharge of gamma-motoneurons (GRANIT *et al.*, 1953). Prolonged after-discharge was also characteristic of rigid tetanus muscle; 2) The low threshold of rigid muscles to electrical stimulation of the central

nervous system is like that of gamma moto-neurons (GRANIT and KAADA, 1953); 3) Deafferentation decreases the activity of rigid muscles. It therefore appears that part of the rigidity of local tetanus is caused not only by augmented discharge of alpha-motoneurons but also by increased gamma-motoneuron activity. This conclusion was further confirmed observing the response of the rigid muscle to stretch (TAKANO and KANO, 1968).

SUMMARY

1. Injection of tetanus toxin into muscles of the cat's hind leg resulted in the signs of local tetanus within 18 to 24 hours.
2. When a pinna-reflex was elicited, long lasting mechanical and electrical activity occurred in the rigid muscle but not in its control.
3. Moderate stimulation of the internal capsule (CI) elicited larger and more prolonged activation of the rigid muscle than the control.
4. With strong stimulating of the CI the rigid muscle was activated by both contra- and ipsilateral stimulation. The control muscle was only activated by contralateral stimulation.
5. When the gamma-fibers were blocked selectively by 0.25% procaine solution the activation effect of CI stimulation on the rigid muscle was markedly diminished.
6. It is suggested that the gamma-motoneurons may be affected by tetanus toxin and that the rigidity of tetanus depends upon activation of both alpha-motoneurons and gamma-motoneurons.

The authors are indebted to Dr. N. SAKURAI, Department of Hygiene, Chiba University for preparation of tetanus animal and wish to express their gratitude to Dr. D. REIS, Department of Neurology, Cornell University for improvements in the manuscript, and to Prof. S. HOMMA, Department of Physiology, and Dr. S. MURAYAMA, Department of Pharmacology, Chiba University for their useful discussion.

REFERENCES

ANDREW, B. L. AND BARR, M. N. (1958). Anaesthesia and local tetanus intoxication. *J. Physiol.*, 141: 40–41P.

BROOKS, V. B., CURTIS, D. R. AND ECCLES, J. C. (1957). The action of tetanus toxin on the inhibition of motoneurones. *J. Physiol.*, 135: 655–672.

ERZINA, G. A. (1961). Effects of the gamma-neurone system on the electrical activity of muscle spindles during local tetanus in the cat. *Sechenov J. Physiol.*, [Eng] 47 (8): 30–33.

GRANIT, R., JOB, C. AND KAADA, B. R. (1953). Activation of muscle spindles in pinna reflex. *Acta physiol. scand.*, 27: 161–168.

GRANIT, R. AND KAADA, B. R. (1953). Influence of stimulation of central nervous structures on muscle spindles in cat. *Acta physiol. scand.*, 27: 130–160.

KANO, M., SAKURAI, N. AND TAKANO, K. (1967). Gamma activity of rigid cat in tetanus intoxication. *J. Chiba Med. Soc.*, 43: 388–389.

KRYZHANOVSKYI, G. N. (1967). The neural pathway of toxin: its transport to the central nervous system and the state of the spinal reflex apparatus in tetanus intoxication. In *Principles on Tetanus*, L. ECKMANN, ed., Hans Huber, Bern, pp. 155–168.

MATTHEWS, P. B. C. AND RUSHWORTH, G. (1958). The discharge from muscle spindles as an indicator of γ-efferent paralysis by procaine. *J. Physiol.*, **140**: 421–426.

SHERRINGTON, C. S. (1905). On reciprocal innervation of antagonistic muscles. VIIIth note. *Proc. Roy. Soc.*, **76B**: 269–297.

SHERRINGTON, C. S. (1917). Reflexes elicitable in the cat from pinna, vibrissae and jaws. *J. Physiol.*, **51**: 404–431.

SCHOMBURG, E. D. (1965). Fusimotorische Effekte bei mechanischer und elektrischer Pinna-Reizung an decerebrierten und narkotisierten Katzen. *Inaugural-Dissertation zur Erlangung des Doktorgrades der Medizinischen Fakultät der Georg-Augst-Universität zu Göttingen.*

TAKANO, K. AND KANO, M. (1968). Reflex activity of the muscle in tetanus intoxication. *J. Physiol. Soc. Japan*, **30**: 122–123.

WILSON, V. J., DIECKE, F. P. J. AND TALBOT, W. H. (1960). Action of tetanus toxin on conditioning of spinal motoneurons. *J. Neurophysiol.*, **23**: 659–666.