Central Effects of the Neurotropic Mycotoxin Fumitremorgin A in the Rabbit (II) Effects on the Brain Stem

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Abstract—In order to determine the main site of the convulsant action of the neurotropic mycotoxin fumitremorgin A, the role of the brain stem reticular formation was studied. In rabbits lightly anesthetized with urethane and chloralose, electrical stimulation of the reticular formation elicited burst discharges in the common peroneal nerve and in the tibial nerve. This facilitatory effect of electrical stimulation was markedly potentiated by intravenous administration of a small dose of FTA, before onset of actual convulsion. Under the influence of FTA, a remarkable increase in the spontaneous electrical activity of the midbrain reticular formation was observed. The firing pattern of some neurons in the reticular formation corresponded very well with abnormal burst discharges in the common peroneal nerve. These effects of FTA were inhibited by chlorpromazine (0.1–1.0 mg/kg, i.v.), diazepam (0.1–1.0 mg/kg, i.v.), mephenesin (5–10 mg/kg, i.v.) and pentobarbital (5–15 mg/kg, i.v.). It was concluded that FTA might activate some neurons in the midbrain reticular formation and that convulsive burst discharges in peripheral motor nerves resulted from abnormal activation of these neurons, although the role of the medullary reticular formation could not be excluded.

Fumitremorgin A (FTA), a tremorgenic mycotoxin isolated from Aspergillus fumigatus, is known to cause severe tremor and generalized tonic-clonic convulsion in experimental animals by oral and parenteral administration (1, 2). Curiously, however, this convulsion is never accompanied by an apparent electroencephalographic seizure pattern (3). The main site of the convulsant action of FTA has been suggested to be in restricted areas in the brain stem (3, 4). However, so far, there has been no direct evidence of FTA-induced abnormal activity of the brain stem, to which this severe convulsion might be attributable. Therefore, it may be of value to examine more precise effects of FTA on the brain stem in order to elucidate roles of the brain stem in the pathophysiology of generalized convulsion. The present experiments aimed to determine the effects of FTA on the brain stem in the rabbit.

Materials and Methods

1. Materials: Male white rabbits, weighing 2.2–2.8 kg, were used in this study. All experiments were carried out under anesthesia with urethane and α-chloralose (400 mg/kg and 40 mg/kg, i.p., respectively), because it is known that tonic-clonic convulsions can take place under this anesthetic condition if a large dose (100–200 μg/kg, i.v.) of FTA is administered (3). In order to avoid the violent body movement caused by FTA, the animals were immobilized by pancuronium bromide (0.1 mg/kg, i.v., initially and repeated as needed) under artificial ventilation. FTA was dissolved in absolute dimethylformamide in a concentration of 0.1%. FTA and all other drugs were administered intravenously through a polyethylene cannula inserted in the femoral vein.

2. Recording of spontaneous discharges in peripheral motor nerves: After tracheal can-
nulation and insertion of an intravenous tubing into the femoral vein, the common peroneal nerve and the tibial nerve were exposed in the left popliteal fossa and cut distally. The desheathed central end of each nerve was placed on the respective bipolar platinum electrodes and maintained within a liquid paraffin pool. Spontaneous discharges of each nerve and their integrated curves were recorded on a polygraph (Nihon Kohden, RMP 6008) and also stored on a magnetic tape recorder (SONY, DFR 3715).

In the present study in immobilized rabbits, the FTA-induced convulsive state was indicated by abnormal burst discharges of these two peripheral motor nerves. Namely, the onset of convulsion could be shown usually as the first explosive increase in the common peroneal nerve discharges. The phase of alternative excitation in the common peroneal nerve and the tibial nerve might correspond to the state of clonic convulsion, and very strong simultaneous excitation in these two nerves might represent tonic convulsion (4).

3. Electrical stimulation of the brain stem: After anesthesia and cannulation in the trachea and the femoral vein, the rabbit was fixed in a stereotaxic instrument and a part of the cerebral cortex was exposed. The spontaneous discharges of the common peroneal nerve and the tibial nerve were also recorded. Then a stimulating electrode (MT Giken, A2-2008) was inserted into the brain stem stereotaxically, taking Sawyer's map (5) as a guide. A stimulus comprising 10 pulses at 50 Hz (1 msec duration, 5-8 V) was applied every 90 sec. If the tip of the stimulating electrode was located within a particular area of the brain stem, this electrical stimulation caused a transitory increase in discharges of the common peroneal nerve and/or the tibial nerve. This facilitatory area ranged from P7.4 to P12.3, from L1.5 to L4.5 and from H(-)1.0 to H(-)6.5 (Fig. 1). It almost corresponded to the midbrain reticular formation. When these results were mapped on Gerhard's atlas of the rabbit brain (6), this area seemed to correspond mainly to the "formatio reticularis tegmenti pars centralis".

The first increased discharges were often observed more clearly in the common peroneal nerve than in the tibial nerve, and there seemed to be a mechanism of reciprocal innervation in such cases, because the tibial nerve discharge was inhibited during the first distinct short-lasting excitation of the common peroneal nerve and then, after cessation of the common peroneal nerve discharges, the tibial nerve showed weaker excitation with longer duration.

Influence of a small dose (20–50 μg/kg, i.v.) of FTA on the effectiveness of the stimulation of this area was examined in order to know the change in excitability of this area of the brain stem.

At the end of each experiment, a minute amount of black dye was injected stereotaxically into the same site as the stimulation point, to confirm its location. In some cases, a small electrolytic lesion was made at the stimulation site by passing a constant current of 2 mA for 15 sec through the stimulating electrode. Then the precise location of the stimulation site was confirmed by histological examination.

4. Experiments about spontaneous discharges in the brain stem reticular formation: A recording electrode (MT Giken, K2-0601) was inserted stereotaxically into the brain stem reticular formation, the same area as mentioned above. Spontaneous activity of this area was displayed on the oscilloscope (Nihon Kohden, VC-10) and recorded on both the polygraph and magnetic tape recorder. The effect of a convulsant dose of FTA (100–200 μg/kg, i.v.) on the spontaneous activity of the reticular formation was examined. If necessary, data on the oscilloscope were photographed.

5. Experiments about single or multiple unit activity in the brain stem reticular formation: Spontaneous single or multiple unit activity in the brain stem reticular formation was recorded extracellularly by a metal microelectrode (MT Giken, J-3002). Simultaneously, spontaneous discharges of the common peroneal nerve and the tibial nerve were recorded, and electrical stimulation of the ipsilateral mesencephalic reticular formation was also performed as described above. The electrical stimulation caused a transitory increase in spike frequency of some neurons in this area, and the spontaneous discharges of these neurons appeared to be correlated
Effects of FTA on the Brain Stem

Fig. 1. Facilitatory effect of the brain stem electrical stimulation on the common peroneal nerve discharges. Stereotaxic coordinates are based on the data from Sawyer et al (5). Results from 12 animals were summarized. In A, results in the range of L2.5–L3.5 were plotted together by projection upon the midsagittal plane; and in B, results in the P9 plane were summarized. ●: effective stimulation site for eliciting the common peroneal nerve discharges. ▽: ineffective stimulation site. CORT: cerebral cortex, SC: superior colliculus, AO: aqueduct, CG: central gray, MG: medial geniculate body, RF: reticular formation, LL: lateral lemniscus, LM: medial lemniscus, CS: corticospinal tract, III: oculomotor nerve.

with the spontaneous activity of the common peroneal nerve or the tibial nerve. Neurons with these properties were selected for this study. The effects of FTA and some other drugs on these neurons were examined. All data were recorded on both the polygraph and magnetic tape recorder.

6. Drugs used: Purified FTA from the mycelium of Aspergillus fumigatus was kindly supplied by Prof. M. Yamazaki of Chiba University. Other drugs used were urethane (Wako Pure Chemicals Industries, Ltd., Tokyo), α-chloralose (Nacalai Tesque, Ltd., Kyoto), chlorpromazine hydrochloride (Yoshitomi Pharmaceutical Industries, Ltd., Osaka), diazepam (Takeda Pharmaceutical Co., Osaka), sodium pentobarbital (Dainippon Pharmaceutical Co., Osaka) and mephenesin (Chugai Pharmaceutical Co., Tokyo).

Results

1. Effects of the brain stem electrical stimulation on peripheral motor nerves and influence of FTA: Effect of FTA on the excitability of the brain stem reticular formation was examined, using 20–50 μg/kg of FTA. Since this dose was nearly equal to or less than the minimum convulsant dose, convulsive discharges of the common peroneal nerve or the tibial nerve were not elicited in many cases. However, this dose of FTA could increase the discharges of these motor nerves evoked by electrical stimulation of the brain stem (Fig. 2). A few minutes after intravenous injection of this small dose of FTA, the evoked discharges began to slightly increase. Then they were enhanced progressively until their intensity became nearly comparable with that
Fig. 2. Influence of FTA on the common peroneal nerve discharges evoked by electrical stimulation of the reticular formation. Upper trace: discharges of the common peroneal nerve. Middle trace: integrated curve of the upper trace. Lower trace: 10 train pulses were applied at "St", through the stimulating electrode inserted into the mesencephalic reticular formation. The facilitatory effect of the electrical stimulation was markedly potentiated 20 min after intravenous injection of 20 µg/kg of FTA.

of weak convulsive discharges. When this facilitatory effect of FTA was fully developed, these two motor nerves showed intense repetitive grouping discharges in response to the electrical stimulation of the brain stem. This potentiation was observed in all cases tested, indicating increased excitability of the reticular formation before the onset of convulsion.

This FTA-induced facilitation reached a maximum 10–20 min after injection of 20–50 µg/kg of FTA, and followed by weak convulsive discharges in a few of the cases. In such cases, electrical stimulation of the brain stem reticular formation sometimes triggered the convulsive discharges of the peripheral motor nerves.

2. Effects of FTA on the spontaneous discharges in the brain stem reticular formation: Before injection of FTA, relatively tonic spontaneous activity was recorded from the mesencephalic reticular formation, with an occasional small increment. Intravenous injection of FTA (200 µg/kg) increased the discharges in the reticular formation explosively after a latent period of a few minutes (Fig. 3). This abnormal excitation lasted for several minutes, and then intermittent grouping discharges of irregular duration were observed over 1 hr, superimposed upon continuous activity. Intervals between these abnormal grouping discharges tended to be prolonged gradually. The entire course of excitation was similar to that of peripheral motor nerves, corresponding well to the course of convulsion in the intact conscious rabbit, as described previously (3, 4).

3. Effects of FTA on single or multiple unit activity in the reticular formation: Although the firing patterns of reticular neurons were not uniform, there were some neurons whose spontaneous activity appeared to be correlated with the spontaneous discharges of the common peroneal nerve or the tibial nerve. Many of these neurons increased their spike rates in response to the electrical stimulation of the brain stem reticular formation. FTA (100–200 µg/kg, i.v.) caused a marked increase in discharges of these neurons a few minutes after its administration. In these cases, the FTA-induced typical burst discharges of the reticular neurons often preceded slightly the grouping discharges of the common peroneal nerve or the tibial nerve, as shown in Fig. 4.

Although not all neurons encountered in the mesencephalic reticular formation responded to FTA, there were other types of neurons which showed an increase in discharges after injection of FTA. However,
Fig. 3. Effect of FTA on the electrical activity of the mesencephalic reticular formation. A: before injection of FTA, B: 2 min after intravenous injection of 200 μg/kg of FTA, C: 4 min, D: 8 min.

Fig. 4. Effect FTA on the single unit activity in the mesencephalic reticular formation and the discharges of the common peroneal nerve. a₁: single unit activity in the reticular formation during abnormal excitation induced by FTA (100 μg/kg, i.v.), b₁: discharges of the common peroneal nerve, a₂: integrated curve of a₁, b₂: integrated curve of b₁. The upper and the lower traces are continuous recordings. Burst discharges of the reticular neuron corresponded well to the increase in the common peroneal nerve discharges, the former slightly preceding the latter.
their firing patterns had no apparent relation to the discharges of either the common peroneal nerve or the tibial nerve.

If diazepam (0.1–1.0 mg/kg) was given intravenously during the convulsive phase, it inhibited the abnormally increased discharges both in the common peroneal nerve and in the reticular formation (Fig. 5). Chlorpromazine (0.1–1.0 mg/kg, i.v.) and pentobarbital (5–15 mg/kg, i.v.) also had a similar inhibitory effect. On the other hand, 5–10 mg/kg of mephenesin, a centrally acting muscle relaxant, also inhibited the FTA-induced excitation (Fig. 6). However, this effect was transitory and was more potent on the peripheral motor nerves than on the brain stem reticular formation, in contrast with the potent inhibitory effect of diazepam, chlorpromazine.

![Fig. 5](image1.png)

**Fig. 5.** Inhibitory effect of diazepam on the FTA-induced convulsion. A: 20 min after intravenous injection of 100 μg/kg of FTA, before injection of diazepam; B: 1 min after injection of 0.1 mg/kg of diazepam. a₁: multiple unit activity in the reticular formation, b₁: discharges of the common peroneal nerve, c₁: discharges of the tibial nerve, a₂: integrated curve of a₁, b₂: integrated curve of b₁, c₂: integrated curve of c₁.

![Fig. 6](image2.png)

**Fig. 6.** Inhibitory effect of mephenesin on the FTA-induced excitation of the mesencephalic reticular formation and of the peripheral motor nerve. a₁: single unit activity in the reticular formation, a₂: integrated curve of a₁, b₁: discharges of the common peroneal nerve, b₂: integrated curve of b₁. Mephenesin (5 mg/kg) inhibited the abnormal excitation induced by FTA (100 μg/kg).
and pentobarbital on the brain stem reticular formation.

**Discussion**

In previous studies, the main site of convulsant action of FTA has been suggested to be in some restricted areas in the brain stem, from the mesencephalon to the medulla oblongata (3, 4). In the present study, the role of the brain stem in the convulsant effect of FTA was investigated in anesthetized rabbits.

The results obtained in the present experiments demonstrated that the mesencephalic reticular formation might play an important role in FTA-induced convulsion. FTA increased discharges of the mesencephalic reticular formation dramatically (Fig. 3), and the entire course of its abnormally increased activity was closely similar to FTA-induced convulsion. Furthermore, after administration of FTA, some neurons in the mesencephalic reticular formation showed a characteristic pattern of firing, which was well-correlated with the convulsive discharges of peripheral motor nerves (Fig. 4). Moreover, the peripheral motor nerve discharges evoked by electrical stimulation of the reticular formation were markedly enhanced prior to the onset of actual convulsion by such a small dose of FTA that it could scarcely elicit tonic-clonic convulsions (Fig. 2). These observations suggest that the excitability of the mesencephalic reticular formation is unusually increased by FTA. Since this area of the reticular formation is considered to be a part of the brain stem facilitatory areas proposed by Lindsley et al. (7), it is likely that the FTA-induced generalized tonic-clonic convulsion is a consequence of excessively increased activity of the reticulospinal facilitatory pathways. This assumption is also supported by the previous observation that FTA failed to cause convulsion after spinalization (4).

In this study, pentobarbital, chlorpromazine and diazepam were able to antagonize the effect of FTA on the brain stem and on the peripheral motor nerves (Fig. 5). Since these agents are known to have potent inhibitory effects on the brain stem, these findings also support the view that the function of the brain stem may play a key role in FTA-induced convulsion.

So far, there is no precise explanation about the mechanism by which FTA increases the excitability of the reticular formation. Only a few indirect findings are available at present. Our previous study has shown that the FTA-induced EEG arousal response is quite different from the typical seizure pattern caused by pentylentetrazol (3). Therefore the mechanism of action of FTA may be different from that of pentylentetrazol, although this drug is assumed to increase neuronal excitability through its direct action on the cell membrane (8), and the most important site of its action is also considered to be in the mesencephalon (9).

As mentioned above, diazepam and pentobarbital potently inhibited FTA-induced convulsion. Since the binding sites of these two drugs are known to be closely related to the \( \gamma \)-aminobutyric acid (GABA) receptor, it may implicate the involvement of a central GABAergic neuronal mechanism in the action of FTA. Yamazaki and his co-workers have also suggested this possibility (1, 2). There is another report about the involvement of GABA in regards to the convulsant effect of verruculogen, a tremorgenic mycotoxin whose chemical structure is very similar to that of FTA. It has been reported that the decrease in GABA level in the central nervous system may be an important pathophysiological process in the convulsion induced by verruculogen (10).

On the other hand, involvement of excitatory amino acids, such as glutamate and aspartate, is also suggested in these mycotoxin-induced convulsions. FTA-induced convulsion has been found to be antagonized selectively by D,L-2-aminophosphonovalelic acid, a specific antagonist for N-methyl-D-aspartic acid (NMDA) receptors (11). It has also been reported that these amino acids might play an important role in the effects of verruculogen (12, 13). Although these findings seem to be very meaningful, further studies are needed to clarify the real involvement of various neurotransmitters in FTA-induced convulsion.

If the primary site of action of FTA is assumed to be in the brain stem reticular formation, this convulsion might be regarded as the so-called “centrencephalic” convulsion. In
the centrencephalic hypothesis, it has been suggested that generalized seizures occur as the result of discharge in the "centrencephalic" system, a neuronal pool in the upper brain stem, usually including the nonspecific thalamus and the reticular formation of the mesencephalon and upper pons. Both clinical convulsion and epileptic discharge in the forebrain are considered to originate from the abnormal excitation of these areas. However, since a typical EEG seizure pattern was never observed in the case of FTA, this convulsion differs from the ordinary type of centrencephalic seizures in this aspect. It is difficult to explain this difference, but, at least, a generalized convulsion without an EEG seizure pattern may not be a very rare case. In fact, it has been reported that electrical stimulation of the brain stem reticular formation caused convulsion not associated with an epileptic discharge (14). Then FTA-induced convulsion may be considered to be a unique model of such a convulsion elicited by a chemical substance.

Based on the results in the present experiment, it may be concluded that the origin of FTA-induced convulsion is located within the brain stem, in particular, in the mesencephalic reticular formation, and that abnormal excitation of the reticulospinal facilitatory pathways results in generalized convulsion. However, the underlying detailed mechanisms remain to be investigated.

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