Central Effects of the Neurotropic Mycotoxin Fumitremorgin A in the Rabbit (I) Effects on the Spinal Cord

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Abstract—Effects of a potent neurotropic mycotoxin, fumitremorgin A (FTA), on the spinal cord were studied, using rabbits lightly anesthetized with urethane and chloralose. Spontaneous discharges of L₇ spinal ventral roots and common peroneal nerves were increased after intravenous injection of 100–200 μ g/kg of FTA. Their abnormal discharge pattern represented the convulsive effect of FTA. Spinal monosynaptic reflexes became irregular in amplitude, with a slight predominance of smaller reflexes. Polysynaptic reflexes were inhibited in many cases. These FTA-induced changes in ventral root discharges and spinal reflexes were abolished by spinal transection at a segment of the upper level. These results suggest that FTA may have no direct facilitatory action on spinal motoneurons and that its remarkable motor effect has its origin in the supraspinal central nervous system.

Fumitremorgin A (FTA) is a potent neurotropic mycotoxin isolated from *Aspergillus fumigatus* (1). When injected intravenously in experimental animals, FTA causes tremor and generalized tonic-clonic convulsion (2, 3). The autonomic nervous system is also severely affected by this toxin (4), and all these effects may result from abnormal excitation of the central nervous system (CNS).

However, a characteristic feature of FTA-induced convulsion was the absence of an electroencephalographic seizure pattern. Since this convulsion appears in decorticated or decerebrated rabbits, the main site of action of FTA has been suggested to be in some restricted areas in the lower part of the CNS, i.e., the brain stem or the spinal cord (4). The purpose of the present study was to determine the role of the spinal cord in FTA-induced convulsion by examining changes in spontaneous activities of ventral roots and spinal reflexes under the influence of FTA in rabbits.

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 tonic-clonic convulsion could take place under this anesthetic condition if a large dose (100–200 μ g/kg) of FTA was given (4). In order to avoid violent body movement induced by FTA, animals were immobilized with pancuronium bromide (0.1 mg/kg, i.v., initially and repeated when required) and ventilated artificially. FTA was dissolved in absolute dimethylformamide in a concentration of 0.1%. FTA and all other drugs were administered intravenously through the polyethylene cannula inserted in the femoral vein.

2. Experiments on spontaneous discharges in peripheral motor nerves: After the trachea and the right femoral vein were cannulated in the anesthetized rabbit, the left common peroneal nerve and the left tibial nerve were exposed in the popliteal fossa and cut distally. The nerves were maintained in a liquid paraffin pool, and bipolar platinum electrodes were attached to the desheathed central end of each nerve. Spontaneous discharges of each nerve and their integrated curves were recorded on a polygraph (Nihon Kohden, RMP 6008). All data were also stored on a magnetic

tape recorder (SONY, DFR 3715).

3. Experiments on ventral root discharges and spinal reflexes: After anesthesia and cannulation in the trachea and the femoral vein, the rabbit was fixed in the prone position and laminectomy was performed by the conventional method, usually at levels from L₄ to S₂. Then the spinal cord was exposed, and one L₇ ventral root and the ipsilateral L₇ dorsal root were transected. The exposed area of the spinal cord was maintained in a liquid paraffin pool. In order to record spontaneous discharges in the ventral root, a platinum bipolar electrode was attached to a bundle of the cut ventral root fibers. In some cases, the fiber bundle was further dissected into smaller bundles containing a few active fibers in order to record multiple unit discharges.

In experiments about spinal reflexes, the stimulating electrode was attached to the cut end of the L₇ dorsal root and that of the ventral root was placed on the recording electrode. Monosynaptic and polysynaptic spinal reflexes were elicited by stimulating the dorsal root every ten seconds, by a single square wave pulse of supramaximal voltage and 0.5 msec in duration.

Spontaneous discharges and spinal reflexes were displayed on the oscilloscope (Nihon Kohden, VC-7 or VC-9) and recorded

on the polygraph and on the magnetic tape recorder. If necessary, data on the oscilloscope were photographed.

In some experiments, the spinal cord was transected at the level of Th₆ or L₃ in order to examine the influence of descending pathways from the upper regions in the CNS.

4. Drugs used: Purified FTA from the mycelium of *Aspergillus fumigatus* was kindly supplied by Prof. M. Yamazaki of Chiba University. Urethane (Wako Pure Chemicals Industries, Ltd., Tokyo), α -chloralose (Nakarai Chemicals, Ltd., Kyoto), sodium pentobarbital (Dainippon Pharmaceutical Co., Osaka), strychnine nitrate (Tokyo Kasei Kogyo Co., Tokyo), chlorpromazine hydrochloride (Yoshitomi Pharmaceutical Industries, Ltd., Osaka) and diazepam (Takeda Pharmaceutical Co., Osaka) were purchased.

Results

1. Effects of FTA on spontaneous discharges in peripheral motor nerves: When FTA (100–200 μ g/kg) was injected intravenously, efferent discharges of the common peroneal and the tibial nerves were increased suddenly after a latent period of 2–10 min (Fig. 1). In many cases, this first explosive increase in discharges was observed in the common peroneal nerve, but at the next mo-

Fig. 1. Effect of FTA on spontaneous discharges in peripheral motor nerves. a₁: discharges in the common peroneal nerve. a₂: integrated curve of a₁. b₁: discharges in the tibial nerve. b₂: integrated curve of b₁. After intravenous injection of FTA (100 μ g/kg), spontaneous discharges in both nerves were markedly increased.

ment, the increased discharges appeared only in the tibial nerve, and then appeared again only in the common peroneal nerve. This condition of alternating excitation in these two nerves continued for usually some ten seconds. It might correspond to the state of clonic convulsion and a reciprocal innervation mechanism might be still functioning. Then, both nerves showed strong excitation simultaneously, which might represent tonic convulsion. After some minutes, this abnormal excitation was replaced by intermittent discharges of smaller amplitude which lasted at least 3 hr, sometimes mingled with strong excitation as seen in the first phase.

When pentobarbital (5–15 mg/kg) (Fig. 2), chlorpromazine (0.1–1.0 mg/kg) (Fig. 3) or diazepam (0.1–1.0 mg/kg) was injected intravenously during the convulsive phase induced by FTA, the abnormal excitation in these peripheral motor nerves was inhibited or abolished, although the inhibitory effect of this small dose of pentobarbital was only transitory.

2. Effect of FTA on the ventral root discharges: Discharges in the L₇ spinal ventral root were increased explosively by FTA (100–200 μ g/kg, i.v.) after a similar latent period (Figs. 4 and 5A–C), and this condition continued for several minutes. After that, intermittent grouping discharges of slightly smaller amplitude were observed over a few hours, although intervals between grouping discharges prolonged gradually. This entire course of excitation corresponded well to that of convulsion in the intact rabbit, as described in the previous paper (4). Pentobarbital (15–20 mg/kg, i.v.) abolished the ventral root discharges, although this effect was only transitory.

If the spinal cord was transected at the Th₆ or L₃ level during the convulsive phase, the increased ventral root discharges were completely abolished (Fig. 5D). On the contrary, strychnine (100 μ g/kg, i.v.) was able to induce convulsive discharges in the same rabbit after transection of the spinal cord (Fig. 5E).

3. Effect of FTA on the spinal reflexes: After

Fig. 2. Inhibitory effect of pentobarbital on FTA-induced convulsion. A: convulsive discharges in the common peroneal nerve observed 40 min after intravenous injection of FTA (100 μ g/kg). Before injection of pentobarbital. B: immediately after intravenous injection of 5 mg/kg of pentobarbital. C: 5 min after B. In each set of A, B and C, the upper trace shows discharges in the common peroneal nerve and the lower trace is its integrated curve.

Fig. 3. Inhibitory effect of chlorpromazine on FTA-induced convulsion. a_1 : discharges in the common peroneal nerve. b_1 : discharges in the tibial nerve. a_2 : integrated curve of a_1 . b_2 : integrated curve of b_1 . A: 10 min after intravenous injection of 100 $\mu\text{g}/\text{kg}$ of FTA and before injection of chlorpromazine. B: 1 min after intravenous injection of 1.0 mg/kg of chlorpromazine. Convulsive discharges observed in A were inhibited by chlorpromazine.

injection of FTA (100–200 $\mu\text{g}/\text{kg}$, i.v.), the amplitude of the L_7 monosynaptic reflex varied from time to time, but there was a tendency for smaller monosynaptic reflexes to be more frequent (Fig. 6A, B).

On the other hand, polysynaptic reflexes almost disappeared under the influence of FTA. Furthermore, during FTA convulsion, basal activity in the oscilloscope became very unstable, possibly because of abnormally increased ventral root discharges. These changes in reflexes returned to normal after spinal transection at the L_3 or Th_6 level (Fig. 6C). In contrast with FTA, strychnine potentiated polysynaptic reflexes, even in the same spinalized rabbit (Fig. 6D). When the thoracic spinal cord (Th_6) had been transected 1 hr prior to intravenous injection of FTA, 200 $\mu\text{g}/\text{kg}$ of FTA failed to cause any change in L_7 mono- and polysynaptic reflexes.

Discussion

Although many of the neurotropic mycotoxins have been discovered originally as causative substances of diseases of domestic animals, progress of research in this field revealed that some of these toxins have physiologically or pharmacologically interesting unique features (5–7). It implies also

Fig. 4. Effect of FTA (100 $\mu\text{g}/\text{kg}$, i.v.) on spontaneous discharges in the L_7 ventral root. A: before injection of FTA. B: 3 min after injection of FTA. C: 10 min after injection. D: 30 min after injection.

Fig. 5. Effect of FTA on the L_7 ventral root discharges (multiple unit discharges) and influence of spinal transection. A: before injection of FTA. B: 4 min after intravenous injection of FTA ($100 \mu\text{g/kg}$). C: 6 min after injection of FTA. D: 8 min after spinal transection at the L_3 level. E: 2 min after intravenous injection of strychnine ($100 \mu\text{g/kg}$) in the same rabbit as D.

their potential usefulness as experimental tools for neuroscience research. For instance, study of FTA might contribute to a better understanding of the pathophysiological mechanism of convulsion. A characteristic property of FTA-induced convulsion is the absence of EEG seizure in spite of behavioral resemblance to generalized convulsion in a grand mal type of epilepsy, suggesting the importance of the brain stem and the spinal cord as the primary origin of convulsion (4).

FTA-induced convulsion is able to take place even in decorticated or decerebrated rabbits (4). Therefore, at first, it would be necessary to know whether the spinal cord is of major importance in the mechanism of this convulsion. FTA-induced increase in spontaneous discharges in peripheral motor nerves and spinal ventral roots (Figs. 1 and 4) corresponded well to behavioral observations on generalized convulsion (4). Consequently the violent motor effect of FTA should be attributable to abnormal excitation of the CNS. However, FTA may have no direct excitatory effect on the spinal cord, because it failed to facilitate spinal reflexes. In fact, on the contrary, spinal reflexes were rather inhibited (Fig. 6).

Abnormal increase in spontaneous dis-

Fig. 6. Effect of FTA on spinal reflexes. A: before injection of FTA. B: 10 min after intravenous injection of $200 \mu\text{g/kg}$ of FTA. C: 15 min after spinal transection at the L_3 level. D: 3 min after intravenous injection of $100 \mu\text{g/kg}$ of strychnine in the same rabbit as C. Successive five traces were superimposed in each photograph.

charges in the ventral root, as well as changes in spinal reflexes, returned to the normal state completely after transection of the spinal cord at an upper level (Figs. 5D and 6C). In contrast to FTA, the well-known spinal convulsant strychnine could evoke convulsive ventral root discharges (Fig. 5E) and potentiate the polysynaptic reflex (Fig. 6D), which are well-established effects of this drug, even in the same spinalized animals in which FTA failed to show any effect. Therefore, mechanisms of action of FTA should be quite different from those of strychnine. These results suggest that FTA-induced abnormal motor hyperactivity is derived from the supra-spinal CNS and that FTA has no direct excitatory effect on spinal motoneuron. Considered together with the results in experiments of decortication and decerebration, the main site of action of FTA should be some regions in the brain stem, from the mesencephalon to the medulla.

There are a few lines of evidence to support this view. The first is the fact that FTA caused abnormal excitation of vagal and sympathetic nerves and marked changes in blood pressure and heart rate (4). These effects in the autonomic nervous system may be easily explained if it is assumed that the brain stem reticular formation is strongly affected by FTA. Secondly, spinal reflexes tended to be inhibited by FTA. This phenomenon might also be attributable to excitation of the brain stem, if Magoun's inhibitory areas of brain stem reticular formation (8), as well as intra-spinal motor control mechanisms, are taken into consideration. Furthermore, pentobarbital, chlorpromazine and diazepam could antagonize the effect of FTA (Figs. 2 and 3). These observations are also in agreement with our assumption, because these drugs are known to have potent inhibitory action on the brain stem.

There are some attractive findings concerning verruculogen, which is another tremorgenic mycotoxin very similar to FTA (9). Norris et al. reported that this toxin caused substantial increase in the miniature-endplate potential frequency at the locust neuromuscular junction where glutamic acid is employed as a neurotransmitter and that verruculogen increased the spontaneous release

of glutamate and aspartate from rat cerebrocortical synaptosomes, but not from spinal cord/medullary synaptosomes (7). Assuming that these excitatory amino acids play key roles in the motor effects of verruculogen, these results suggest that its main site of action is not the spinal cord, but a certain part of the higher CNS. This finding may be noteworthy because FTA, whose chemical structure is closely similar to that of verruculogen, also showed no direct action on the spinal cord in our experiments.

So far, it has not been fully known whether the medulla is essentially involved in the mechanism of action of FTA, although there are a few indirect affirmative findings in relation to autonomic functions, as mentioned above. On the other hand, the release of amino acids from spinal cord/medullary synaptosomes was not influenced by verruculogen (7). However, the involvement of the medulla cannot be excluded in this case, because a fairly large part of the results might represent the effect of verruculogen on the spinal cord. Further studies are required to clarify the role of the medulla in the mechanism of action of these mycotoxins.

As to the involvement of upper parts of the CNS, Peterson et al. performed *in vivo* experiments in the rat, using a smaller dose of verruculogen. In their experiments, the increase in glutamate and aspartate content was observed in the superfusate of the lateral ventricle, but not of the cerebral cortex (10). Based on these results, they suggested a subcortical action of verruculogen. Although these results seem to be incompatible with those of Norris et al., with respect to the involvement of the cerebral cortex, it is possible that the subcortical CNS may be more easily affected by a small dose of verruculogen than the cerebral cortex. If the mechanism of action of FTA is assumed to be similar to that of verruculogen, these data about verruculogen are particularly interesting, because the convulsant action of FTA in decorticated or decerebrated animals (4) might be explained by a similar subcortical action.

Our results in the present study indicate that FTA does not directly facilitate spinal motoneurons, but that its remarkable motor effect has its origin in the brain stem, from the

medulla oblongata to the mesencephalon. The details about the direct effect of FTA on the brain stem neurons remain to be elucidated in order to understand the precise mechanism of its action.

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