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STUDIES ON VOLVATOXIN

I. EFFECT OF VOLVATOXIN ON THE ELECTROENCEPHALOGRAM OF LIGHTLY ANESTHETIZED RAT[1]

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Yu-Lun Yang, Lih-Fen Chang and Chin-Yih Wu (1980) Studies on volvatoxin *Bull. Inst. Zool., Academia Sinica* **19**(2): 31-39. Volvatoxin A (MTA), consisting of two toxic protein components: volvatoxin A_1 (VT-A_1) and volvatoxin A_2 (VT-A_2), was isolated from the edible mushroom, *Volvariella volvaceae*. To understand the neural mechanism of writhing reaction induced by the volvatoxin, the changing of EEG patterns on the light anesthetized rat were observed. The following results were obtined.

1. within 1 to 7 minutes after the administration of VT-A_2 or mixed with VT-A_1 and VT-A_2 (MTA), the EEG exhibited abnormal discharge, possibly resulting from severe excitation of the cerebral cortex.

2. the large dose of VT-A_1, injected both in vein and in intracerebral ventricle, did not elicit the abnormal discharge of EEGs.

The volvatoxin A (MTA), a cardiotoxic protein which composed of volvatoxin A_1(VT-A_1) and volvatoxin A_2 (VT-A_2), was first isolated by Lin *et al.*[14]. It has three major biological activities, namely, causing hemolysis of human blood cells, eliciting the writhing reaction and changing the electrocardiogram (ECG) in experimental animals[5,15,16].

The toxicity of VT-A_1 or VT-A_2 is separately lower than that of the MTA. However, the toxicity increased when VT-A_1 and VT-A_2 were mixed at the ratio of 1 : 2 by weight[5,14,15,16].

The aim of this study is to understand the changing of the electroencephalogram (EEG) pattern, and the relationship between the EEG changes and the writhing reaction after the administration of VT-A_1, VT-A_2 and MTA to laboratory rats.

Since the EEG is remarkably affected by the partial pressures of blood gases and the blood acidity[6,8,11,17,18,23,25], the value of blood gases was recorded along with the EEG in each experiment.

MATERIALS AND METHODS

General preparations

42 adult Sprague-Dawley rats with an average weight of 280-300 g, were used. Surgical procedures were accomplished while the condi-

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tions in which the rat was anesthetized by chloralose treatment (50 mg/kg i. p.). The following operations were performed: a tracheotomy for the measurement of the respiratory movement, a carotid cannulation for the determination of the arterial blood pressure, a femoral arterial cannulation for blood sampling, and a craniotomy for EEG recording. The animal was fixed on a standard stereotaxic appratus for its brain localization. A set of infrared lamps were used for maintaining the normal brain temperature. According to the map of Pellegrino and Cushman[22], an injection needle was inserted into the cerebro-lateral ventricle.

The toxic proteins were isolated by using the method of Lin *et al.*[14] and their purities were tested by the writhing method in the mice[14]. All the drugs were dissolved in Ringer's solution, and 30 μl was administrated to the rat's cerebro-lateral ventricle (i. c. v.), 200 μl to the femoral vein (i. v.), respectively.

Treatment and Recording

During this experiment, all the animals were kept under the above described anesthetic condition. Therefore, determination of the effect of the anethetic drug on the animal was necessary. For controls, the animals were treated with the following solutions:

a) a 200 μl of Ringer's solution by i. v. and 30 μl by i. c. v.

b) a toxic protein which has been heated at 55°C for 30 minutes.

For recording, a monopolar ball electrode was placed on the exposed parietal part, and an indifferent screw electrode was attached to the nasal bone of the animal. These electrodes were then connected to a multipurpose polygraph (RM-150, Nihon Kohden), but most mesurments were carried out by using an 8-channel electroencephalograph (EEG 5-109, Nihon Kohden) and EEG frequency analyzer (MAF-5, Nihon Kohden). In this case, the EEG in each 10 seconds duration was integrated and divided into five frequency bands. Their bandwidth (Hz) were: 2-4, 4-8, 8-13, 13-20 and 20-30. The signals of the ECG for calculation of heart rate, respiratory movement and carotid arterial pressure were recorded from the EEG apparatus. But only 2 channels were used for their recording among the ECG, respiratory movement and blood pressure (other 6 channels were recorded for an original EEG and 5 analyzing EEGs). Thus, some graphic data could not appear simultaneously.

During the experiment, a 150 μl blood sample was collected from the femoral artery for the determination of the partial pressure of oxygen (P_{O_2}), carbon dioxide (P_{CO_2}) through a special gas analyzer (IL 213, Instrumetation Lab.)[25].

RESULTS

Records of the control groups

(a) Eight animals injected with chloralose (50 mg/kg i. p.) were used as control. All of their EEGs showed an amplitude of about 200μV and synchronized waves. The slow waves (2-4 Hz) were predominant (40-44%), followed by 4-8Hz (29-32%), 8-13Hz (14-17%), 14-20Hz (5-6%), 20-30Hz (4-5%) in this order. Their five analytical sequences are were very stable for 2 hours, and these stabilities were found significant by t-test ($p<0.05$).

(b) Rats with i. v. or i. c. v. injection of Ringer's solution, or heated toxic protein dissolved in the Ringer's solution, showed no distinct change in their EEG patterns and analytical sequences. The blood pressure, respiratory rate as well as heart rate remained at the original level in the control groups.

Records of the test groups

(a) Intravenous injection of 5-10 mg/kg of VT-A_1 had no effect on the EEG (Fig. 1A and 3A, and Table 1), but a dosage of 1 mg/kg of VT-A_2, produced within 3 minutes "sporadic polyspike and wave"[21]. The duration of this abnormal discharge was about 15 min., and the maximal response of discharge appeared within 5 to 10 minutes after administration of VT-A_2 (Fig. 1B and 3B, and Table 1). A 0.075 mg/kg of MTA could cause a polyspike and wave within 7 minutes, and it was followed by low

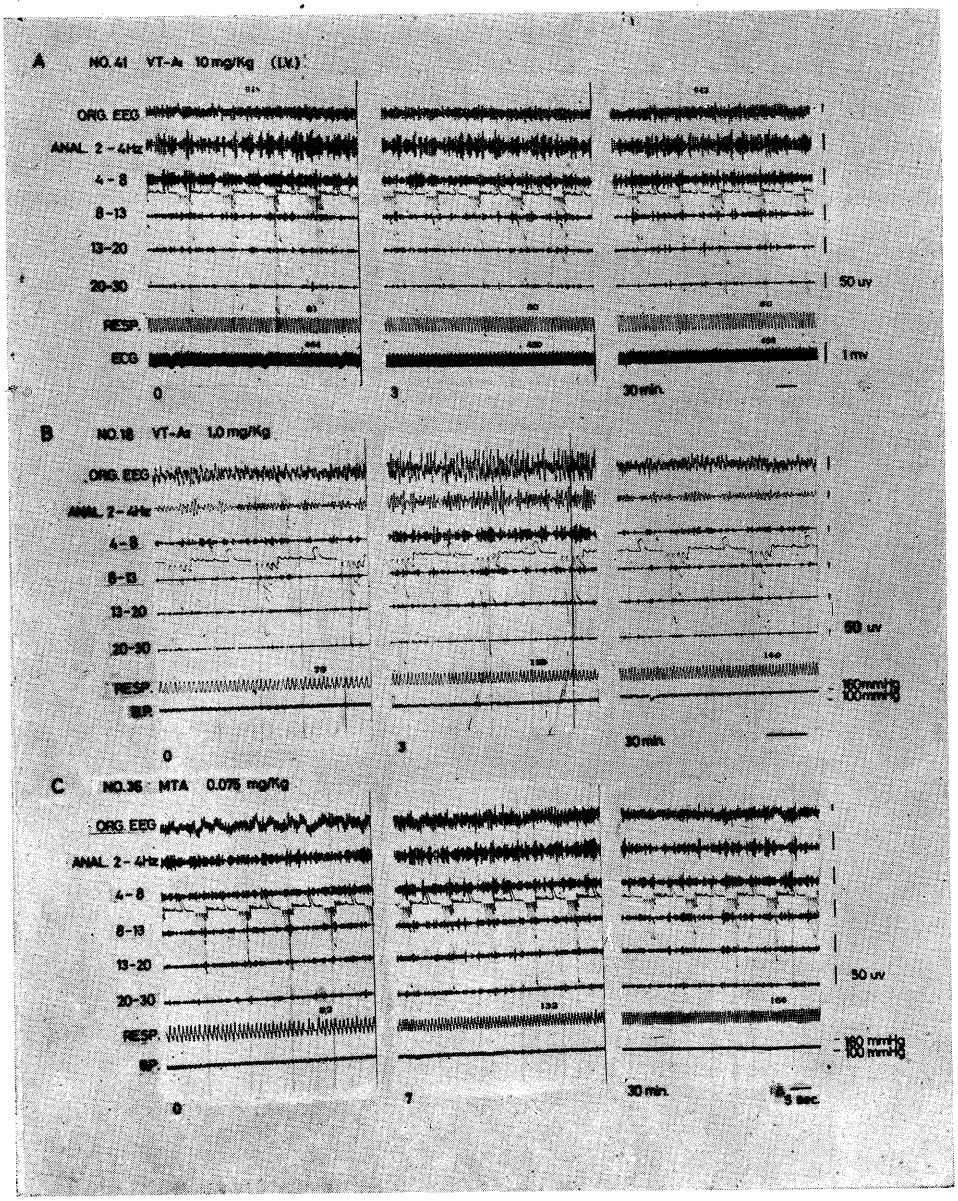

Fig. 1. Polygraphic recordings showing effect of VT-A_1 (A), VT-A_2 (B) and MTA (C) by i. v. injection.
O: control. 3, 7, 30 min: time after the administration of volvatoxin. Each section consists of the original EEG (ORG. EEG), the analyzed EEG (2-4, 4-8, 8-13, 13-20 and 20-30 Hz), the respiratory movement (RESP) and the ECG with heart rate/min or blood pressure. In this figure, the percentage of each EEG within 10 sec, is shown by the height of the column.

frequency and low amplitude ones (Fig. 1C and 3C, and Table 1).

The return of the EEGs back to normal condition usually took 20 minutes in most experiments. In the responses to the injection of 0.1 mg/kg of MTA, the above mentioned phenomena were more pronounced.

Minimal effective dosage of the VT-A_2 and MTA on EEG changes was 1 mg/kg and 0.075 mg/kg respectively (Table 1). The EEG patterns of 10 out of 14 (71.4%) and 7 out of 12 rats (58.3%) were changed and recovered to original stages within 30 to 45 minutes. In these cases, the VT-A_2 increased percentage of

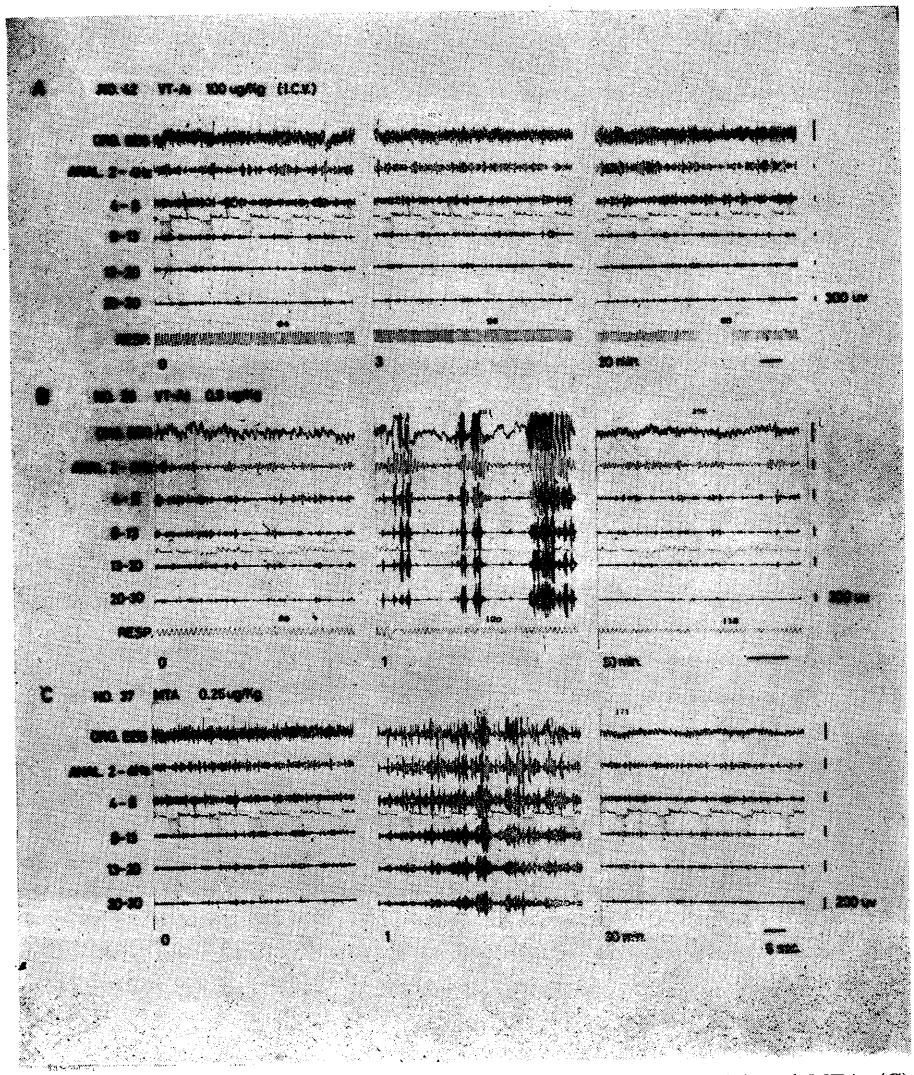

Fig. 2. Polygraphic recordings showing effect of VT-A_1 (A), VT-A_2 (B) and MTA (C) by i. c. v. injection.
O: control. 1, 3, 30 min: time after the administration of volvatoxin. Each section consists of the original EEG (ORG. EEG), the analyzed EEG (2-4, 4-8, 8-13, 13-20 and 20-30 Hz) and the respiratory movement (RESP) except C. In this figure, the percentage of EEG within 10 sec. is shown by the height of the column.

the low frequencies (2–4 and 4–8 Hz), but the MTA increased percentage of the high frequencies (13–20 and 20–30 Hz), but no effect on the medial frequencies (8–13 Hz) (Fig. 3B and 3C).

(b) There was no effect on the EEGs by i. c. v. injection with 100 μg/kg of VT-A_1. However, a 0.5 μg/kg of VT-A_2 or a 0.25 μg/kg of MTA showed the sporadic polyspike and wave, and their, latent times were 1 or 2 minutes, respectively. Their latencies were shortened when large dosags of toxic proteins were administered continuously, and the mortality was increased (Fig. 2A, 2B, 2C, 3D, 3E and 3F, and Table 2).

Effect of toxic protein on respiration, blood pressure and heart rate

There was no effect on the animals respiration, blood pressure and heart rate even when 10 mg/kg of VT-A_1 was administrated by i. v. injection in the i. v. injection of 1 mg/kg of VT-A_2 caused a phasic and slight decrease of blood pressure within 3 minutes. When the sporadic polyspike and wave could be found in the EEGs, the respiratory movement was also

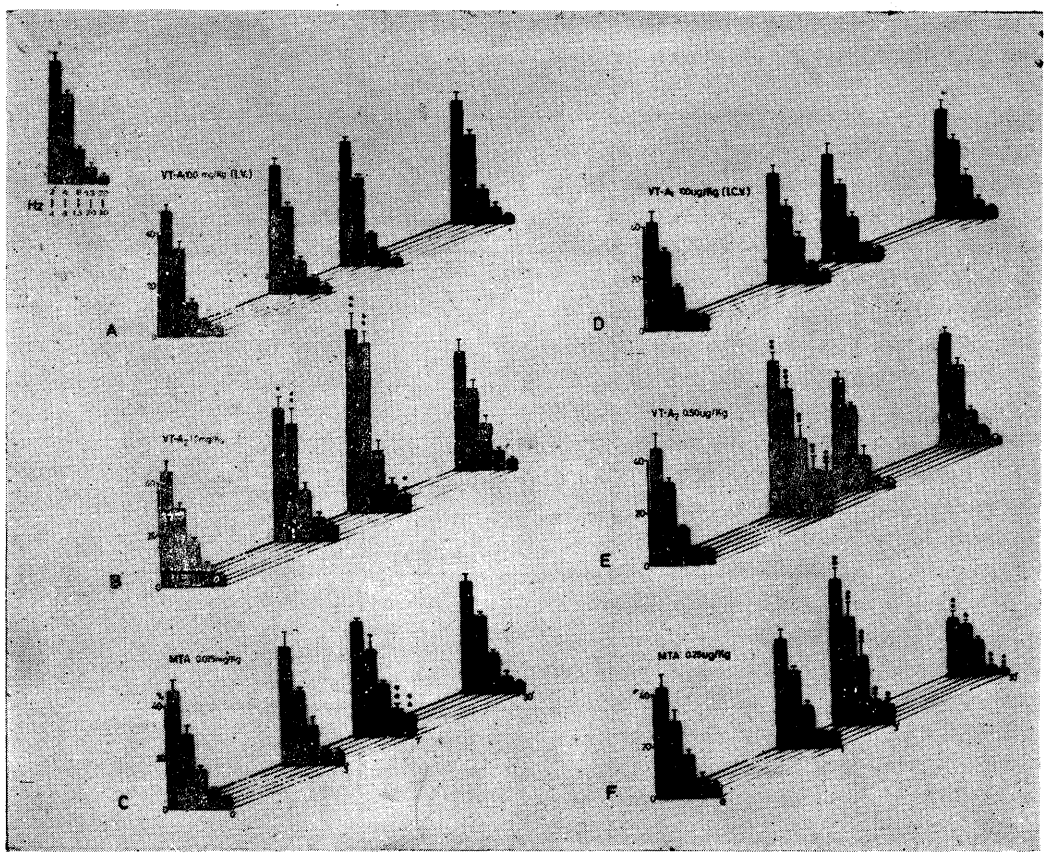

Fig. 3. Properity and decay of EEG patterns elicited by volvatoxin injection within 10 sec. O: control. 3, 7, 30 min: time after i. v. (A, B, C) and i. c. v. (D, E, F) injection. The changes of EEG frequency are shown by percentage. Upper left corner: frequency of each EEG pattern.
i. v. injection: VT-A_1: 100 mg/kg; VT-A_2: 10 mg/kg; MTA: 0.075 mg/kg.
i. c. v. injection: VT-A_1: 100 μg/kg; VT-A_2: 0.5 μg/kg; MTA: 0.25 μg/kg.
• $p<0.05$: $p<0.01$

increased. After an i. v. injection of minimal effective dosage of MTA, the respiratory movement always increased but not the blood pressure (Fig. 1A, 1B and 1C).

With i. c. v. injections, a 100 μg/kg of VT-A_1 still has no effect, but 0.5 μg/kg of VT-A_2 caused an increase in frequency of respiratory movement within 1 minute. The same dosage of VT-A_2 showed no effect on the heart rates and the blood pressure in the treated animals (Fig. 2A, B). The minimal dosage of the MTA also had no effect on the respiration, blood pressure and heart rate.

The relative changes of blood pH, blood gases and EEGs after administration of the toxic protein

During the experiment, EEGs showed abnormal discharges after the toxic protein was administrated. The hyperventilation, increase of pH and P_{O_2} and the decrease of P_{CO_2} were always detected (Fig. 4).

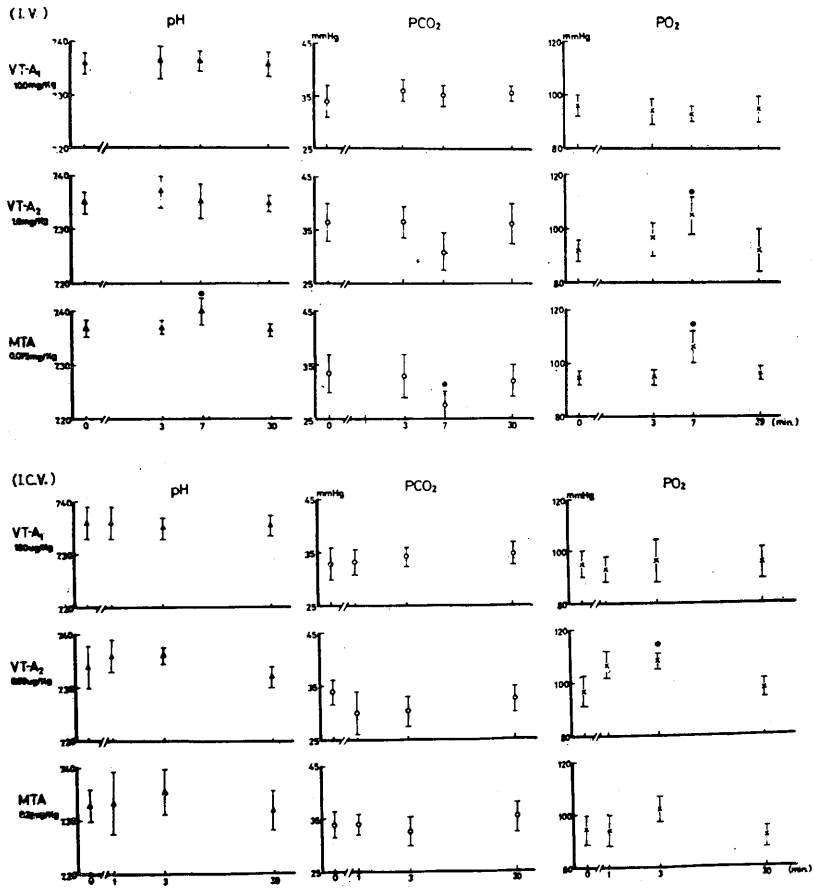

Fig. 4. Effects of toxic protein on the pH, PCO_2 and PO_2 of blood elicited by i. v. and i. c. v. injection.
O: control 1, 3, 7, 30 min: time after the injection
i. v. injection: VT-A_1: 100 mg/kg; VT-A_2: 10 mg/kg; MTA: 0.075 mg/kg.
i. c. v. injection: VT-A_1: 100 μg/kg; VT-A_2: 0.5 μg/kg; MTA: 0.25 μg/kg.
• $p<0.05$

TABLE 1
Relationships between intravenous injection of volatoxin and their dosage, time of onset and survivor

Treatment (i. v.)	Number of experiments	Dosage (mg/kg)	Time of onset (min)	Survivor#
VT-A_1	3	1.0	—	—
	3	3.0	—	—
	6	10.0	—	—
VT-A_2	2	0.5	—	—
	5	0.7	—	—
	3	1.0*	2.6±0.3	—
	5	1.5	1.7±0.3	—
	5	2.0	1.0±0.2	±
	4	4.0	0.4±0.2	+
MTA	2	0.05	—	—
	4	0.075*	5.2±0.6	—
	3	0.10	5.0±0.5	—
	4	0.25	4.0±0.5	±
	5	0.50	3.4±0.2	+
	4	1.0	3.2±0.5	+

#: — existence, + death
*: minimal effective dosage

TAELE 2
Relationships between intracerebral ventricular injection of volvatoxin and their dosage, time of onset and survivor

Treatment (i. c. v.)	Number of experiments	Dosage (mg/kg)	Time of onset (min)	Survivor#
VT-A_1	3	100	—	—
VT-A_2	4	0.25	—	—
	3	0.5*	0.8±0.2	—
	3	1.0	0.3±0.1	—
	5	10.0	0.2±0.1	±
	5	25.0	0.2±0.1	+
MTA	5	0.2	—	—
	4	0.25*	2.3±0.2	—
	3	0.5	1.0±0.1	±
	3	1.0	0.8±0.2	+
	4	2.0	0.7±0.1	+

#: — existence, + death
*: minimal effective dosage

DISCUSSION

Anesthetic drugs have inhibitory action on the central nervous system and consequently the EEG is disturbed[7,12,20]. The different anesthetic drugs elicit different EEG patterns. According to Okuma[20], the EEGs of the anesthetized animals could be divided into five steps. In this experiment, all of the animals were studied at step II which showed the skeletal muscle relaxation, regular movement of respiration, loss of the eye-lid reflex and slight synchronization with 200-500 μV in EEG's amplitude. Thus, all tested animals were in the same slightly anesthetized condition, and the individual variations of anesthetic level could be minimized.

Chloralose is a long-lasting anesthetic drug, its activity could last for 4-6 hours[13]. In steady condition, it has few effects on the cardiovascular system, respiratory system, P_{CO_2} and P_{O_2} consumption[1,9,24,25].

Tables 1 and 2 showed that large dose of VT-A_1, regard less of i. v. or i. c. v. injection, could not cause any change in EEGs, respiratory movement and blood pressure. With i. c. v. injection of VT-A_2 or MTA, it could elicit significant change in EEG. It could be assumed that they had been taken up by the cerebro-cortical cells and directly induced their abnormal deporalization. The synchronized sporadic polyspikes also were shown.

Since the struture of the toxic proteins is not clear[14], it is difficult to explain that the onset of i. c. v. response is shorter than that of i. v. administration. More detailed experimental research is needed.

Use of the radio-isotopic tracer method could indicate (and even directly measure) if the toxic protein can diffuse through the blood brain barrier or not[2,3,19]. But, in this experiment, the toxic proteins were infused directly into cerebral ventricle, therefore, it will not be necessary to consider out the blood brain barrier[3,17].

The i. c. v. injection of the heat treated toxic protein always had no effect. Evidently the changes inEEG patterns were caused by the injection of toxic protein, and the elevation of cerebral ventricular pressure was not due to the i. c. v. injection of 30 μl toxic proteins.

According to Carroll and Lim[4], the writhing reaction have been related to spinal cord, cerebellum and viscera such as stomach, intestine and peritoneum in the rat. The transection, ablation or procain administration of these organs could eliminate the writhing syndrome. The i. v. injected toxic proteins, since they have large molecular weights[14], were unable to pass blood brain barrier by diffusion, except if they can be transferred in by pinocytosis. However, after injection, the toxic protein was diluted in blood vessels and transported by blood to the targets where they accumulated and might elicit the abnormal excitation. These excitation would ascend to the cerebral cortex and caused the abnormal discharge. Thus, the change of EEG pattern caused by i. v. injection would be secondary.

In free moving mice, the intraperitoneal injection of MTA induces the writhing reaction within 10 minutes[14]. In this experiment, all of the animals studied were under slight anesthesia and the minimal effective dose of MTA could induce the abnormal discharge within 10 minutes. The coincidence in the onset of these two responses could be explained by assuming that the occurrence of abnormal discharge was in correspondence with the writhing reaction. These varied onset of discharge compared with that of the moving mice might be attributed to the different way of administration of drugs and different physiological conditions of the experimental animals. But this assumption needs to be substantiated by more detailed EEG studies of the free moving animals.

EEG pattern is easily affected by changes in pH and P_{CO_2} of the blood[8,10]. During the experiment, the EEG had sporadic polyspike and waves, and the respiratory movement, P_{O_2} and pH of the blood are increased, but P_{CO_2} decreased.

If the P_{CO_2} decreased quickly, it could cause the constriction of brain blood vessels. Therefore, the cerebral ischemic anoxia and respiratory alkalosis appeared[8,10,17]. This induced the increase of low amplitude waves in EEGs.

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草菇毒蛋白的研究

第一報　草菇毒蛋白在輕度麻醉老鼠腦波上的影響

楊幼倫　張麗芬　吳京一

草菇毒蛋白是自草菇 (*Volvariella volvaceae*) 中分離出的毒蛋白，含有兩種成分，即草菇毒蛋白 A_1 及 A_2 (VT-A_1, VT-A_2)，並已證實對小白鼠有扭曲反應。

玆欲瞭解草菇毒蛋白產生扭曲反應之神經性機制，本研究就草菇毒蛋白對輕度麻醉之大白鼠腦波加以探討。

分別以腦室內直接注入及靜脈內注射之方法，給與 VT-A_1, VT-A_2，及以 A_1 和 A_2 重量比 1：2 的混合物 (MTA)，觀察腦波頻率之變化，並以 *t*-test 分析其有意性，結果如下：

1. VT-A_2 及 MTA 分別注入後，在 1 至 7 分鐘內，老鼠腦波呈不正常之放電現象。

2. 分別以腦室內或靜脈內投與 VT-A_1，其注入量雖是甚大（靜脈內即 10 至 200 倍，腦室內即 200 至 400 倍），亦無法誘起不正常之放電現象。