[原著]Behavioral Effects of Intraspinally Injected Habu (Trimeresurus flavoviridis) Venom on Rats

琉大保医誌 1 (I) : 30～34, 1978.

Behavioral Effects of Intraspinally Injected Habu (Trimeresurus flavoviridis) Venom on Rats

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Introduction

Peng (1952) reports that Formosan cobra venom injected into the cerebellomedullary cistern of the rabbit causes a rise in blood pressure and a transient stimulation of respiration followed by a depression, and that the lethal dose is much smaller than that by the peripheral application. Bhargava et al. (1970) reveals that an application of a neurotoxic fraction prepared from Naja naja venom to the exposed cerebral cortex of the rat produces a long-lasting convulsant effect.

Although histopathological changes in the central nervous system (CNS) of the cat caused by a Habu snake bite (induced experimentally) have been demonstrated by Sakae (1963), pharmacological effects of Habu venom on the CNS remain to be clarified. This investigation was made in order to study the effects of intraspinally injected Habu venom on the CNS.

In an attempt to determine the mode of action of the venom on the CNS, a preliminary experiment was conducted, designed to observe whether or not formation of a pharmacologically active substance takes place under *in vitro* incubation of the cerebrospinal fluid (CSF) with the venom.

Methods

The animals used in this experiment were rats of the Wistar strain weighing 200 to 250 g. The venom obtained from Okinawa Prefectural Public Health Laboratory was crude. It was dissolved into a normal saline solution, which was injected intraspinally at a volume of 0.025 ml containing 50 μg of the venom.

For the technical method of spinal injection we referred to Nishi (1955). The animal was placed in the prone position with the head and shoulders pressed into an appropriate box, and the coxa was fastened with the operator's hand to prevent an escape and body movements. A thin needle with syringe was inserted into the vertebral canal at an angle of 40 to 45° to the vertebral line, through the space between the two spinous processues of the fourth and the fifth lumbar vertebra. It is usually recommended to lay a rolled cotten wool under the abdomen and also to make an incision close to the fourth vertebral process. These steps facilitated the spinal puncture. A successful spinal injection is indicated by a transient streching of the leg muscles.

Pharmacologically active substance-forming activity of the venom was tested by *in vitro* incubation with th the CSF which was obtained from human subject whose laboratory studies revealed no changes of the composition of the CSF. One ml of the CSF and 50 μg of the venom (dissolved in 0.025 ml of the normal saline solution) were incubated at 37°C in a teflone tube, and at intervals of 5, 15, 30 and 60 minutes, each of the incubates was examined by the biological method of assaying the uterine contraction. The contraction of the uterus was recorded by using a high

sensitive strain guage (RP-3, Nihon Kohden). The uterus was obtained from a virgin rat weighing about 150 g, which had been given estradiol benzoate at the ratio of 100 μg per Kg body weight 18 hours before the use in the experiment. The isolated uterus was stored at 4°C in de Jalon solution for 5 hours to get a stable sensitivity. The uterus was suspended in an organ bath at 25°C containing 5 ml of oxygenated de Jalon solution to which atropine sulfate and chlorpheniramine malate had been added at the final concentration of 10 μg per ml respectively. Then, 0.1 ml of the incubated CSF was introduced into the bath. Before starting the assay, the uterus was made to contract several times by adding from 5 to 50 μg of synthetic bradykinin until it gave a steady response to the same dose.

Results

Behavioral effects of the venom after its spinal injection in rats: The animal exhibited a series of characteristic signs after the spinal injection of the venom. The respiraton showed a transient stimulation followed by a slow frequency, brought sometimes with strong abdominal contractions resembling vomitting. No disorder of the animal posture could be observed but walking diminished. At a slight pinch of the foot or tail, both responses of withdrawing and squeaking were much more pronounced than those of the normal animal. This state lasted from 10 to 15 minutes.

The animal grew more sensitive with the passage of time and then the squeaking was seen even at a slight touch of the body and/or spontaneously from 20 to 30 minutes later. At the same time, the animal showed aggressive reactions against the instrument used for pinching.

Figure 1. Habu venom given by spinal injection produces characteristic behavioral phenomena in the rat, A: squeaking with out-stretched fingers and erected fur, B and C convulsions.

From 50 to 70 minutes later, the animal became extremely restless, began running and suddenly got into a violent convulsion resembling that of the picrotoxin-induced type. The convulsion was clonic and it was repeated at intervals of about 10 minutes. The characteristic states of the recurrent convulsions are shown in Figure 1. The animal usually died in the tonic extension of the convulsion accompanied by the respiratory paralysis within 90 to 120 minutes.

Formation of a pharmacologically active substance after in vitro incubation of the CSF with the venom: In so far as the CSF was left in a teflon tube at the room temperature (25°C) for 1 hour, formation of an active substance could not be seen by assaying the rat uterine contraction after the introduction of 0.1 ml of the CSF into the bath containing 5 ml of de Jalon solution.

On the contrary, the CSF incubated with the venom at the same concentration as the untreated CSF caused powerful uterine contractions but slow in the onset, whose level grew higher in

proportion to the length of the incubation time, as shown in Figure 2. The contractile force of the CSF incubated for 30 minutes was comparable to that of 100 ng per ml of bradykinin.

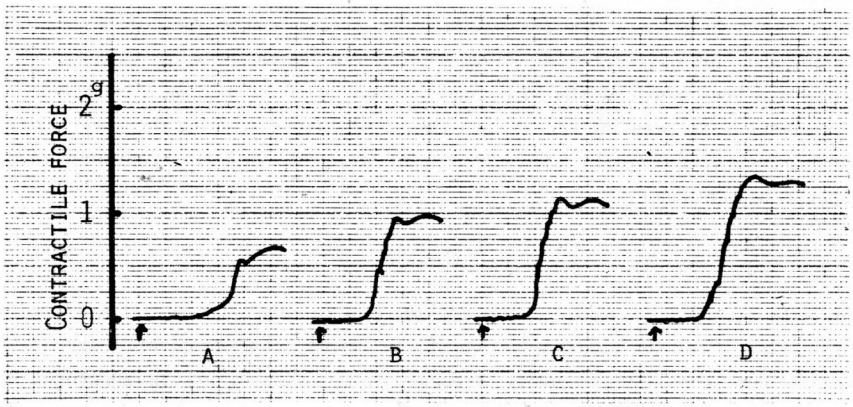

Figure 2. Tracing of uterine contraction.
At 5(A), 15(B), 30(C) and 60(D) minutes of incubation at 37°C. 0.1 ml of the incubates was assayed on the isolated rat uterus.

Discussion

The effects of Habu venom on the CNS after the spinal injection in rats were characterized by squeaking and repeated clonic convulsions with the maximum latency of approximately 60 minutes, and by the animal's death in a tonic convulsive extension accompanied by respiratory paralysis within 90 to 120 minutes.

Lee (1971, 1972) states that whether the convulsive effect is caused by a direct action of whole Cobra venom or by other venom component such as neurotoxin is not completely elucidated. Lee and Chen (1977) concludes that cardiotoxin and phospholipase A_2 rather than neurotoxin, which are isolated from Cobra venom, produce recurrent convulsions on their central application. However, we assumed in the case of Habu venom that a newly formed pharmacologically active substance may play a part in producing the behavioral effects on the animal because this new substance can be released in the *in vitro* incubation of the CSF with the venom. The assumption that an active substance is formed *in vivo* by contamination of the CSF with the injected venom in the spinal canal of the rat is reasonable.

Although the pharmacologically active substance was not identified specifically, it is inferred to be a sort of kinin since snake venoms possess kinin-releasing activity (Rocha e Silva, 1949; Mebs, 1970; Suzuki and Iwanaga, 1970). Even though Oshima et al. (1969) report that Habu venom did not show an appreciable bradikinin-releasing activity, Matsusaki and Aniya (unpublished) reveal that either the intact or the heat treated venom can release a potent uterine contractive substance, by incubating it with the heparinized rabbit plasma and that the characteristic behavioral effects such as squeaking and recurrent convulsions are observed after its spinal injection. In the present experiment, it has been proved that the CSF incubated with the venom caused powerful uterine contractions but slow in the onset, and that the contractions were not antagonized by atropine or antihistamine. Therfore, it is reasonable to expect that the formation of a sort of kinin is possible by incubation of the CSF with the venom.

Sicuteri's report (1970) shows that bradykinin (2 to 5 μg per Kg) injected into the cisterna magna of the rabbit produces squeaking and squeaking-flight reactions after 10 to 20 seconds of latency. Thus, if a sort of kinin is formed *in vivo* after the contamination of the CSF with the spinally injected venom, then it may be the cause of squeaking or convulsions. Making a comparison between the behavioral effects of the venom in rats and those of bradykinin in rabbits, we have found a few differences, such as delayed onset, extended duration and severeness of the reactions in the case of the venom, as compared with the case of bradykinin. One of the causative factors of these differences seems to be a deficient formation of a kinin in the early period and an increase of its formation with the passage of time. This inference is easily understood from the result shown in Figure 2. As another factor, it is conceivable for kininase inhibitor to be in the venom, which makes it possible for kinin to remain in an active form.

Summary

Behavioral effects of intraspinally injected Habu venom on rats were characterized by squeaking and repeated clonic convulsions. These effects are caused at least in part by a sort of kinin formed by contamination of the CSF with the venom *in vivo*.

Acknowledgement

The authors are indebted to Okinawa Prefectural Public Health Laboratory for generous supply of Habu venom and also to Dr. S. Takagi for the cerebrospinal fluid used in this study.

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