# ACTION OF THE PRESYNAPTIC NEUROTOXIN NOTECHIS II-5 FROM THE VENOM OF Notechis scutatus scutatus ON MOUSE MOTOR NERVE ENDINGS

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The neurotoxin notechis II-5 (N-II-5) from the venom of the Australian tiger snake (Viperidae family) gave rise to changes in the frequency of miniature end-plate potentials (MEPP) in the mouse diaphragm in three distinct phases: An initial decrease in frequency, then an increase, and finally a decrease in the frequency of MEPP or even a complete block. The effect of N-II-5 was enhanced with a rise in the temperature of the solution from 20 to 30 and 35°C. There was no effect if $Ca^{++}$ was removed from the solution. Addition of $Ca^{++}$ to the solution after washing the muscle to remove N-II-5 with calcium-free solution was accompanied by development of the typical N-II-5 effect. In the presence of N-II-5, an increase in the $K^+$ concentration in the solution to 20 mM did not lead to a sharp increase in the frequency of MEPP typical of depolarized nerve endings. Agents which increased the intracellular $Ca^{++}$ concentration, not through depolarization of the nerve endings (hypertonic solution, ionophore A 23187), preserved the ability to increase the frequency of MEPP. It is suggested that the presynaptic effect of N-II-5 is connected with its phosphorylase activity and can be explained by the disturbance of the activity of the liberation sites and not by exhaustion of mediator reserves.

KEY WORDS: presynaptic neurotoxins; mamalian motor nerve endings; spontaneous liberation of mediator; calcium; phospholipases.

The neurotoxin notechis II-5 (N-II-5) is a fraction of the venom of the Australian tiger snake (Notechis scutatus scutatus) belonging to the Viperidae family. N-II-5 was isolated in 1976 in Eaker's laboratory in the University of Uppsala (Sweden) and it is a polypeptide with a molecular weight of 13,500; it possesses type $A_2$ phospholipase activity (1000 μmoles fatty acids/min/mg); $LD_{50}$ for intravenous injection into albino mice is 45 μg/kg body weight [3]. Investigations on frog muscle have shown that N-II-5 gives rise to phasic changes in the frequency of miniature end-plate potentials (MEPP), which end with a complete block of mediator liberation [6].

The object of this investigation was to study the characteristics of the action of N-II-5 on motor nerve endings of a mammalian muscle; namely the mouse diaphragm.

The experimental method was described previously [1].

## EXPERIMENTAL RESULTS

N-II-5 in a concentration of 5 μg/ml did not change the resting potential of the muscle fibers during incubation for 3-4 h. The mean amplitude of MEPP remained constant, evidence of the normal sensitivity of the postsynaptic membrane was preserved.

Just as in experiments on frog muscle [6], N-II-5 gave rise to changes in the frequency of MEPP in three phases (Fig. 1, Table 1): Phase I) an initial decrease in the frequency of MEPP, phase II) an increase in frequency, phase III) a final decrease in the frequency of MEPP or even their complete block. This effect was irreversible. It follows from Table 1 that the effect of N-II-5 depended essentially on the temperature

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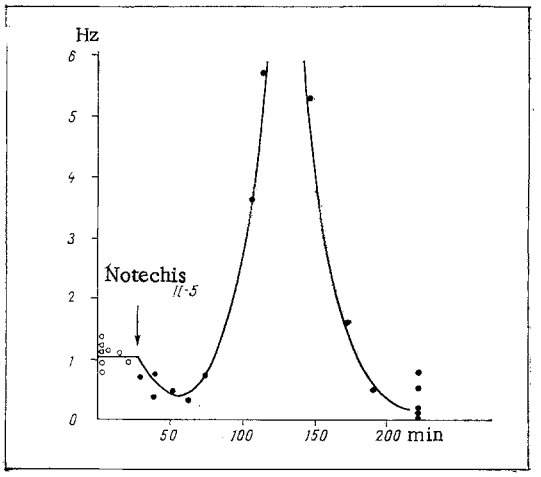


Fig. 1

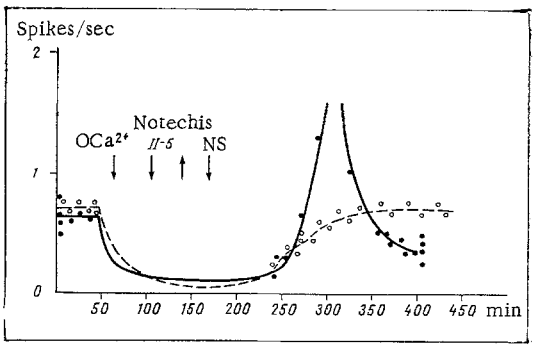


Fig. 2

Fig. 1. Time course of effect of notechis II-5 in a concentration of 5 μg/ml at 30°C. Time of addition of toxin to normal physiological saline (NS) shown by arrow. Distribution of frequencies of MEPP in different synapses of same preparation before (in NS) and after action of notechis II-5 (in the phase of block) shown on left and right. Open circles – control, filled circles – experiment. Each circle denotes mean frequency of MEPP in one synapse during 1 min. Abscissa, time (in min); ordinate frequency of MEPP (in Hz).

Fig. 2. Effect of notechis II-5 after incubation of preparation in calcium-free solution with EGTA at 20°C. Times of replacing normal physiological saline (NS) by calcium-free solution ($OCa^{++}$) of addition of toxin, of beginning of washing preparation with calcium-free solution, and replacing calcium-free solution by normal saline indicated by arrows. Filled circles – experiment; open circles – control; replacement of solutions without addition of notechis II-5. Abscissa and ordinate: as in Fig. 1.

TABLE 1. Effect of Notechis II-5 (5 μg/ml) on Frequency of MEPP in Mouse Diaphragm (M ± m)

| Change in frequency of MEPP in phase II | Number of experiments | Temperature, °C | Normal value, spike/sec | % | Phase I (initial block) | | Phase II (increase in frequency of MEPP) | | Phase III (secondary block) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | spikes/sec | % | spikes/sec | % | spikes/sec | % |
| Slight increase | 10 | 20 | 0,60±0,02 (29) | 100 | 0,19±0,02 (27) | 31,67 | 2,49±0,30 (15) | 415,00 | 0,15±0,02 (32) | 25,00 |
| | 12 | 30 | 1,07±0,09 (29) | 100 | 0,29±0,05 (35) | 27,10 | 4,57±0,40 (18) | 427,10 | 0,20±0,04 (35) | 18,69 |
| Sharp increase | 7 | 20 | 0,57±0,03 (18) | 100 | 0,18±0,03 (21) | 31,58 | 10,04±0,65 (7) | 1761,40 | 0,14±0,02 (24) | 24,56 |
| | 23 | 30 | 0,95±0,03 (70) | 100 | 0,25±0,03 (56) | 26,32 | 38,15±3,59 (39) | 3973,96 | 0,18±0,02 (92) | 18,95 |
| | 7 | 35 | 1,97±0,04 (23) | 100 | 0,41±0,09 (21) | 20,81 | 83,61±5,80 (12) | 4244,16 | 0,31±0,07 (29) | 15,74 |

Legend. Number of synapses shown in parentheses.

of the physiological saline: With an increase in temperature there was an increase in the number of cases when a sharp rise in the frequency of MEPP was observed during phase II, the maximal increase in the frequency of MEPP became higher, and the degree of the decrease in the frequency of MEPP during phases I and III also was increased (Table 1). Potentiation of the effect was perhaps attributable to the higher enzymic activity of the toxin with a rise in temperature.

In medium without $Ca^{++}$ phospholipases $A_2$, as a rule, cannot exhibit their enzymic activity [8]. During incubation of the muscle in calcium-free solution with 1 mM EGTA, N-II-5 caused no change in the frequency of MEPP (Fig. 2). The effect thus depended on the phospholipase activity of the toxin, in agreement with observations made previously on frog muscle [6]. After the addition of $Ca^{++}$ to the solution, phasic changes in the frequency of MEPP typical of the effect of N-II-5 began to develop, in spite of the fact that the muscle had first been washed with calcium-free solution to remove the toxin. Blocking enzymic activity evidently did not prevent binding of the toxin with the target, although realization of the toxic effect on mediator liberation be-

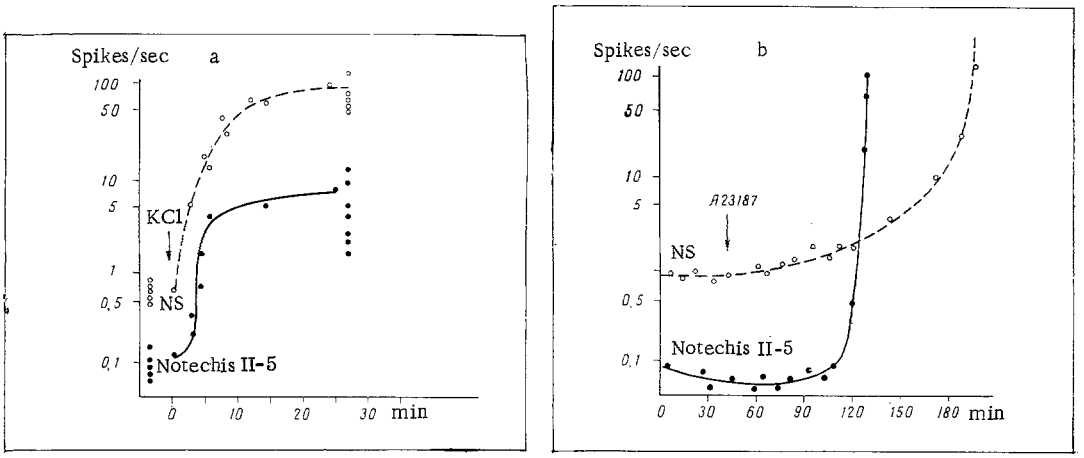


Fig. 3. Changes in frequency of MEPP in normal preparations and after poisoning with notechis II-5, under the influence of agents raising $Ca^{++}$ concentration in axoplasm (30°C). Times of addition of 20 mM KCl (a) and A 23187 (b) indicated by arrows. In a) Distribution of frequencies of MEPP before and after addition of KCl shown on left and right. Scatter of frequencies of MEPP in different synapses is increased in poisoned preparation compared with control, where distribution of frequencies of MEPP is displaced sharply upward. Ordinate, logarithmic scale; remainder of legend as in Fig. 1.

came possible only after restoration of enzymic activity by the addition of $Ca^{++}$ to the solution. After washing the muscle with calcium-free solution, the presynaptic effect of N-II-5 was rather weaker than in the experiments with a normal $Ca^{++}$ concentration in the solution. Binding of the toxin with the target is evidently partly reversible.

To elucidate the causes of blocking the liberation of mediator under the influence of N-II-5 the following agents, which increase the $Ca^{++}$ concentration inside nerve endings were used: 1) an increase in the $K^+$ concentration in the solution to 20 mM, which leads to depolarization of nerve endings [5] and to a corresponding increase in the inflow of $Ca^{++}$ from the external medium; 2) ionophore A 23187, which carries $Ca^{++}$ into the cell [7]; 3) increase in the osmotic pressure of the solution by 2.5 times by the addition of sucrose which, is considered [4] to liberate $Ca^{++}$ from mitochondria; 4) 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole (TTFB) [2], an uncoupler of oxidative phosphorylation, which causes a sharp rise in the frequency of MEPP under normal conditions [6].

As Fig. 3a shows, an increase in the $K^+$ concentration in the solution was accompanied by a smaller increase in the frequency of MEPP in the poisoned preparations than in the control. This can be explained by disturbance of coupling between depolarization of the presynaptic membrane and liberation of mediator. Ionophore A 23187 caused a considerable increase in the frequency of MEPP in both control and poison preparation (Fig. 3b); in the latter case the increase in frequency of MEPP appeared as a rule earlier than in the control. In poisoned nerve endings, the intracellular system of regulation of the $Ca^{++}$ concentration is evidently disturbed. The hypertonic solution caused a small increase in the frequency of MEPP, equally in the control and poisoned preparations. TTFB in both cases led to a strong increase in the frequency of MEPP; blocking spontaneous mediator secretion by N-II-5 thus cannot be explained by exhaustion of the reserves of mediator in the nerve endings.

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## LITERATURE CITED

1. M. A. Kamenskaya, L. G. Magazanik, E. R. Kotova, et al., Byull. Éksp. Biol. Med., No. 5, 396 (1979).
2. R. B. Beechej, Biochem. J., 98, 284 (1966).
3. J. Halpert and D. Eaker, J. Biol. Chem., 251, 7343 (1976).
4. H. Kita and W. Van der Kloot, J. Neurophysiol., 40, 212 (1977).
5. A. W. Liley, J. Physiol. (London), 134, 427 (1956).
6. L. G. Magazanik and T. I. Slavnova, Physiol. Bohemoslov., 27, 421 (1978).
7. H. E. Statham and C. J. Duncan, Life Sci., 17, 1401 (1975).
8. J. F. Wenicke, A. D. Vanker, and B. D. Howard, J. Neurochem., 25, 483 (1975).