BLOCKING ACTION OF SNAKE VENOM POLYPEPTIDES ON CHOLINERGIC MECHANISMS OF THE LEECH DORSAL MUSCLE

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The blocking action of α-polypeptides isolated from the venom of Bungarus multicinctus and Naja naja siamensis was investigated in experiments on the isolated dorsal muscle of the leech. These neurotoxins (NTs), in a concentration of 1×10^{-5} g/ml, did not block responses to monoquaternary cholinomimetics (acetylcholine, carbachol, nicotine, monocholine ester of succinic acid). The ability of NT to block responses to biquaternary cholinomimetics depended on the length of their molecule. Only responses to dicholine esters of malonic, succinic and glutaric acids were blocked by NT. Dicholine esters of adipic, pimelic, suberic, azelaic, and sebacic acids retained the whole of their activity after treatment of the leech muscle with NT. The possible causes of the selective action of NT are discussed.

KEY WORDS: polypeptides of snake venom; cholinergic receptors; dorsal muscle of the leech; cholinomimetics.

Polypeptides isolated from the venom of snakes of the corba family can selectively and irreversibly block the sensitivity of certain vertebrate tissues to acetylcholine (AC) and cholinomimetics [8, 9, 14]. Tissues with nictoine-sensitive cholinergic receptors are susceptible to this action [13]. On the other hand, evidence has been obtained to suggest that neurotoxins (NTs) selectively block the key mechanisms of electrogenesis of cholinergic receptor membranes and not the active centers of those receptors [1-5].

In order to study to what extent the effectiveness of NT correlates with the presence of nicotine-sensitive cholinergic receptors in the tissue an investigation was carried out on the dorsal muscle of the leech, the cholinergic receptors of which are very similar in their pharmacological characteristics to the nicotine-sensitive cholinergic receptors of vertebrate skeletal muscles [7].

EXPERIMENTAL METHOD

A piece of the dorsal muscle of the leech (12-14 segments) was carefully cleaned on its inner surface (to remove internal organs, traces of the nerve chain, and connective tissue) and placed in a 3-ml bath. A solution of the following composition (in millimoles/liter) was used: Na^+ 116, K^+ 2.5, Ca^{++} 1.8, HCO_3^- 2.4, Cl^- 122; pH 7.4. In some experiments the solution also contained neostigmine ($2 \cdot 10^{-6}$ g/ml) to inhibit cholinesterase. The cholinomimetics were added to the bath in a volume of 0.06 ml. The contractions were recorded on a kymograph. The contractile strength of the muscle was 2 g. Contact between the muscle and the agonist continued for 5-10 min and the interval between exposures was 20-30 min. The constancy of the responses to certain cholinomimetics was first verified. Next, for 1 h, the muscle was exposed to the action of NT in a concentration of $1 \cdot 10^{-5}$ g/ml, after which treatment with the same cholinomimetics was repeated in the same concentrations as before the action of NT. Each cholinomimetics was tested on at least four muscle preparations. Purified α-polypeptides were used: α-bungarotoxin from the venom of Bungarus multicinctus and polypeptide No. 3 from the venom of Naja naja siamensis.

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EXPERIMENTAL RESULTS AND DISCUSSION

NT was used in a concentration 50-100 times higher than the threshold level in experiments on frog or rat muscles. Nevertheless, the NTs studied did not prevent the effect of the monoquaternary cholinomimetics (AC, carbachol, and nicotine). Responses to these cholinomimetics not only were not reduced after treatment with NT, but as a rule they were acutally enhanced. Consequently, the presence of nicotine-sensitive cholinergic receptors is not always responsible for the effectiveness of NT.

The contractile responses induced by the biquaternary cholinomimetics succinyldicholine were blocked by NT, in agreement with the observations of Ross and Triggle [16]. The next step was to investigate in more detail the relationship between the structure of biquaternary cholinomimetics and the ability of NTs to block their effects. It was first necessary to discover how a change in the distance between the cationic heads of the biquaternary cholinomimetics is reflected in the blocking effect of NTs. For this purpose cholinomimetics of two homologous series were used: dicholine esters of dicarboxylic acids from malonic to sebacic $(CH_3)_3NCH_2CH_2OOC(CH_2)_nCOOCH_2CH_2N(CH_3)_3$ and members of the polymethylene-bis-trimethylammonium series. The NTs blocked only the responses to the dicholine esters of malonic, succinic, and glutaric acids (n=1, 2, and 3). With an increase in the distance between the cationic heads of the cholinomimetics (n=4, 5, 6, 7, and 8) the effect of the NTs disappeared. Shortening the molecule of the biquaternary cholinomimetic had no effect on the ability of NTs to block the responses to these cholinomimetics, as was shown by experiments with members of the polymethyle-bis-trimethylammonium series $(CH_3)_3N-(CH_2)_n-N(CH_3)_3$ (from n=12 to n=7).

The question of the importance of the presence of the second cationic head in the molecule of the cholinomimetic for the blocking action of NT was next considered. Experiments were carried out with monoquaternary analogues of succinyldicholine: the monocholine ester of succinic acid $(CH_3)_3^+NCH_2CH_2CH_2OOC(CH_2)_2COOH$ and the asymmetrical choline-ethyl ester of succinic acid $(CH_3)_3N^+CH_2CH_2OOC(CH_2)_2COOCH_2CH_3$. The responses of the leech dorsal muscle to neither cholinomimetic was blocked by NT. Clearly, therefore, NTs block sensitivity only to biquaternary cholinomimetics with a definite distance between their nitrogen atoms: not more than 17-18 A.

These results are in agreement with the hypothesis that the leech dorsal muscle contains two different cholinergic receptors: one sensitive to monoquaternary cholinomimetics and one sensitive to biquaternary cholinomimetics such as succinyldicholine and decamethonium [10, 11, 15]. It might be supposed [16] that these two cholinergic receptors represent two different degrees of oligomerization of the subunits of the cholinergic receptor [6, 7, 12]. However, the high specificity of the NTs – why they block only cholinergic receptors aggregated into tetramers without having the slightest effect on dimers and monomers – remains unexplained. The interpretation of the results in terms of the hypothesis of the oligomeric structure of the cholinergic receptors also fails to take into account the fact that NTs block the action of AC on the somatic muscle of the lamprey [3], although the cholinergic receptors of this muscle are not aggregated into tetramers. Other correlations must evidently be sought between the effect of the NTs and the characteritics of the tissue cholinergic mechanisms. Susceptibility to the action of NT or its absence may perhaps reflect differences in the ionic mechanisms of the AC effect, i.e., NTs are inhibitors of the ionic carriers of cholinergic receptor membranes rather than of the active centers of the receptors. There is reason to suppose that the effects of AC and succinyldicholine are carried out by different ionic mechanisms. For instance, AC induces contraction of the leech muscle when completely polarized by the action of 122 mM KCl, whereas succinyldicholine has no such action [10]. If NTs blocks only the ionic mechanism of the effect of succinyldicholine and structurally similar compounds, the selective action of the NTs becomes understandable.

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