Synthetic peptides corresponding to the sequence of noxiustoxin indicate that the active site of this K⁺ channel blocker is located on its amino-terminal portion*

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Summary. A nonapeptide Thr-Ile-Ile-Asn-Val-Lys-Cys-Thr-Ser (NTX₁₋₉) and a decapeptide Met-Asn-Gly-Lys-Cys-Lys-Cys-Tyr-Asn-Asn (NTX₃₀₋₃₉) corresponding to the N-terminal and C-terminal sequences respectively of Noxiustoxin (NTX) were synthesized by the solid phase method of Merrifield (1963). The first synthetic peptide (NTX₁₋₉) was shown to be toxic to mice independently of the route of administration: intraperitoneally, subcutaneously or intraventricularly (100–200 µg/20 g mouse weight). The second (NTX₃₀₋₃₉) was not toxic even at higher dose (400 µg/20 g mouse). When the effects of the peptide NTX₁₋₉ and of the authentic toxin (Noxiustoxin) were studied on the liberation of [³H] 4-aminobutyric acid (³H-GABA) from mouse synaptosomes, both gave essentially the same results, except that peptide NTX₁₋₉ was needed at higher concentration. Synthetic peptide NTX₃₀₋₃₉ had no effect in the same preparation at even higher doses. The GABA release produced by toxic peptide NTX₁₋₉ was not affected by tetrodotoxin but was completely abolished by the presence

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Abbreviations used: BOC-amino acids ter-butyloxy carbonyl-amino acids; BOC-amino acid-PAM-resin ter-butyloxy carbonyl-aminoacyl-4-(oxymethyl) phenacetamidomethyl-resin; GABA 4-aminobutyric acid; HPLC high performance liquid chromatography; MSA mouse serum albumin; NTX Noxiustoxin; NTXₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙ$_{1}^{1}$-9 of the sequence according to Fig. 1; TTX tetrodotoxin.
of the K⁺ ionophore valinomycin, mimicking the effect of native NTX in the same system (Sitges et al., 1986). These results indicate that the toxic active site of Noxiustoxin is possibly located in or near the N-terminal amino acid portion of the molecule.

**Keywords:** Scorpion toxins, noxiustoxin, K⁺ channels, synthetic peptide.

**Introduction**

Noxiustoxin, a 39 amino acid peptide isolated from the venom of the scorpion *Centruroides noxius* Hoffmann (Possani et al., 1982), has been shown to be a specific blocker of the voltage-dependent K⁺ channel of the squid giant axon (Carbone et al., 1982, 1987), as well as of the K⁺ permeability in mouse brain synaptosomes (Sitges et al., 1986). Recently, the same toxin was shown to modify the Ca dependent K channels of skeletal muscle reincorporated in artificial bilayers (Valdivia et al., 1988).

Due to the novelty and usefulness of this toxin for neurobiological studies and since the total amino acid sequence of NTX, previously published (Possani et al., 1982), was confirmed recently (Valdivia et al., 1988), we decided to synthetize the whole molecule. Several reasons directed our work. The first was the possibility of obtaining large amounts of a rare peptide by chemical synthesis, rather than by capturing scorpions alive in the field, extracting the venom and finally purifying the toxin. It is worth noting that we need to milk a thousand scorpions in order to obtain 100 mg of crude venom and that NTX corresponds only to 1% of the whole venom (Possani et al., 1981, 1982). The second reason was the possibility of finding a non-toxic sequence of the molecule that could be used eventually as a synthetic vaccine against scorpions stings. Finally, any synthetic active fragment of NTX would serve as a model for the active site of the toxin. Accordingly, in designing a strategy for the complete chemical synthesis of NTX we decided to synthesize seven peptides: NTX1-9, NTX1-20, NTX1-30, NTX1-39, NTX11-39, NTX20-39, and NTX30-39 in such away that we would be able to map the properties of the entire toxin by studying the first quarter of the molecule (N-terminal), the last quarter of the molecule (C-terminal), both half molecules and both three quarters, as well as the total toxin.

In this communication, due to the compared bioactivity of the peptides, we will concentrate our attention on the N-terminal and C-terminal peptides. A complete detailed description of all synthetic peptides and their immunological properties is being prepared for publication elsewhere (Gurrola et al., 1988).

**Material and methods**

**Source of chemicals**

The solvents and chemicals used in this work were analytical grade. All protected amino acids (BOC-amino acids) were from Peninsula Laboratories. Methylene chloride, chloroform, ether, toluene, isopropanol, anisol, acetic acid, acetic anhydride were from J. T.