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Toxicon 45 (2005) 93–99

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Weak neurotoxin from *Naja kaouthia* cobra venom affects haemodynamic regulation by acting on acetylcholine receptors

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Received 25 March 2004; revised 21 September 2004; accepted 24 September 2004

Available online 11 November 2004

Abstract

Recent in vitro studies of weak neurotoxins from snake venoms have demonstrated their ability to interact with both muscle-type and neuronal α7 nicotinic acetylcholine receptors (nAChR). However, the biological activity in vivo of weak neurotoxins remains largely unknown. We have studied the influence of weak neurotoxin (WTX) from the venom of cobra *Naja kaouthia* on arterial blood pressure (BP) and heart rate (HR) in rats and mice. It was found that intravenous injection of WTX induced a dose-dependent decrease in BP and an increase in HR in both species, the rats being more sensitive to WTX. Application of WTX following blockade of nAChRs or muscarinic acetylcholine receptors (mAChR) by hexamethonium or atropine, respectively, showed that both nAChRs and mAChRs are involved in the haemodynamic effects of WTX. Blockade of either nAChRs or mAChRs affected WTX action differently in rats and mice, thus reflecting interspecies differences in haemodynamic regulation.

Keywords: Weak neurotoxin; Blood pressure; Heart rate; Nicotinic acetylcholine receptors; Muscarinic Acetylcholine receptors

1. Introduction

Weak neurotoxins, described about thirty years ago (Carlsson, 1975), have recently attracted attention of toxinologists due to their interesting biological properties. Weak neurotoxins belong to the group of so-called 'three-fingered' toxins and consist of 62–68 amino acid residues with five disulfide bonds. The fifth disulfide bond is located in the N-terminal loop, in contrast to the long-chain α-neurotoxins in which the fifth disulfide is in the central loop. Weak neurotoxins in general are less toxic (LD_{50} in the range from 4 to 80 mg/kg) than α-neurotoxins. Except for acute toxicity measurements, all other data concerning the biological activity of weak neurotoxins were obtained in experiments in vitro including inhibition of acetylcholine-induced contractions in isolated nerve-muscle preparations (Chang et al., 2000; Poh et al., 2002; Nirthanan et al., 2002), radioligand binding experiments with muscle-type and α7 neuronal nicotinic acetylcholine receptors (nAChR) (Utkin et al., 2001b; Poh et al., 2002), and inhibition of acetylcholine-induced currents in nAChRs heterologously expressed in *Xenopus* oocytes (Utkin et al., 2001b; Nirthanan et al., 2002). These experiments demonstrated the interaction of weak neurotoxins with both muscle-type and α7 neuronal nAChR with varying degree of affinity. However, since weak neurotoxins from cobra venoms have low affinity for nAChRs, the role of nAChRs in

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0041-0101/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2004.09.014

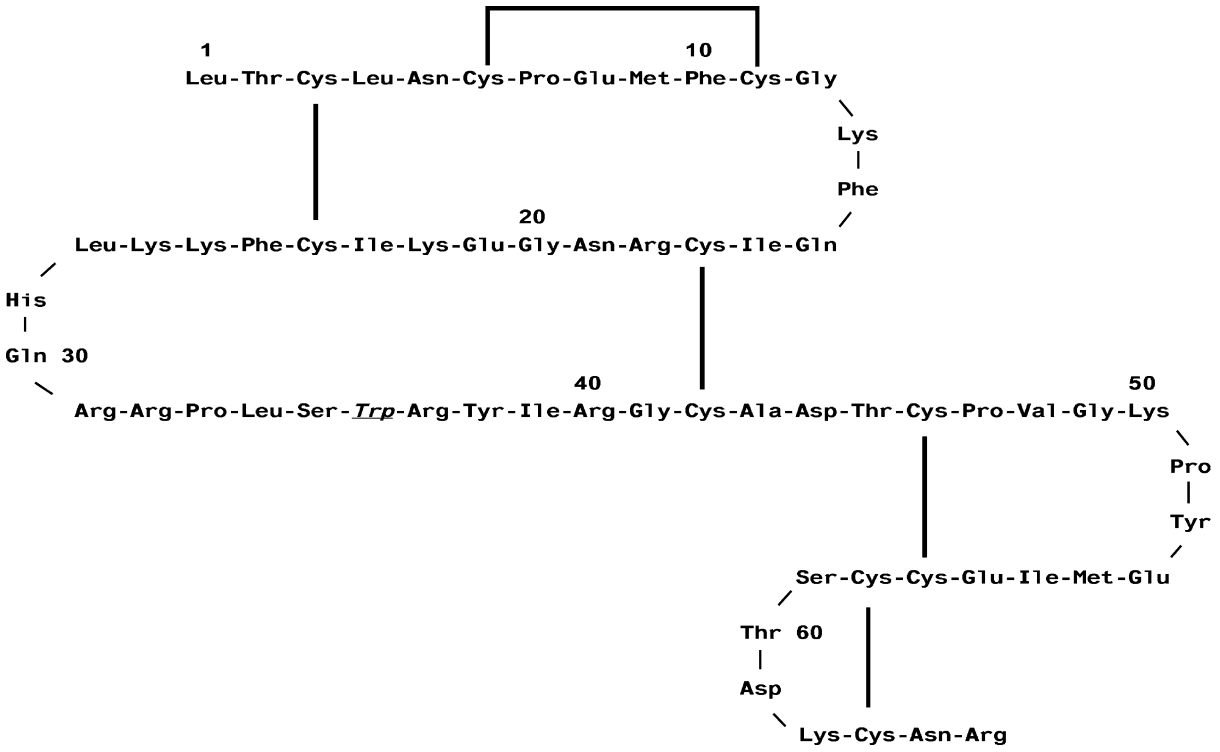

Fig. 1. Amino acid sequence of WTX from *N. kaouthia* (Utkin et al., 2001a). Lines connecting the corresponding cystein residues show disulfide bonds. Tryptophan residue in position 36 unique for WTX is underlined.

the mechanism of their toxicity remains unclear. On the other hand, low toxicity makes weak toxins valuable tools to study their biological activity in vivo. The aim of the present work was to study the effect of the *Naja kaouthia* weak neurotoxin (WTX, Fig. 1) on the cardiovascular system of rats and mice. We have found that intravenous injection of WTX induced a dose-dependent decrease in blood pressure (BP) and an increase in heart rate (HR) in both species. Application of WTX following blockade of nAChRs by hexamethonium or muscarinic acetylcholine receptors (mAChR) by atropine, respectively, indicated the involvement of both nAChRs and mAChRs in the observed haemodynamic effects of WTX.

2. Materials and methods

The experiments were carried out on adult male Wistar rats (300–350 g of body weight) and adult male NMRI mice (30–40 g of body weight). One day before the experiment, catheters were implanted into the femoral artery and femoral vein of rats under Nembutal anesthesia (40 mg/kg, intraperitoneally). In mice, catheters were implanted into the thoracic aorta through the left carotid artery and into the jugular vein under the action of neuroleptanalgesia (ketamine 62.5 mg/kg + droperidol 3.1 mg/kg, intramuscularly). The arterial catheter was used to record BP and the venous catheter—for injection of test drug and toxin.

The experiments were carried out on conscious animals. BP was recorded using an electromanometer (model CP-01, Century Technology, Inglewood, CA). Data was then analysed and mean BP ± SEM and HR ± SEM calculated. In control animals these values were 106 ± 5 mm Hg and 596 ± 12 beats/min for mice and 112 ± 4 mm Hg and 332 ± 10 beats/min for rats.

WTX was purified from cobra *N. kaouthia* venom as described (Utkin et al., 2001a). Briefly, crude venom was first fractionated on Sephadex G-50sf column, the main toxic fraction thus obtained was further separated by ion-exchange chromatography on HEMA-BIO 1000 CM column (Tessek, Czech Republic), and finally WTX was purified by reversed-phase HPLC on Vydac C18 column (Vydac, USA).

To study dose-dependence of WTX effects, it was injected intravenously at doses of 0.5, 1 and 2 mg/kg to rats as described previously (Rzhevsky et al., 2001) and at 2, 4, 6, and 8 mg/kg to mice.

nAChR blockade was achieved using hexamethonium (20 mg/kg, intravenously) while atropine (1 mg/kg, intravenously) was used to block mAChR. The efficiency of blockade was checked by assessing the HR changes in response to sodium nitroprusside (5 μg/kg) or phenylephrine (1 μg/kg) after the injection of acetylcholine receptor