

# AdTx1, a new peptidic $\alpha_1$ -adrenoceptor antagonist with high affinity and selectivity for human $\alpha_{1A}$ -adrenoceptor subtype: pharmacological characterization

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## OBJECTIVES

Venoms are a rich source of ligands for ion channels, but very little is known about their capacity to modulate G-protein coupled receptor activity. We screened green mamba venom fractions, obtained by liquid chromatography, on rat brain synaptosomes in binding experiments with <sup>3</sup>H-prazosin to identify specific ligands targeting  $\alpha_1$ -adrenoceptors. We discovered AdTx1, a 65 amino-acid peptide stabilized by four disulfide bridges, which belongs to the three-finger-fold peptide family (Figure 1). It has subnanomolar affinity ( $K_i=0.35$  nM) and high specificity for the human  $\alpha_{1A}$ -adrenoceptor subtype. AdTx1 is the most selective  $\alpha_{1A}$ -AR antagonist ever described (860 and 1100 fold less affinity for  $\alpha_{1B}$ -AR and  $\alpha_{1D}$ -AR, respectively) and displays an unusually stable  $\alpha_{1A}$ -adrenoceptor/AdTx1 complex ( $t_{1/2diss} = 3.6$  h). It is well known that this receptor subtype is implicated in the contractility of prostatic and urethral smooth muscle in mammals. Indeed, 10-100 nM AdTx1 displayed potent insurmountable antagonism of phenylephrine-induced contractions of rabbit isolated prostatic muscle (1). The aim of the current study was to test the antagonistic effects of AdTx1 *in vivo* on the increase of intraurethral pressure ( $\Delta$ IUP) induced by intravenous administration of the selective  $\alpha_1$ -adrenoceptor agonist, phenylephrine (PHE) in anesthetized male rats.



Figure 1: The sequence obtained for AdTx1 resembles that of a **three-finger fold peptide**. The corresponding peptide was chemically synthesized, purified and refolded, allowing a sufficient quantity to be tested *in vivo*.

## METHODS

Adult male rats were anesthetized with pentobarbital. Catheters were inserted into the jugular vein for drug administration and into the urethra for measuring IUP by a strain gauge and a syringe injection pump. Room temperature saline was infused into the urethra at a flow rate of 0.5 mL/h. IUP (mmHg) was recorded continuously using an electronic interface. Baseline values for IUP were taken from the last minute prior to test substance injection. AdTx1 was administered i.v. 30 min before PHE administration. PHE (10-3000  $\mu$ g/kg, i.v.) was administered as a bolus with a 3 min interval between each dose. Only one dose of AdTx1 was tested in each rat. In each animal and for each PHE dose,  $\Delta$ IUP was determined. Results are given as mean values  $\pm$  s.e.m. One-way ANOVA and Student-Newman-Keuls were used to compare experimental groups.

## CONCLUSIONS

AdTx1 is active following i.v. administration in rats. At the lowest dose tested (0.1 mg/kg), it acted as a competitive antagonist, whereas at 1 mg/kg, the effect of PHE on IUP was nearly abolished. Therefore at higher doses *in vivo*, AdTx1 was found to act as an insurmountable antagonist, similar to its actions in the rabbit isolated prostate *in vitro* (1). The high selectivity of AdTx1 for  $\alpha_{1A}$ -AR could be an advantage vs classical  $\alpha_1$ -AR antagonists (terazosin, doxazosin) which cause hypotension through  $\alpha_{1B}$ -AR blockade (2). Moreover, tamsulosin-induced anejaculation is likely due to its high affinity for D<sub>3</sub> dopamine receptors ( $K_D=0.28$  nM; 3) which have been shown to play a key role in ejaculation (4). AdTx1 (up to 3  $\mu$ M) has no affinity for D<sub>3</sub> dopamine receptors, therefore it should be devoid of effects on ejaculatory function.

**We conclude that AdTx1 is a promising template for the development of new  $\alpha_1$ -AR antagonists for the treatment of benign prostatic hyperplasia and lower urinary tract symptoms.**

## RESULTS

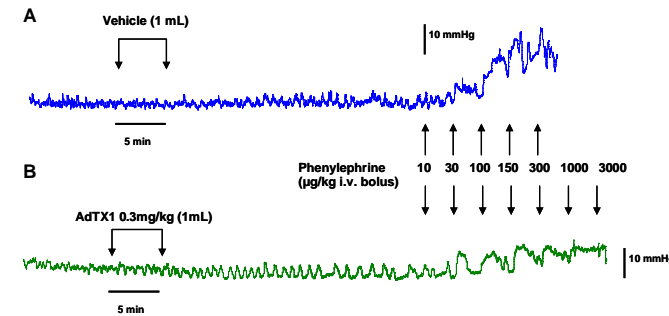


Figure 2: Typical recordings of intraurethral pressure in anesthetized rats and the effect of intravenous injections of cumulative doses of PHE following administration of saline (A) or AdTx1 at 0.3 mg/kg i.v. (B). Note that PHE (even at 1000-3000  $\mu$ g/kg) was not able to significantly increase intraurethral pressure.

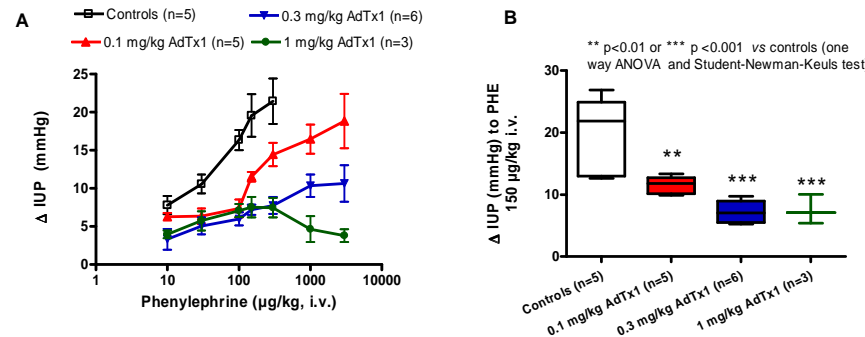


Figure 3:  $\Delta$ IUP to PHE in the absence and presence of 3 doses of AdTx1 (A) and  $\Delta$ IUP at the PHE dose of 150  $\mu$ g/kg i.v. (B)

## REFERENCES

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