

Comparison of the effects of AdTx1 and tamsulosin on the increase in intra-urethral and arterial pressures induced by phenylephrine in anesthetized male rats

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Objectives

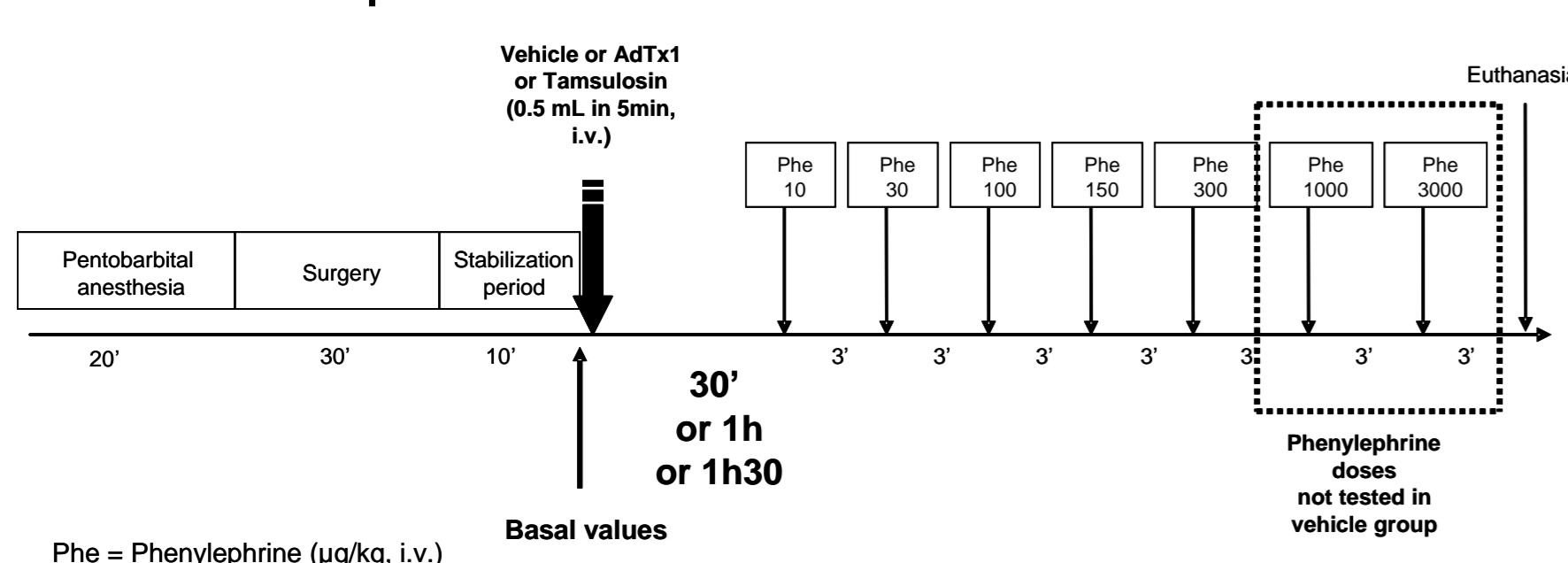
Venoms are a rich source of ligands for ion channels, but very little is known about their capacity to modulate G-protein coupled receptor (GPCR) activity. At CEA laboratories, a strategy to identify novel toxins targeting GPCRs was developed. Screening of green mamba venom for natural peptides led to the discovery of AdTx1, a 65 amino-acid peptide stabilized by four disulphide bridges. In binding studies, this peptide has high affinity and selectivity for human α_{1A} -adrenoceptors (α_{1A} -ARs). AdTx1 inhibits ³H-prazosin binding to human α_{1A} -ARs expressed on yeast membranes, with a K_i value of 0.33 nM. Conversely, AdTx1 has low affinity for human α_{1B} -adrenoceptor and rat- α_{1D} adrenoceptor subtypes, with K_i values of 317 and 1250 nM, respectively. AdTx1 has previously been shown to produce potent insurmountable antagonism of phenylephrine-induced contractions of rabbit isolated prostatic muscle at concentrations between 10 and 100 nM⁽¹⁾.

The aim of this study was to compare the effects of tamsulosin (selective α_1 -adrenoceptor antagonist, commonly used for BPH treatment)⁽²⁾ and AdTx1 on phenylephrine (PHE)-induced intra-urethral (IUP) and arterial pressures (AP) increases in anesthetized male rats.

Methods

- Adult male Wistar rats were anesthetized with pentobarbital (40 mg/kg, i.p. and 10 mg/kg, s.c. post surgery).
- Catheters were inserted into the jugular vein for drug administration, into the urethra for measuring IUP and carotid artery for measuring AP.
- Vehicle, AdTx1 and tamsulosin were administered intravenously (i.v.) 30 min, 1 h and 1 h 30 before PHE administration.
- PHE (10-3000 μ g/kg, i.v.) was administered as a bolus with a 3 min interval between each dose.
- In each animal and for each dose of PHE, delta IUP and AP from basal values (values pre PHE administration) were calculated.
- Results are given as mean values \pm s.e.m. Statistical analysis was performed using a one-way ANOVA followed by Newman-Keul's test.

Schematic Experimental Protocol:



Conclusions

AdTx1 (administered i.v.) is able to produce a long-lasting antagonism of PHE-induced contractions of rat urethra, probably acting on α_1 -ARs located on the smooth muscle. Importantly, AdTx1 (0.3 mg/kg) exhibited a relevant effect on IUP and a small effect on AP. In contrast, tamsulosin reduced PHE-induced increases in both IUP and AP.

In this study, the *in vivo* potency of tamsulosin ($pK_B = 8.89$) is very similar to the potency ($pK_B = 9.23$) reported in the rat isolated prostate⁽⁴⁾ whereas the *in vivo* potency of AdTx1 is similar to the value ($pA_2 = 8.38$) reported in the rabbit isolated prostate⁽¹⁾. A direct comparison between the *in vivo* potencies of AdTx1 and tamsulosin shows that the peptide is only 5-times less potent than tamsulosin. Recently tamsulosin has been demonstrated to be efficacious and safe for the treatment of non-neurogenic voiding dysfunctions in women⁽⁵⁾. When administered orally, AdTx1 was devoid of effects on IUP (data not shown). These results suggest that AdTx1 has the potential to be a treatment for BPH in men and for Lower Urinary Tract Symptoms in both men and women, but only when administered locally (*i.e.* by intraprostatic, intravesical or intravaginal routes).

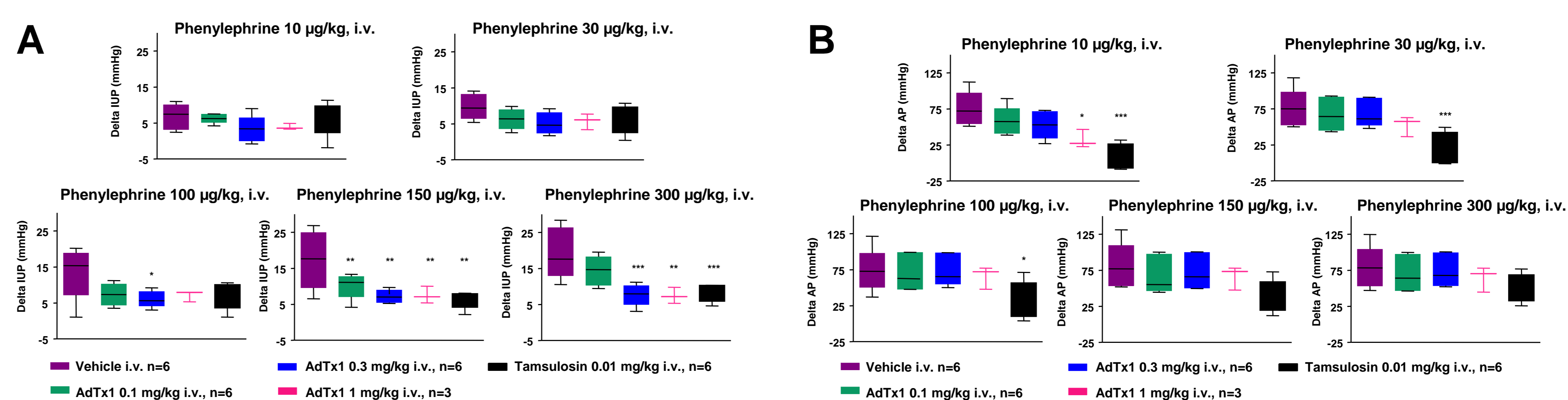
References

- (1) Quinton *et al*, Br J Pharmacol, 159:316-325, 2010. (4) Pulito *et al*, J Pharmacol Exp Ther, 294:224-229, 2000.
 (2) Chapple *et al*, Eur Urol, 29:129-144, 1996. (5) Lee *et al*, J Korean Med Sci, 25:117-122, 2010.
 (3) Gaddum, Pharmacol Rev, 9:211-218, 1957.

Results

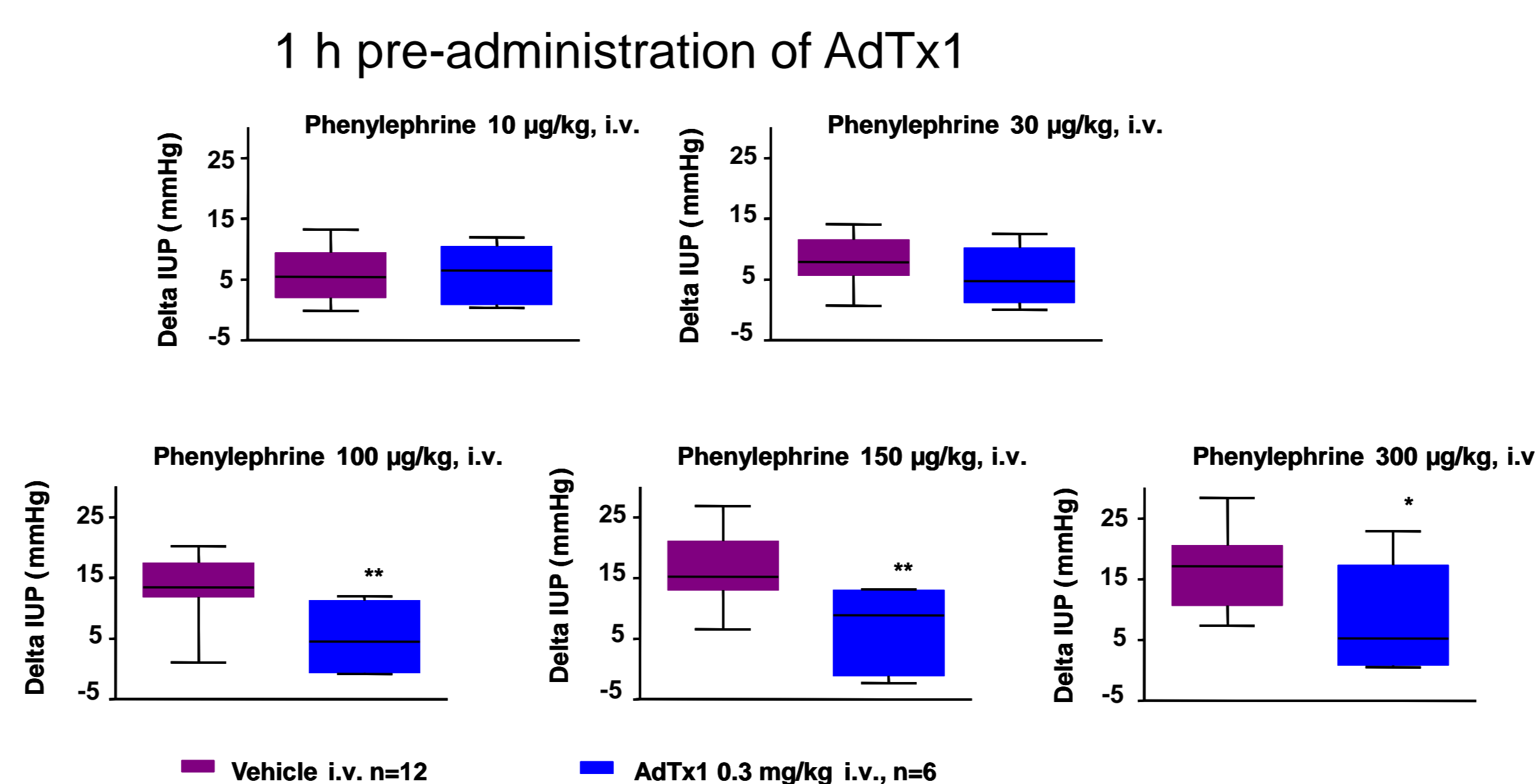
Effects of 30 min pre-administration of AdTx1 or tamsulosin on the increases in IUP (A) and AP (B) induced by PHE in anesthetized male rats

- On IUP, AdTx1 (0.3 and 1 mg/kg, i.v.) significantly decreased the effects of PHE doses of 100, 150 and 300 μ g/kg (i.v.) whereas tamsulosin (0.01 mg/kg, i.v.) significantly reduced the effects of PHE doses of 150 μ g/kg and 300 μ g/kg.
- On AP, tamsulosin significantly decreased the effects of PHE doses of 10, 30, 100 μ g/kg (i.v.). AdTx1 had no effect at 0.3 mg/kg whereas at 1 mg/kg it reduced the effect of PHE at 10 μ g/kg.



* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus vehicle; one way ANOVA followed by Newman-Keul's test.

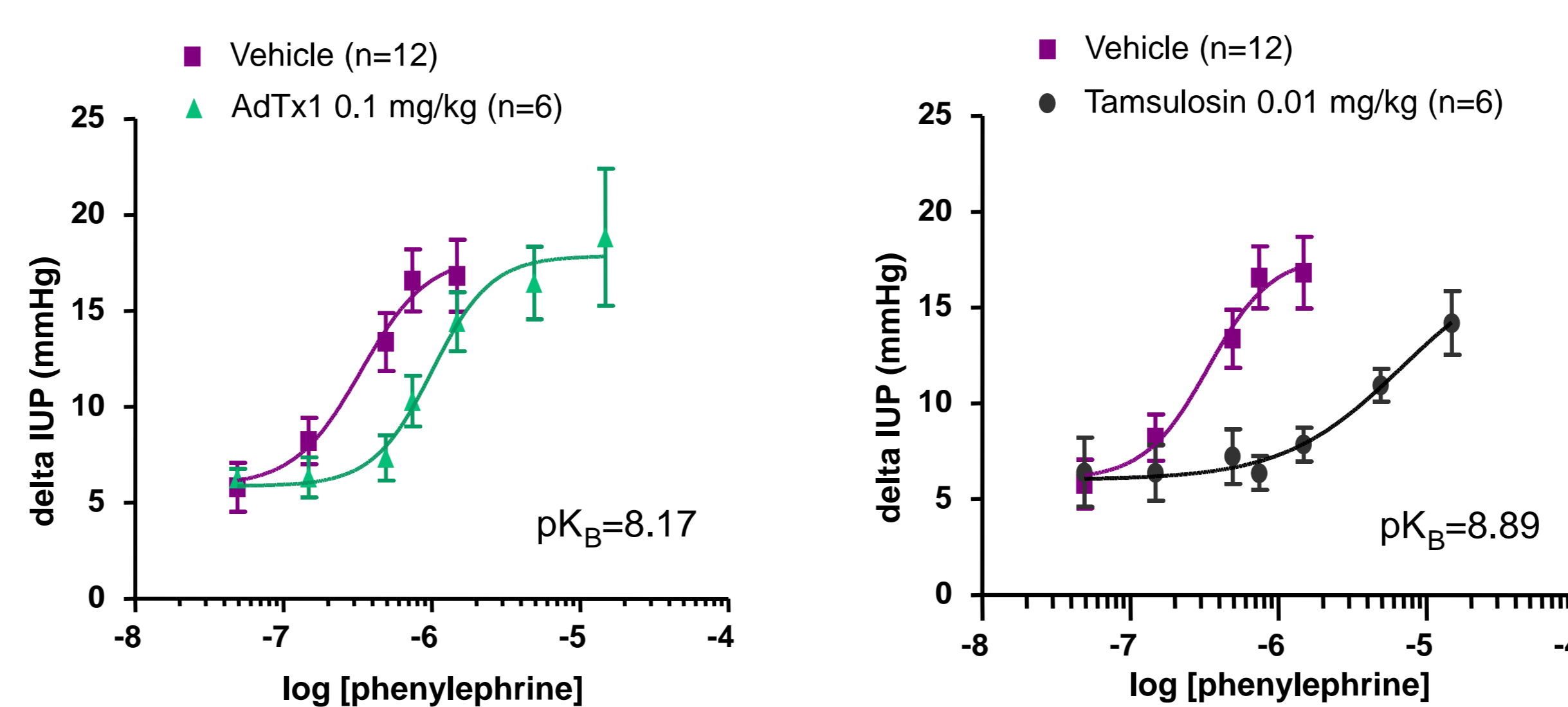
Effects of 1 h pre-administration of AdTx1 and tamsulosin on the increase in IUP induced by PHE in anesthetized male rats



* $p < 0.05$, ** $p < 0.01$ versus vehicle; unpaired Student *t*-test.

- On IUP, when administered 1h before PHE, AdTx1 (0.3 mg/kg, i.v.) significantly decreased the effects of PHE doses of 100, 150 and 300 μ g/kg. When administered 1h30 before PHE, AdTx1 (0.3 mg/kg, i.v.) and tamsulosin (0.01 mg/kg, i.v.) did not reduced the effects of PHE on IUP (data not shown).
- On AP, AdTx1 (0.3 mg/kg i.v., administered 1h and 1h30 before PHE) and tamsulosin (0.01 mg/kg, i.v., administered 1h30 before PHE) slightly decreased the effects of a low dose of PHE (10 μ g/kg i.v.) (data not shown).

Comparison of the *in vivo* potencies (pK_B values) for AdTx1 and tamsulosin (administered 30 min before PHE doses)



- Agonist and antagonist concentrations were expressed as mol/kg. Data were fitted with a sigmoidal dose-response equation using GraphPad Prism and the Dose Ratio was calculated as EC_{50} (with antagonist)/ EC_{50} (without antagonist). The antagonist potency was calculated using the classical Gaddum equation⁽³⁾:

$$pK_B = \log (\text{Dose Ratio} - 1) - \log ([\text{antagonist}])$$