siRNA-mediated Silencing of Angiotensin Type 1a Receptor ($AT_{1a}R$) Splice Variants Reveals that $AT_{1a}R$ Expression is Translationally Controlled by $AT_{1a}R$ Alternative Splicing in Rat Vascular Smooth Muscle Cells

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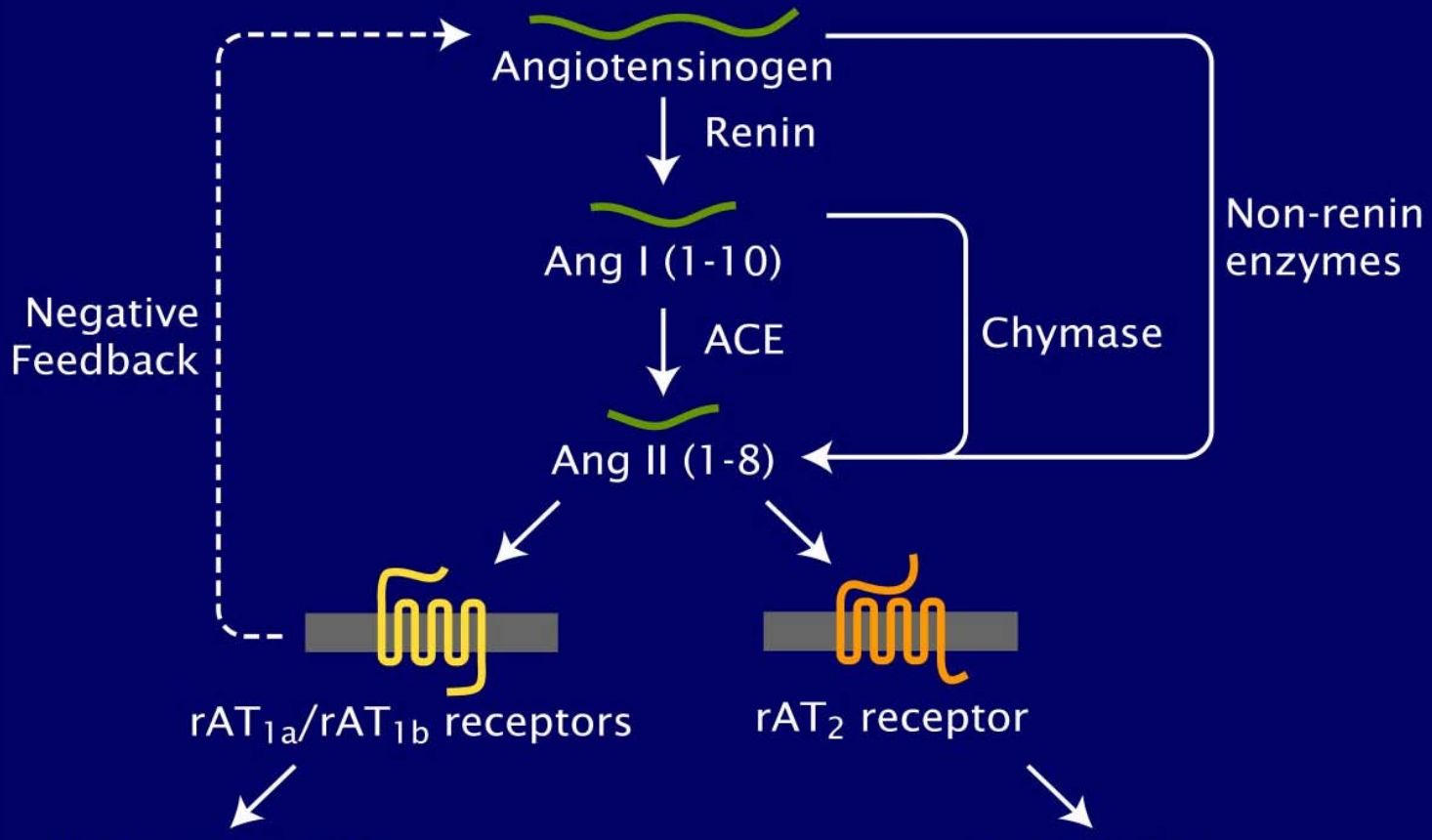
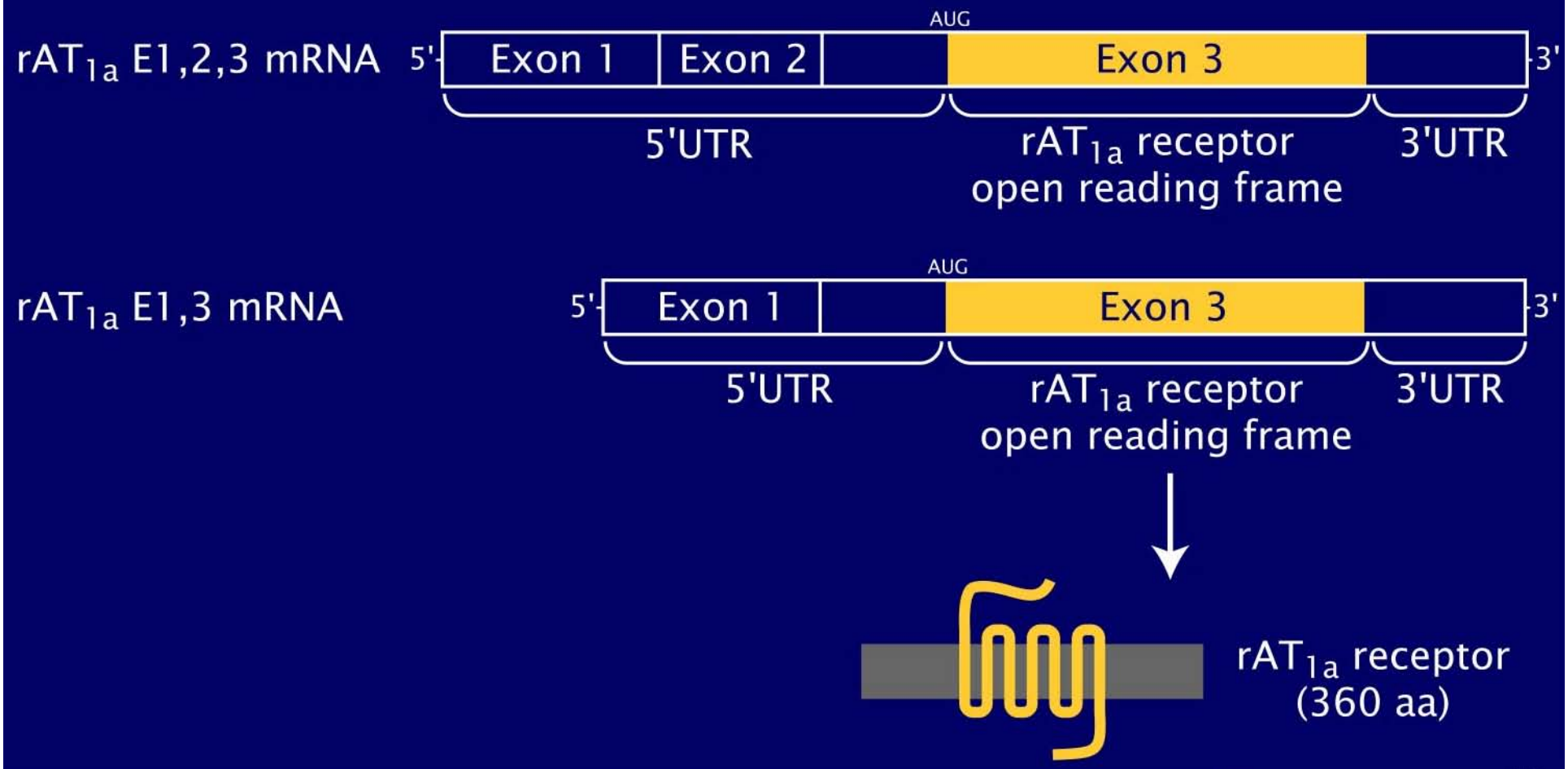
Presenter Disclosure Information

No relationships to disclose

The Renin-Angiotensin System



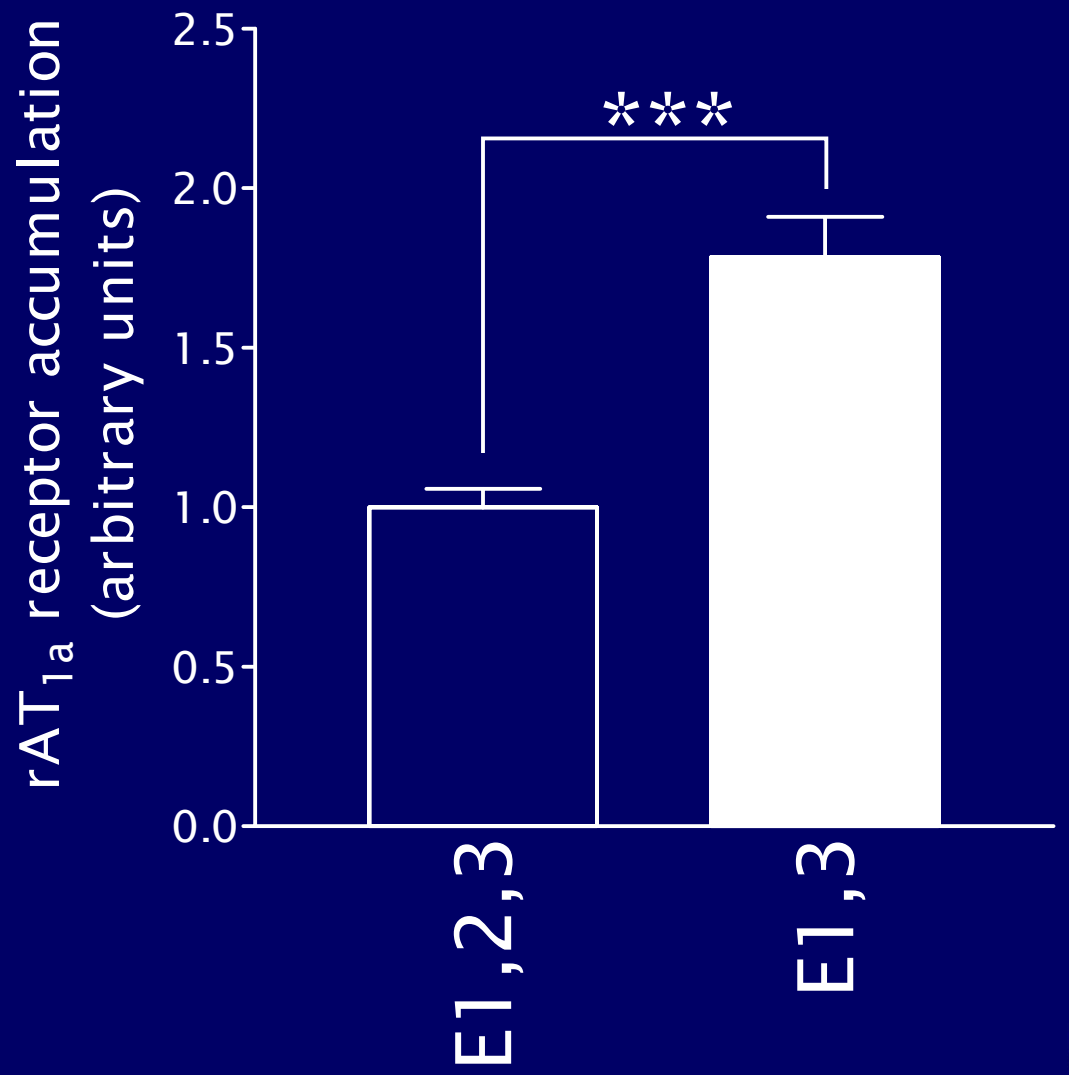
There are two rat AT_{1a} receptor mRNA splice variants



Are the E1,3 and E1,2,3 splice variants differently translationally regulated?

Hypothesis: Alternative splicing in the 5'UTR yields rAT_{1a} receptor mRNA splice variants that exhibit differences in translational regulation.

The E1,3 splice variant is more efficiently translated *in vitro* than the E1,2,3 mRNA



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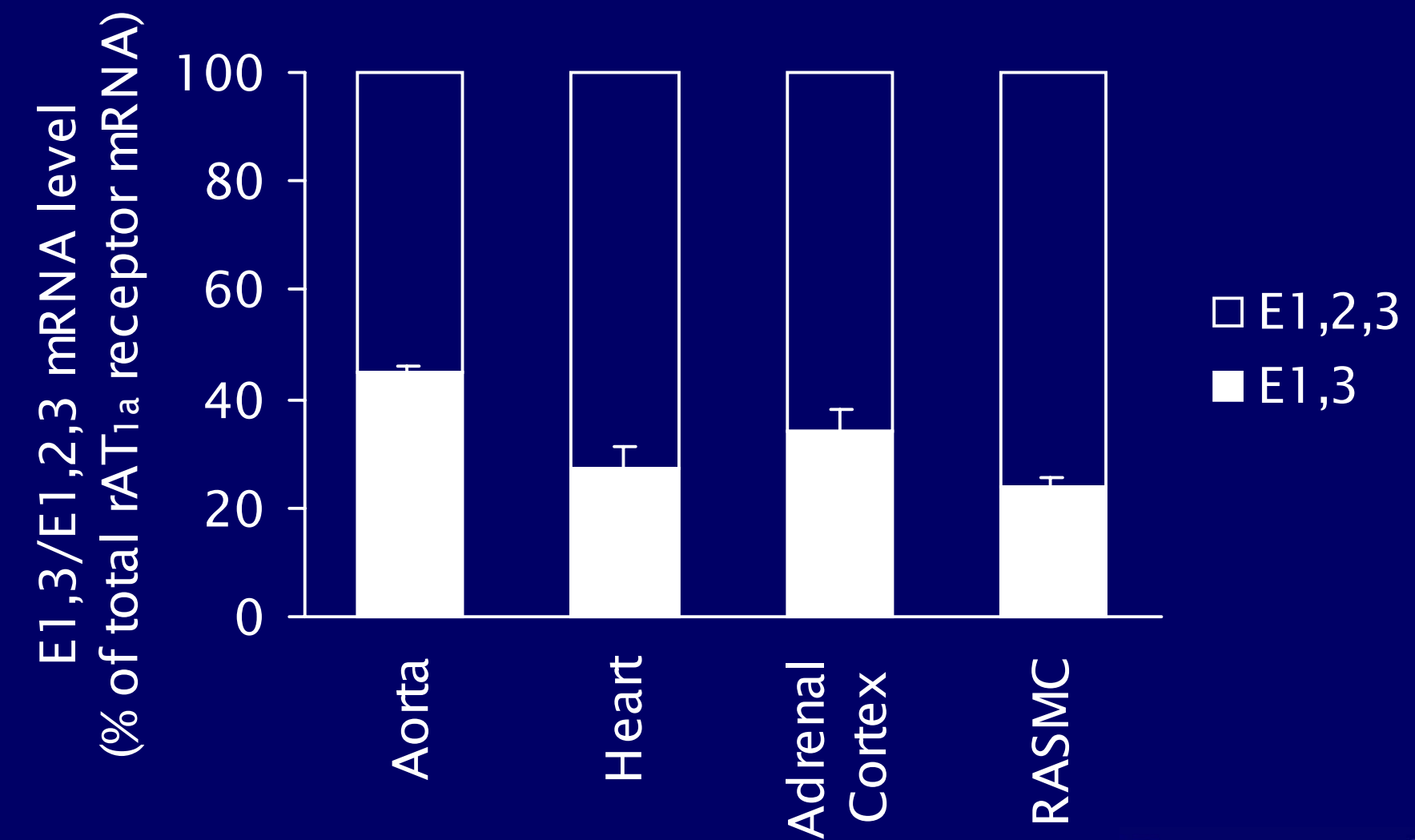
Question

1. Is the E1,3 mRNA translated more efficiently than the E1,2,3 mRNA in cells endogenously expressing the rAT_{1a} receptor?

E1,2,3 is the predominant rAT_{1a} receptor mRNA splice variant in tissues



Questions

1. Is the E1,3 mRNA translated more efficiently than the E1,2,3 mRNA in cells endogenously expressing the rAT_{1a} receptor?

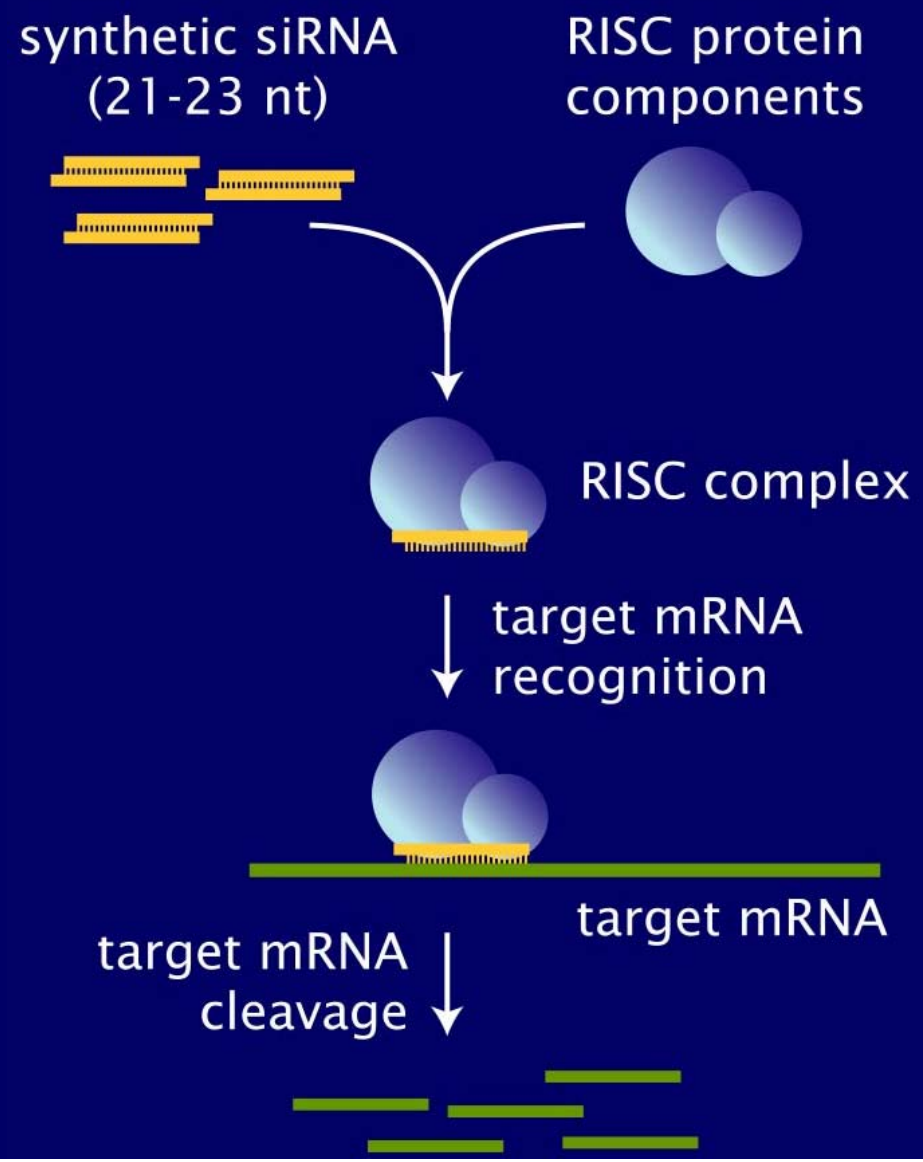
2. What is the relative contribution of the E1,3 and E1,2,3 mRNAs to synthesis of rAT_{1a} receptor protein?

Experimental Approach

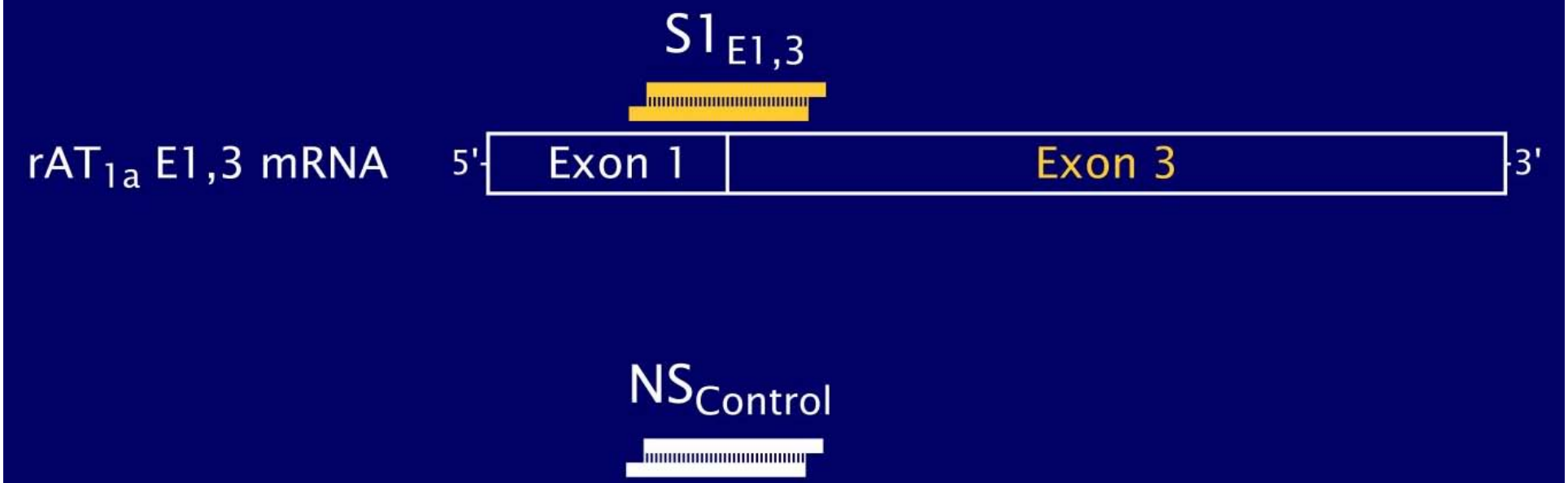
1. Use small interfering RNA(siRNA)-mediated RNA interference to specifically knockdown the E1,3 splice variant in Rat Aortic Smooth Muscle Cells (RASMC)

2. Measure the effect of E1,3 knockdown on AT_1 receptor binding

Small interfering RNA(siRNA)-mediated RNA interference



Design of siRNA selectively targeting the rAT_{1a} receptor E1,3 splice variant



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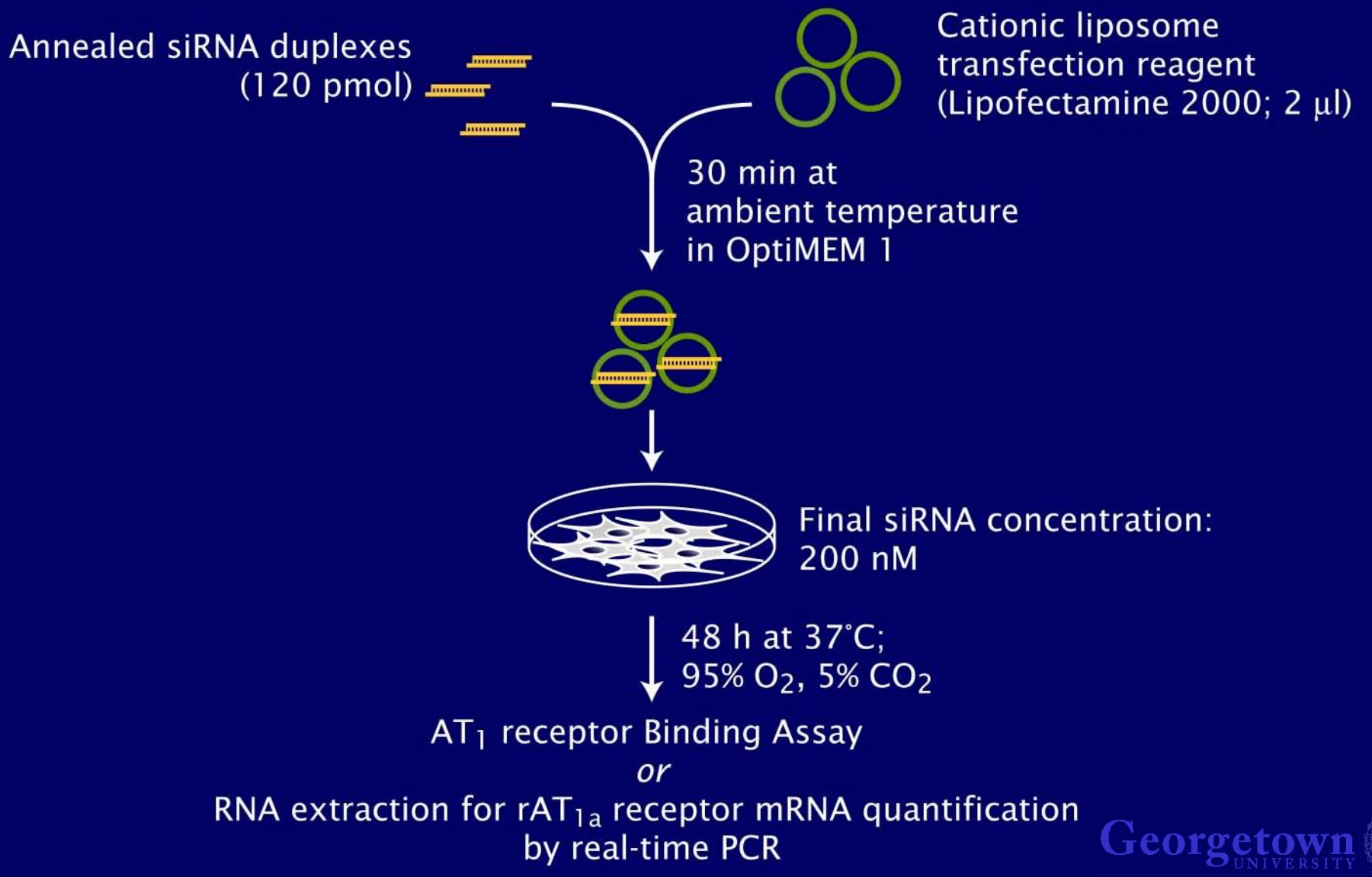
Transfection of Rat Aortic Smooth Muscle Cells (RASMC) with siRNA



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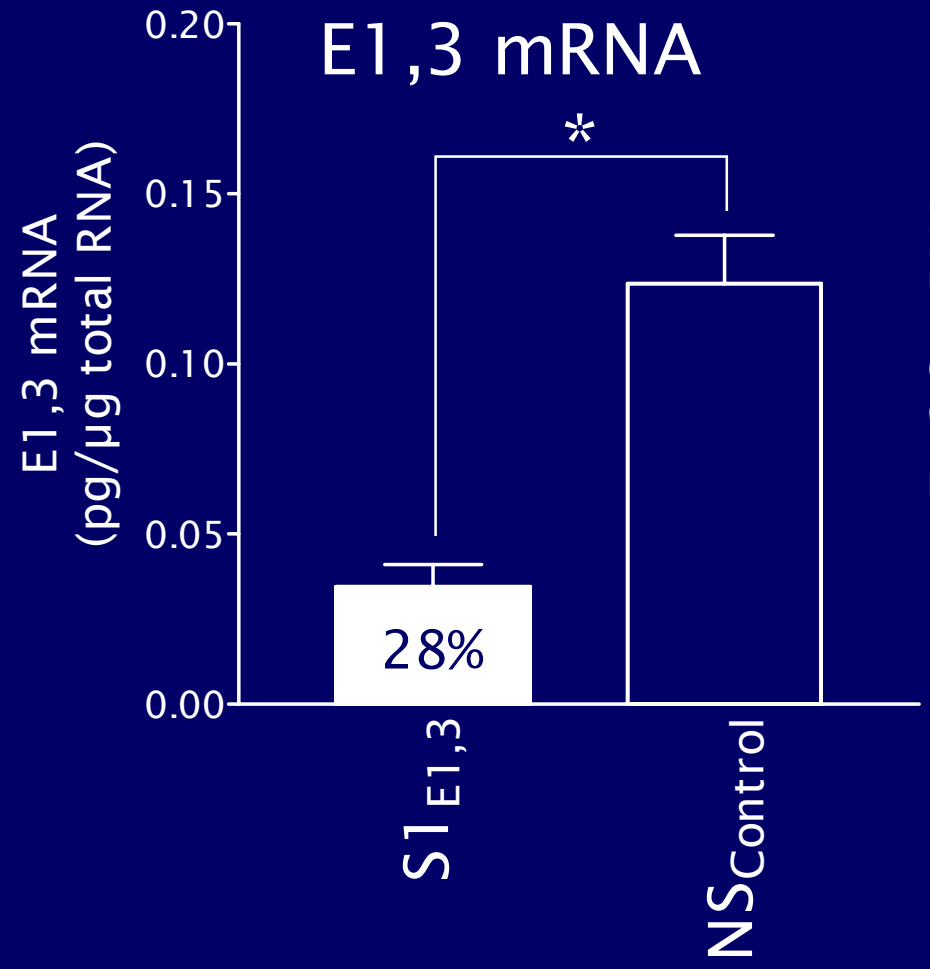
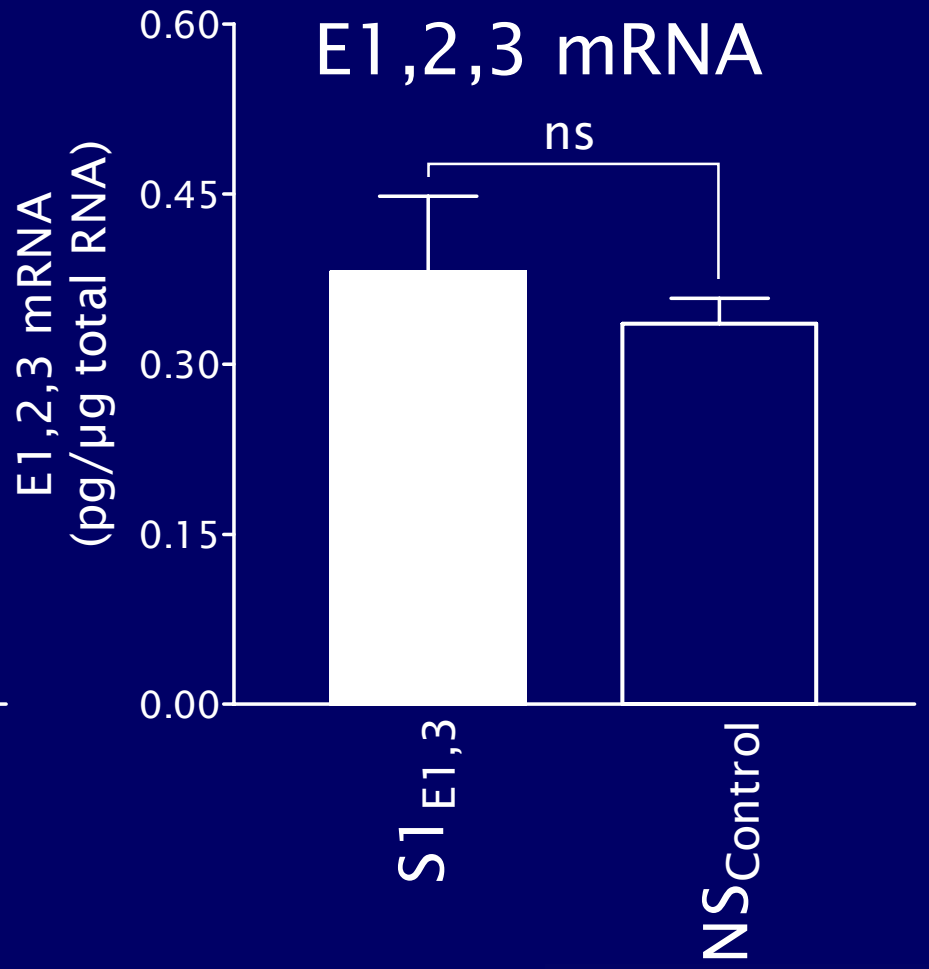
$S1_{E1,3}$ specifically reduces the levels of E1,3 mRNA in RASMC





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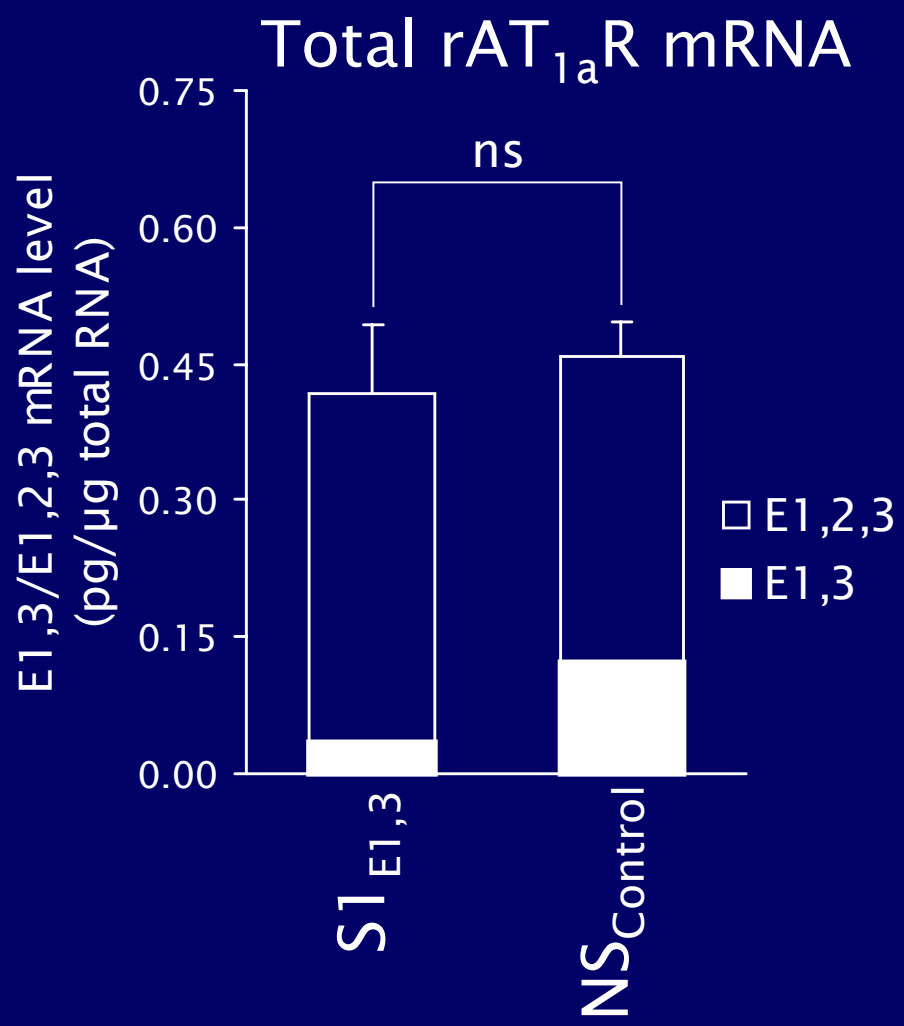
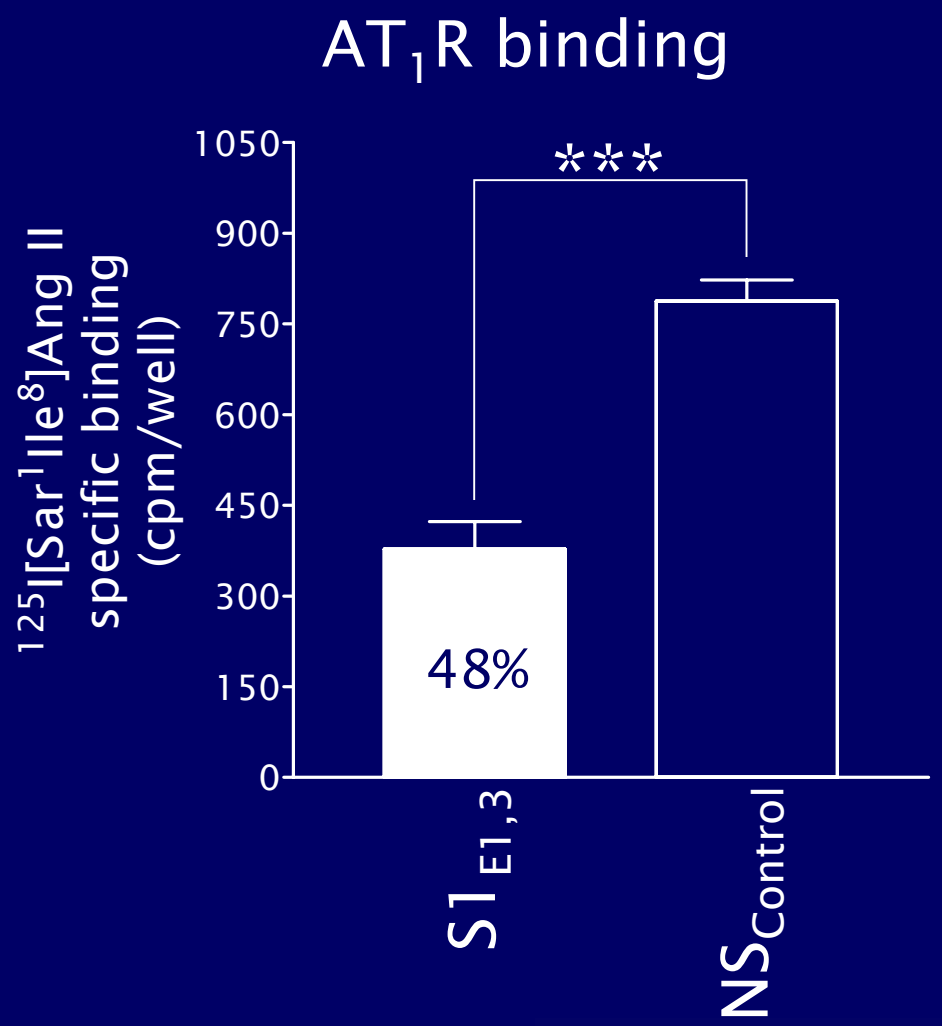
$S1_{E1,3}$ treatment has a disproportionate inhibitory effect on AT_1 receptor binding





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Summary

Results demonstrate that it is possible to use siRNA-mediated RNA interference to specifically knockdown the E1,3 rAT_{1a} receptor mRNA splice variant while leaving the E1,2,3 splice variant intact.

E1,3 mRNA is more efficiently translated than E1,2,3 mRNA in cells endogenously expressing the rAT_{1a} receptor.

Perspectives

These data that suggest that alternative splicing of the rAT_{1a} receptor might play a role in regulation of the expression of the rAT_{1a} receptor.

Potentially, aberrant regulation of rAT_{1a} receptor splicing could lead to pathophysiological states.