

The role of phosphorylation events in regulation of the cachexia mediator Sodium-coupled Neutral Amino acid Transporter (SNAT2) in L6 rat skeletal muscle cells



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Background

Many chronic inflammatory diseases including CKD are characterised by loss of muscle or cachexia, which leads to decreased mobility and quality of life. Metabolic acidosis is common in CKD and stimulates muscle protein wasting which may further enhance chronic inflammation. *In vivo*, this muscle wasting by acidosis also requires the presence of glucocorticoid (GC). Metabolic acidosis is thought to act by inhibiting the pH-sensitive System A amino acid transporter protein SNAT2 (Fig. 1), resulting in impaired global protein synthesis and enhanced global proteolysis. Previous work in this lab showed depletion of free amino acids in skeletal muscle biopsies from exercising CKD patients. Low amino acid level was still detected 24h after exercise (and exercise-induced lactic acidosis) had ceased, possibly reflecting a persistent inhibition of SNAT2 by GC (Watson et al, 2013).

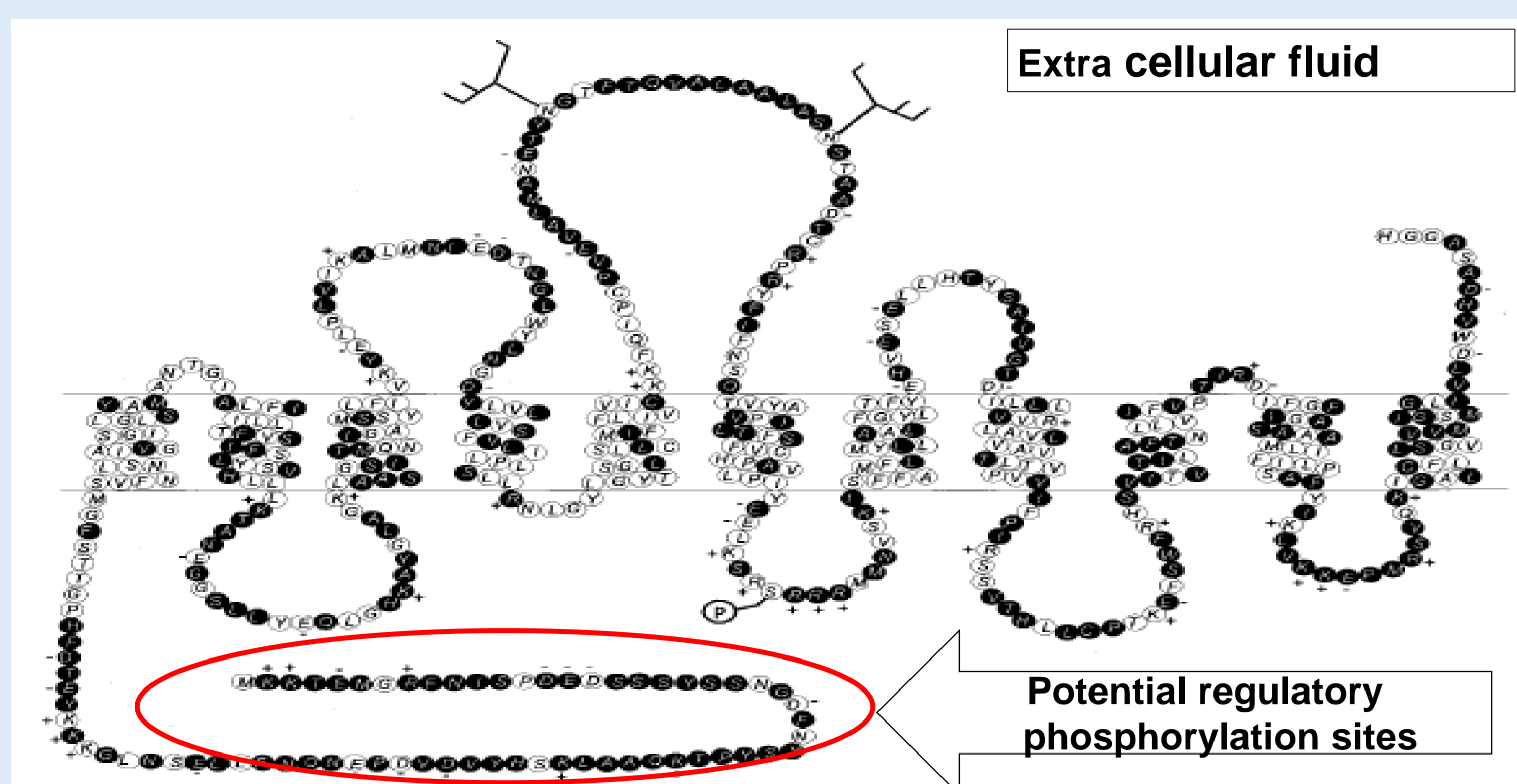


Figure 1. Schematic diagram of the SNAT2 protein (Yao et al., 2000)

Aims

The aim of this study was to investigate (a) how glucocorticoid and amino acid depletion (AAD) influence SNAT2 activity in cultured skeletal muscle cells, and (b) the possible role of phosphorylation events in these effects.

Methods

The L6 rat muscle cell model was used to study the effects of AAD, the glucocorticoid Dexamethasone (DEX), and drugs influencing protein phosphorylation, on SNAT2 transport activity. The activity of this transporter was measured from the rate of α -[1-¹⁴C]-MeAIB transport into the cells and related to the total protein content of the cultures. To obtain an experimental system in which the effect of direct SNAT2 phosphorylation on its function could be assessed; human SNAT2 was cloned and expressed in the readily transfected cell line HEK293A.

Results

In the presence of 2% serum, incubation of L6 myotubes with DEX for 4h at 500nM reproducibly inhibited SNAT2 activity by 60%, and this effect was significantly blunted (but not abolished) by the Phosphoprotein tyrosine phosphatase (PTPase) inhibitor Vanadate (100mM) (Fig 2).

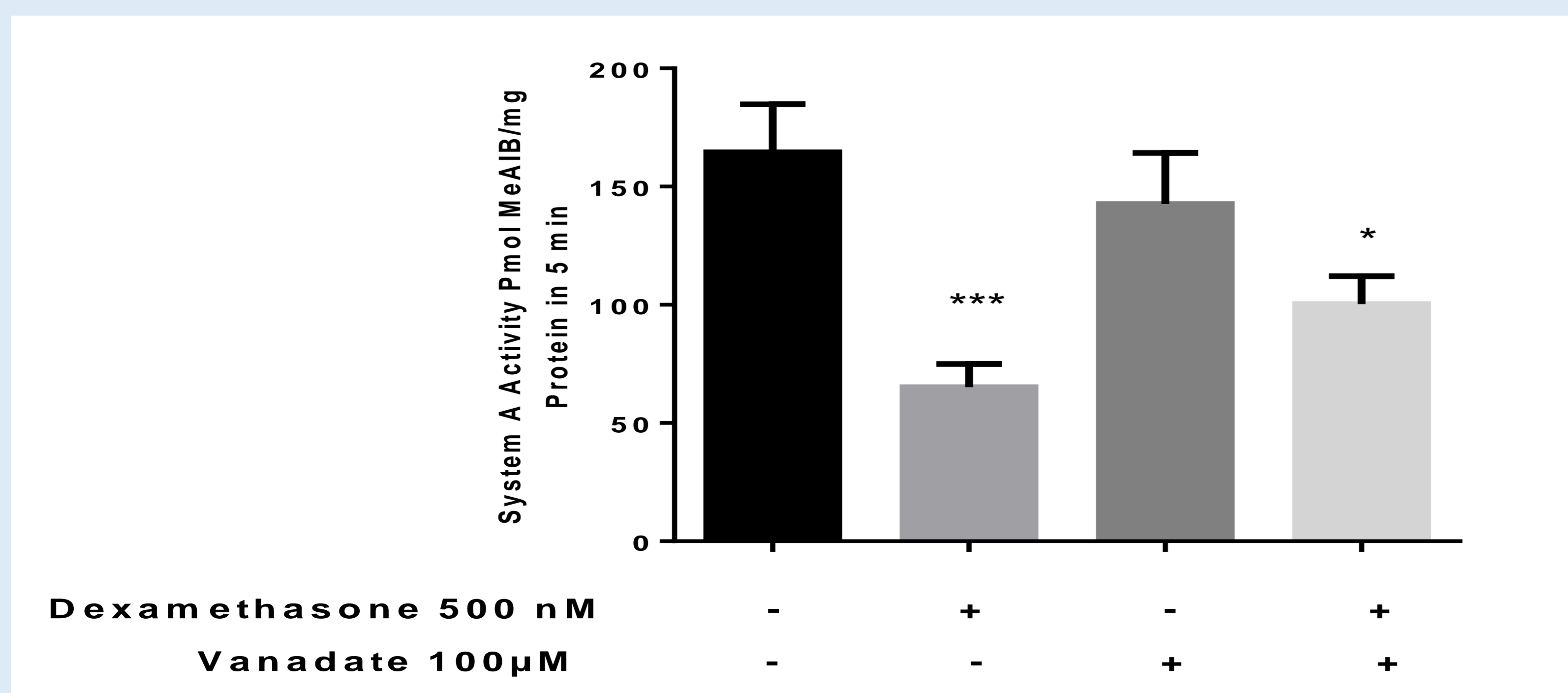


Figure 2. System A amino acid transporter activity in L6 myotubes. Data in the graph presented as Mean \pm SEM. (n=7) *P<0.05, ***P<0.0001.

Results continued

Ser/Thr kinase (MAPK) activation by AAD also blunted DEX's effect on SNAT2 activity (Fig 3). and Vanadate and AAD together abolished DEX's effect on SNAT2 (Fig 4). A C-terminal eGFP-tagged SNAT2 construct was successfully transfected into HEK293A cells and expressed but showed a much weaker response to DEX in these cells (Fig 5).

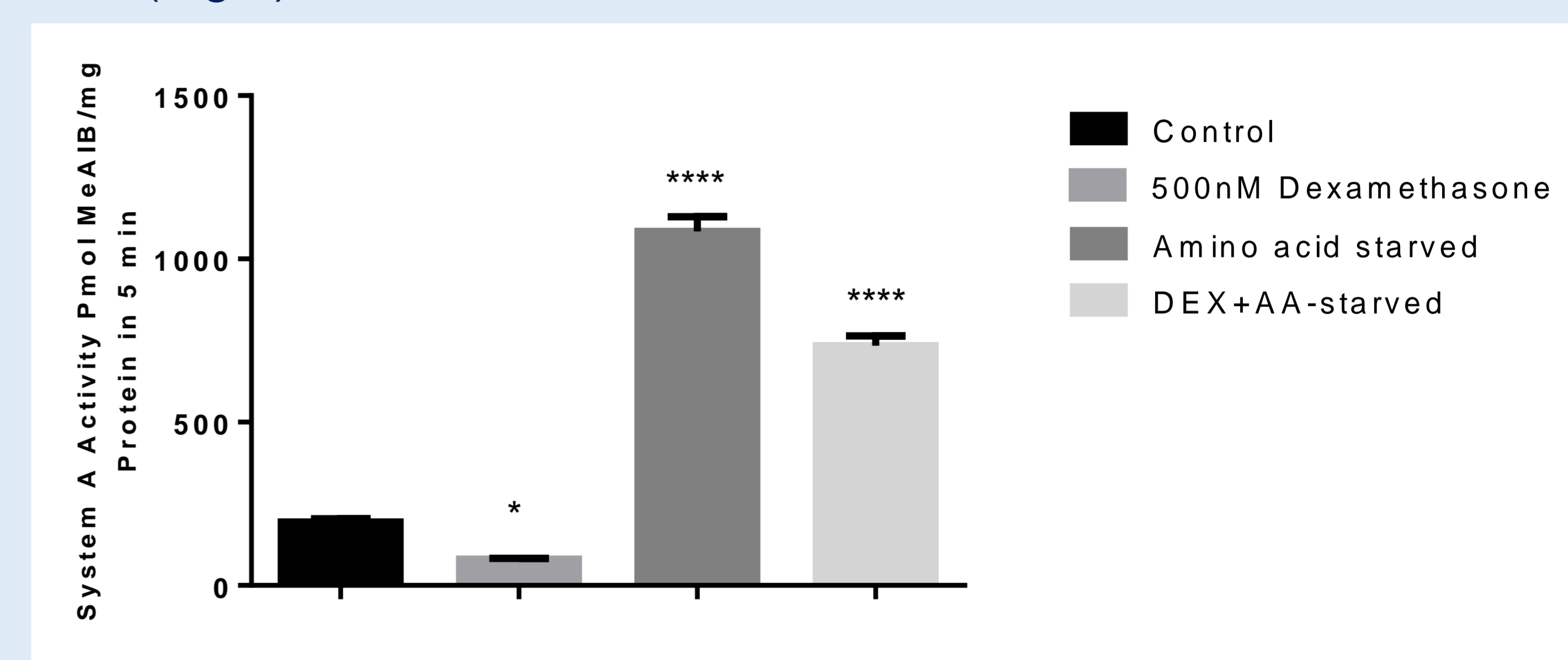


Figure 3. The effect of amino acid depletion on the inhibitory effect of Glucocorticoid (Dexamethasone) on System A activity in L6 cells.

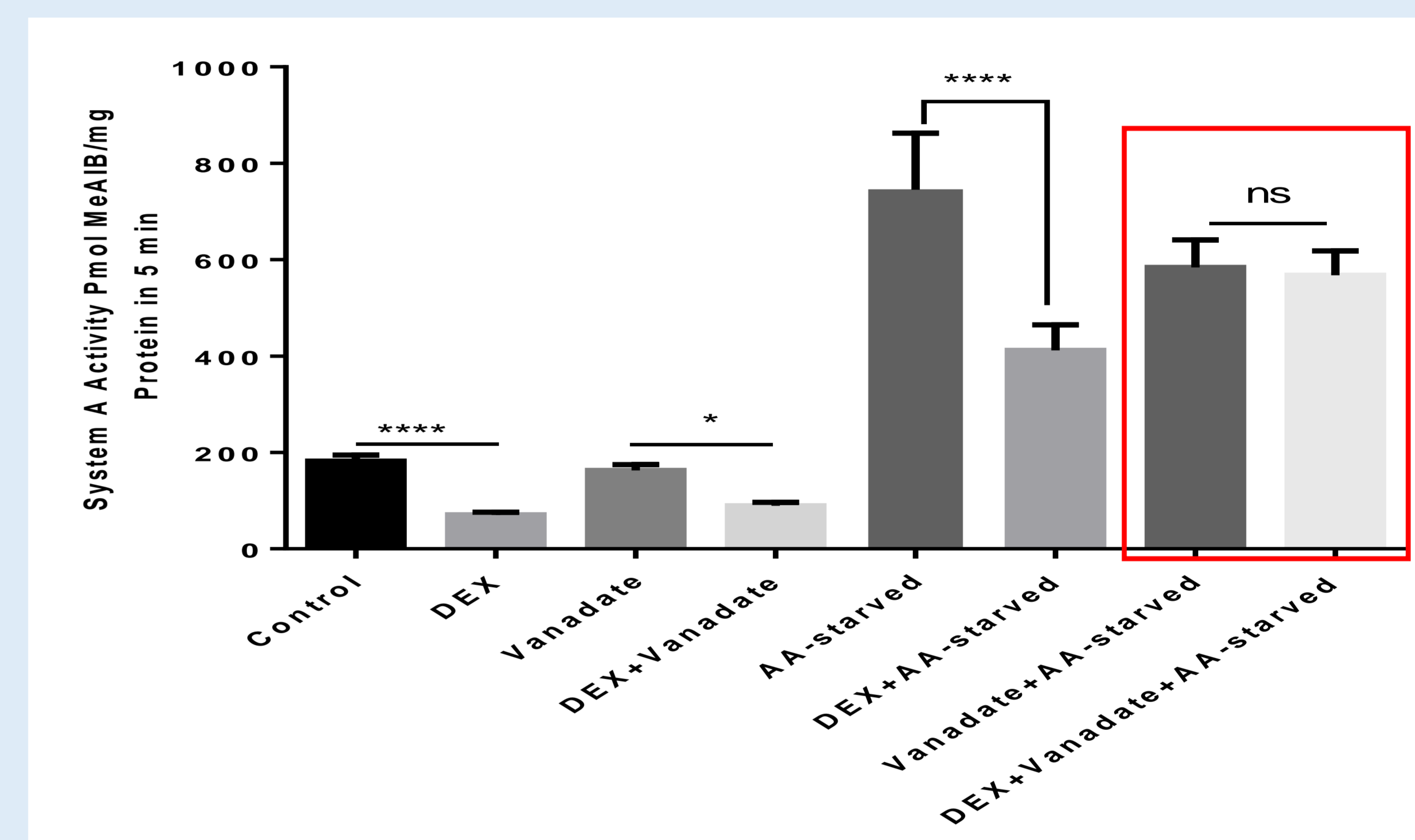


Figure 4. The combined effect of Vanadate and AAD on the inhibitory effect of Glucocorticoid (Dexamethasone) on System A activity in L6 cells.

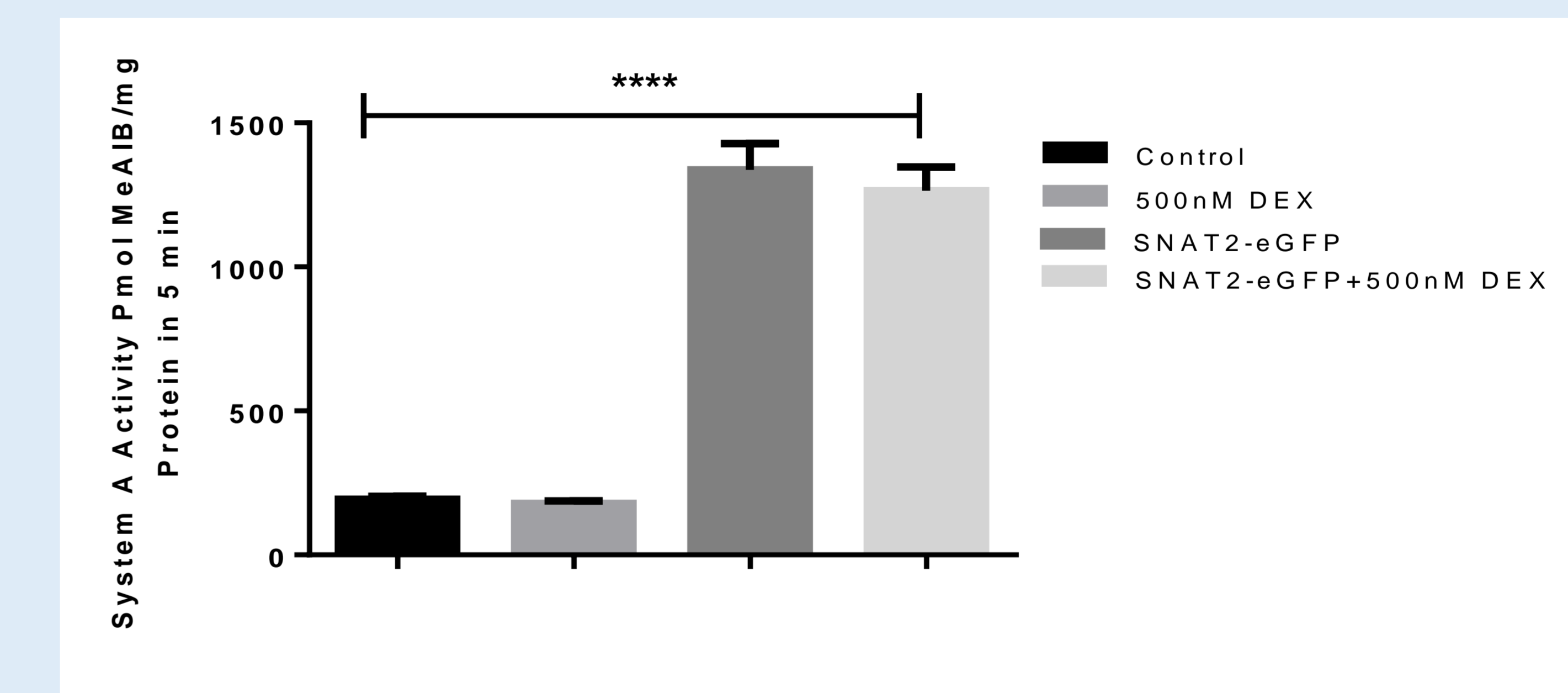


Figure 5. The effect of 500nM Dexamethasone (DEX) on system A amino acid transporter (SNAT2) activity in HEK-293A cells transfected with 1 μg/μl eGFP-tagged SNAT2 cDNA.

Conclusion

SNAT2 regulation by glucocorticoid or AAD may act through a combination of Tyr and Ser/Thr phosphorylation events, possibly on the SNAT2 protein itself. Expression of functionally active SNAT2 in HEK293A cells provides an experimental model in which to test this possibility by isolation of the eGFP tagged protein.

References

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