

ADENOSINE A_{2A} RECEPTORS ARE RESPONSIBLE FOR NECA-INDUCED VASODILATATION IN HUMAN ISOLATED MIDDLE CEREBRAL ARTERIES.

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Introduction

The autocooid, adenosine, is a known vasodilator in many species. During periods of ischaemia, hypoxia or enhanced oxygen demand, adenosine may mediate cerebral artery vasodilatation in humans (Phillis, 1989). The adenosine receptor subtype responsible for cerebral artery dilatation is believed to be of the A₂ class (Hardebo *et al.*, 1987), although it has not been determined whether high affinity A_{2A} or low affinity A_{2B} receptors are responsible. Therefore, the aim of the present study was to determine which of these subtypes is responsible for the dilatation induced by the non-selective A₁/A₂ agonist, 5'-(N-ethylcarboxamido)-adenosine (NECA).

Methods

Human cerebral arteries were obtained from 5 donors (2 female, 3 male, aged between 33 and 79) at *post mortem* with the informed consent of next of kin, and with the approval of local ethics committees. Rings of middle cerebral artery were mounted in 10ml organ baths containing gassed Krebs solution (95% O₂ / 5% CO₂) at 37°C under isometric conditions and an initial tension of 5mN. The integrity of the endothelium in each case was not established. After 60 minutes equilibration, a cumulative concentration-effect curve to the thromboxane A₂ mimetic, U46619, was constructed. After washing, the selective A_{2A} antagonist ZM241385 (Ongini *et al.*, 1999) or the selective A_{2B} antagonist MRS1754 (Ji *et al.*, 2001) was added at 100nM and left in contact with the tissues for 30 minutes. Vessels were constricted with an approximate EC₅₀ concentration of U46619 (10-100nM) and allowed to plateau, after which NECA (100pM – 300µM) was added by cumulative application. After addition of the final concentration of NECA, prostacyclin (1µM) was administered to induce a standard relaxation. The equation $(\log_{10}(\text{concentration ratio} - 1)) - (\log_{10}[\text{antagonist}])$ was used to estimate pA₂ values.

| | A ₁ | A _{2A} | A _{2B} | A ₃ |
|----------|----------------|-----------------|-----------------|----------------|
| MRS1754 | 400 | 500 | 2 | 570 |
| ZM241385 | 260 | 1.4 | 32 | >10,000 |

Table 1.

Binding affinities of MRS1754 and ZM241385 at human adenosine receptor subtypes (Ki's in nM) (Fredholm *et al.*, 2001).

Results

Cumulative administration of NECA to pre-constricted arteries in the absence of antagonist resulted in a concentration-dependant vasodilatation (pEC₅₀ 7.5 ± 0.1). The A_{2B} receptor antagonist MRS1754 (pKi at A_{2B}=8.7) produced no significant shift in the NECA concentration-response curve (pEC₅₀ 7.7 ± 0.3), whereas the A_{2A} antagonist ZM241385 produced a significant rightward shift (pEC₅₀ 5.5 ± 0.3, pA₂ 8.7 ± 0.2, Figure 2). The estimated pA₂ value for ZM241385 compares well with its published affinity (pKi=9.1) for human recombinant A_{2A} receptors (Ongini *et al.*, 1999).

Summary

In conclusion, NECA-induced vasodilatation of human middle cerebral arteries appears to be mediated via adenosine A_{2A} receptors.

References

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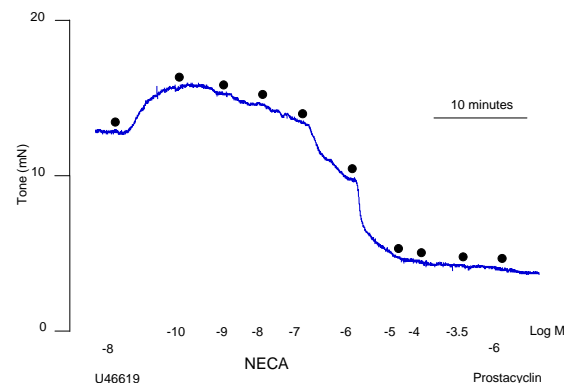


Figure 1.

Typical effect of NECA on isolated human middle cerebral artery following U46619 pre-contraction. Trace shows concentration dependant relaxation to NECA.

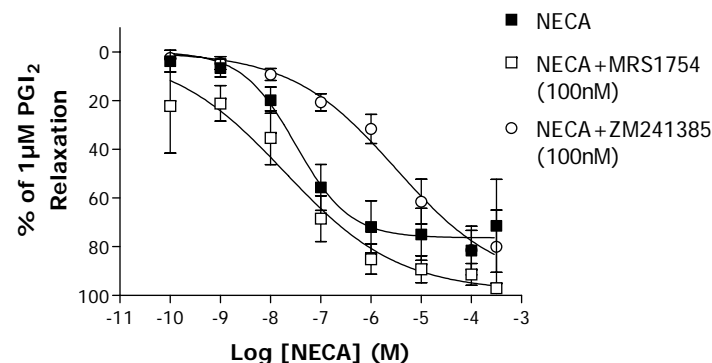


Figure 2.

Concentration-response curves to NECA in U46619 pre-contracted human middle cerebral arteries in the absence and presence of adenosine receptor antagonists. Data are expressed as a percentage of the relaxation caused by 1µM prostacyclin, mean ± s.e.m. (n=4-5).