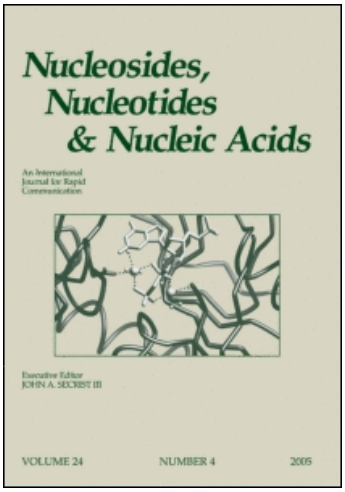

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CHARACTERISTICS OF ADENOSINE-MEDIATED INHIBITION OF TACHYKININ RELEASE FROM PERIFUSED MYENTERIC PLEXUS SYNAPTOSOMES

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Abstract: Adenosine receptor agonists were shown to inhibit evoked release of the tachykinins Substance P and Neurokinin-A from perifused myenteric synaptosomes. The potencies of selective A_1 and A_2 agonists are consistent with activity at adenosine A_1 receptors located on the nerve endings.

Indirect evidence from pharmacological studies suggests that adenosine receptors on enteric nerve endings are coupled negatively to the release of tachykinins.[1] The purpose of the present study was to directly measure adenosine-mediated inhibition of tachykinin release evoked by depolarization from enteric nerve endings and to characterize the receptors responsible for such inhibition. The model system used was a perifused preparation of synaptosomes prepared from guinea pig myenteric plexus. Synaptosomes were prepared as reported,[2] layered on Whatman GF/F filters in low dead-space chambers and perifused with HEPES-Locke's solution (pH 7.2) at 37°C. The flow rate was 0.5 ml/min. Effluent fractions (1ml) were boiled to inactivate peptidases and assayed for substance P-like immunoreactivity (SP-LI) and Neurokinin-A-LI (NKA-LI) by specific RIA's which have been described elsewhere.[1] The RIA for SP-LI used antiserum K-25 which detects 1.5 fmol SP per assay tube and cross-reacts with NKA at 0.3% compared to SP (100%). The RIA for NKA-LI used antiserum NKA-5 which detects 1.5 fmol NKA per assay tube and cross-reacts with SP at 0.3% and NKB at 75%.

Depolarization of the synaptosomes with veratridine or a 50 mM increment in external K^+ concentration ($\uparrow[K^+]_o$) evoked a Ca^{++}-sensitive release of both tachykinins to approximately double the basal levels. Perifusion in the presence of the A_1-selective adenosine agonist N^6-cyclopentyladenosine (CPA, 50 nM) diminished the magnitude of $\uparrow[K^+]_o$-evoked release of SP-LI and NKA-LI in a reversible and repeatable manner. Similarly, the non-selective A_1/A_2 receptor agonist 5'-N-ethylcarboxamido-adenosine (NECA, 100 nM), the marginally A_2-selective agonist CV 1808 (2-phenylaminoadenosine (2-PAA), 100 μM) and the selective A_2 agonist 2-[p-(carboxyethyl)phenylethylamino]-5'-N-ethylcarboxamidoadenosine (CGS 21680, 10μM)

inhibited evoked release of SP-LI and NKA-LI. Perifusion with increasing concentrations revealed the graded nature of the inhibition. Approximate EC_{50}'s were: CPA, 50nM; NECA, 100nM; CGS 21680, 1 μM and 2-PAA, 100μM. The potency order of CPA > NECA > CGS 21680 >> 2-PAA is consistent with action at A_1 receptors.

Perifusion with the agonists in the presence of the A_1-selective antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 1 μM) demonstrated antagonism of the nucleoside-mediated inhibition. The inhibitory responses to CPA and NECA were antagonized by DPCPX while 2-PAA was unable to diminish the release of SP-LI in the presence of DPCPX. It was, however, effective in inhibiting similar release of NKA-LI in the same experiments. These results directly demonstrate a functional link between adenosine receptor occupancy and the release of tachykinins.

Previous studies, using a pharmacological approach, have suggested that the adenosine receptors present on enteric nerve endings may be heterogeneous and comprise both A_1 and A_2 subtypes.[3] This posibility was examined using highly selective adenosine analogs. In separate experiments, the electrically (0.2 Hz, 1 msec) stimulated guinea pig ileum, which is the tissue source for the synaptosomes used above, was used to assess the inhibitory potency of CGS 21680 and agonist N-ethyl-2-[-(4-fluorophenyl)ethoxy]adenosine-5'-uronamide (SHA 116), both of which are highly selective A_2 receptor agonists. The potencies of these analogs for inhibition of the twitch response were: (EC_{50}'s) CGS 21680, 7.7μM; SHA 116, 10.5μM. These potencies are consistent with an action at A_1 receptors as no effects were observable at the nanomolar concentrations expected for activity at A_2 receptors.

It is probable that the actions of adenosine on SP-LI and NKA-LI release are mediated by interaction with an A_1 receptor although the lack of a specific antagonist means that involvement of A_2 receptors in the response cannot be excluded. It is likely that the receptor is similar to that mediating inhibition of acetylcholine release.

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