# $A_3$ ADENOSINE RECEPTORS IN HUMAN NEUTROPHILIS AND PROMYELOCYTIC HL60 CELLS: A PHARMACOLOGICAL AND BIOCHEMICAL STUDY.

**Cattabriga E.**, 2° anno di corso del Dottorato di ricerca in Farmacologia Cellulare e Molecolare, XVI ciclo. Durata del Dottorato in anni: 3. Sede di servizio Università di Ferrara, Dipartimento di Medicina Clinica e Sperimentale, sezione di Farmacologia.

This work compares the pharmacological properties of $A_3$ adenosine receptors in human polymorphonuclear granulocytes (PMNs) and promyelocytic HL60 cells.
The gene expression of $A_3$ receptors was examined by RT-PCR experiments while the amount of $A_3$ subtype on the plasma membrane was quantified by using the high affinity and selective $A_3$ antagonist [$^3$H]5N-(4-methoxyphenil-carbamoyl)amino-8-propyl-2-(2-furyl)pyrazolo-[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine ([$^3$H]MRE3008F20). Saturation experiments reveal a single high affinity binding site with $K_D$ of 2.3±0.3, 2.6±0.4 nM and $B_{max}$ of 430±35, 345±31 fmol/mg of protein, in PMNs and HL60 cells, respectively.
Competition of radioligand binding by adenosine ligands displays a rank order of potency typical of the $A_3$ subtype. $EC_{50}$ values of N6-(3-iodo-benzyl)-2-chloro-adenosine-5'-N-methyluronamide
(Cl-IB-MECA) for inibition of cAMP levels via $A_3$ receptors are in good agreement with the binding data and furthermore the response is potently inhibited by MRE3008F20.
In contrast, the higt micromolar concentration of Cl-IB-MECA and MRE3008F20 is stimulating and blocking, respectively, $Ca^{2+}$ mobilization are not consistentcompletely with the involvement of an $A_3$ receptor. Furtermor, an important finding of this work is that the inhibition of PMNs oxidative burst is predominantly $A_{2A}$-mediated, even though an effect of $A_3$ subtype could not be excluded.
This conclusion is based on potent blockade of Cl-IB-MECA-mediated inhibition of oxidative burst by SCH 58261 and a minor but significant blockade by MRE3008F20.
In conclusion HL60 cells express $A_3$ receptors similar to those in PMNs, thus providing a useful model for investigation of biochemical pathways leading to $A_3$ receptors activation.