Separation of solubilized A2 adenosine receptors of human platelets from non-receptor [3H]NECA binding sites by gel filtration

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Year 1988
Title Separation of solubilized A2 adenosine receptors of human platelets from non-receptor [3H]NECA binding sites by gel filtration
Journal Naunyn-Schmiedeberg's Archives of Pharmacology
Volume 337
Number 1
Pages / **Article-Number** 64-8
Mesh terms Adenosine, analogs & derivatives, metabolism; Adenosine-5'-(N-ethylcarboxamide); Binding Sites; Blood Platelets, metabolism; Cholic Acids; Chromatography, Gel; Humans; Radioligand Assay; Receptors, Adrenergic, alpha, isolation & purification; Solubility
Human platelet membranes were solubilized with the zwitterionic detergent CHAPS (3-[3-(cholamidopropyl)-dimethylammonio]-1-propanesulfonate) and the solubilized extract subjected to gel filtration. Binding of the adenosine receptor agonist [3H]NECA (5'-N-ethylcarboxamidoadenosine) was measured to the eluted fractions. Two [3H]NECA binding peaks were eluted, the first of them with the void volume. This first peak represented between 10% and 25% of the [3H]NECA binding activity eluted from the column. It bound [3H]NECA in a reversible, saturable and GTP-dependent manner with an affinity of 46 nmol/l and a binding capacity of 510 fmol/mg protein. Various adenosine receptor ligands competed for the binding of [3H]NECA to the first peak with a pharmacological profile characteristic for the A2 adenosine receptor as determined from adenylate cyclase experiments. In contrast, most adenosine receptor ligands did not compete for [3H]NECA binding to the second, major peak. These results suggest that a solubilized A2 receptor-Gs protein complex of human platelets can be separated from other [3H]NECA binding sites by gel filtration. This allows reliable radioligand binding studies of the A2 adenosine receptor of human platelets.
Publisher Springer
ISSN/ISBN 0028-1298 ; 1432-1912
edoc-URL https://edoc.unibas.ch/75062/
Full Text on edoc No;
Digital Object Identifier DOI 10.1007/bf00169478
PubMed ID http://www.ncbi.nlm.nih.gov/pubmed/2835689
Document type (ISI) Journal Article