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Aspirin inhibits human bradykinin B_2 receptor ligand binding function

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ABSTRACT

The bradykinin B_2 receptor, a member of the G protein-coupled receptors superfamily, is involved in a variety of physiological functions, including vasodilation, electrolyte transfer in epithelia, mediation of pain, and inflammation. The effect of aspirin on bradykinin binding to cell-surface receptor and on signal transduction were studied in CHO-K1 cells, stably expressing the human B_2 receptor. Cell-surface organization of the receptor was assessed by immunoprecipitation and Western blot analysis in CHO-K1 cells expressing N-terminally V5-tagged B_2 receptor. We found that the widely used analgesic, anti-thrombotic, and anti-inflammatory drug aspirin alters the B_2 receptor ligand binding properties. Aspirin reduces the apparent affinity of the receptor for [^{3}H]-bradykinin by accelerating the dissociation rate of [^{3}H]-bradykinin–receptor complexes. In addition, aspirin reduces the capacity of unlabeled bradykinin or the B_2 receptor antagonist icatibant to destabilize pre-formed [^{3}H]-bradykinin–receptor complexes. Kinetic and reversibility studies are consistent with an allosteric type of mechanism. Aspirin effect on B_2 receptor binding properties is not accompanied by alteration of the cell-surface organization of the receptor in dimers and monomers. Aspirin does not influence the receptor ability to transduce bradykinin binding into activation of G-proteins and phospholipase C. These results suggest that aspirin is an allosteric inhibitor of the B_2 receptor, a property that may be involved in its therapeutic actions.

1. Introduction

Bradykinin has a variety of physiological effects including vasodilation and endothelial activation, modulation of water and electrolyte transports in epithelia, and mediation of pain [1,2]. The physiological role of the kallikrein–kinin system has been well documented in the cardiovascular system, where kinins produced in arteries through the action of kallikrein participate in the control of arterial blood flow, and are involved in vascular remodeling, and in angiogenesis [3–6]. Through its vascular, chemotactic and pain producing effects, BK is also involved in inflammation [7].

BK exerts its effects by interacting with two different subtypes of G protein-coupled receptors, B_1 and B_2 [8]. The B_2 receptor is constitutively synthesized in tissues, contrary to the B_1 receptor, and mediates most of the BK effects described

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Abbreviations: GPCR, G protein-coupled receptor; BK, bradykinin; B_2 receptor, bradykinin receptor B_2 subtype; B_1 receptor, bradykinin receptor B_1 subtype; CHO-K1 cells, K1 type of Chinese hamster ovary cells; IPs, inositol phosphates.

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so far. We have previously shown that BK binds to the B_2 receptor in a negative cooperative manner, as a consequence of interaction between receptor molecules triggered by occupancy of the ligand binding site [9–11]. This phenomenon constitutes an early desensitization mechanism. The specific B_2 receptor antagonist icatibant also triggers allosteric interaction resulting in BK–receptor complex dissociation [9]. However, whether other classes of pharmacological agents can modulate B_2 receptor function is not known. Recently, several GPCRs, have been reported to be positively or negatively modulated by compounds acting on sites topographically distinct from the orthosteric site used by the endogenous ligands [12–14]. These compounds are termed allosteric modulators. We tested several candidate compounds for interaction with the B_2 receptor and found that aspirin (acetylsalicylic acid), influenced the binding of BK to the receptor.

Aspirin is a widely used drug with analgesic, antiplatelet, and anti-inflammatory properties. It is often used in pathological situations where kinin receptors are activated, such as ischemic heart disease or inflammation. It has been reported previously that aspirin is an allosteric inhibitor of the endothelin ETA receptor, a member of the GPCR family [15]. In the present study, we show that aspirin, at therapeutic concentration, is a negative modulator of the B_2 receptor ligand binding function, bringing a second example of a GPCR influenced by this compound.

2. Materials and methods

2.1. Materials

The CHO-K1 cells were from American Type Culture Collection, Rockville, MD, USA. Lipofectamine 2000 and monoclonal anti-V5 antibody were from Invitrogen, Leek, Netherlands. Fetal calf serum (lot no. S01190S0180) was from Biowest, ABCYS-Paris, France. Antibiotic cocktail for cell culture, igepal, protease inhibitor cocktail, bovine serum albumin (A-4378), aspirin (acetylsalicylic acid) were from Sigma Aldrich Chimie, Saint Quentin Fallavier, France. BK was from Alexis, San Diego, California. Icatibant was a generous gift from Hoechst, Germany. [^{3}H]-bradykinin (64 Ci/mmol), [^{3}H]-myoinositol (14 Ci/mmol), [^{35}S]-GTPγS (1065 Ci/mmol) and ECL kit were from Amersham Biosciences, Buckinghamshire, UK. Protein G magnetic beads were from Dynal, Oslo, Norway. Protein markers were from New England BioLabs-Ozyme, Saint-Quentin en Yvelines, France. Polyacrylamide was from Interchim, Montluçon, France. Peroxydase-conjugated secondary antibody was a goat anti-mouse IgG from Jackson ImmunoResearch Laboratories, Pennsylvania, USA.

2.2. Cell culture and receptor expression

The experiments described below were performed on CHO-K1 cells transfected with previously cloned human B_2 receptor cDNA placed under the control of cytomegalovirus promotor into the eucaryotic expression vector pcDNA3 [9,16]. A human B_2 receptor cDNA construct, having an exogenous V5 epitope coding sequence at the aminoterminal extremity of the receptor, just after the methionine start codon, was used in experiments involving receptor immunoprecipitation [17]. Lipofectamine 2000 reagent was used for transfection according to manufacturer instructions. G418-resistant cell clones expressing the recombinant receptors were selected on the basis of their ability to bind [^{3}H]-BK. These clones as well as parental CHO-K1 cells were grown at 37 °C in Ham's F12 medium, supplemented with 10% (v/v) fetal calf serum, antibiotics (penicillin 0.2 unit/ml, streptomycin 20 pg/ml and amphotericin B 0.5 mg/ml) and 0.5 mM glutamine, in a humid atmosphere of 95% air and 5% CO_2. Cells were used at confluence, *i.e.* 48–72 h after cell passage.

2.3. Cell membrane preparation

After washing three times with PBS, cells grown in 10 cm Petri dishes were resuspended in lysis buffer (50 mM Tris–HCl, pH 7.4, 2 mM $MgCl_2$, 0.3 ml EDTA, protease inhibitor cocktail (1:500 dilution), 10 mM captopril, 0.08 unit/ml aprotinin), homogenized by 10 strokes of a "B" glass Dounce homogenizer, and centrifuged (100 × *g* for 5 min) at 4 °C. Supernatants were recovered and centrifuged (20,000 × *g* for 20 min) at 4 °C. Membranes were recovered in appropriate volume of membrane buffer (50 mM Tris–HCl, pH 7.4, 5 mM $MgCl_2$, protease inhibitor cocktail (1:500 dilution), 10 mM captopril, 0.08 unit/ml aprotinin), and stored at −80 °C until use. Protein concentration was determined using the method of Bradford.

2.4. Radioligand binding assay in intact cells

All experiments were started by washing cells grown in 48-well plates with 0.25 ml of modified Hank's balanced salt solution (HBSS: 127 mM NaCl, 5 mM KCl, 0.33 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 20 mM $NaHCO_3$, 20 mM HEPES, 5 mM glucose, 10 mM sodium acetate, 0.8 mM $MgSO_4$, 1 mM $MgCl_2$, 1.5 mM $CaCl_2$, 0.1% BSA, pH 7.4) and by pre-incubation for 30 min at 25 °C in 0.1 ml of solution A (HBSS containing 0.4 M sucrose to block receptor internalization [9,18], and protease inhibitors (10 mM captopril, 0.08 unit/ml aprotinin). When tested, aspirin was added during the pre-incubation period and during additional incubation step(s).

In a first set of experiments, [^{3}H]-BK binding was determined after incubating cells in 0.1 ml of solution A in the absence or presence of increasing concentrations of aspirin (0.3–20 mM) with a fixed concentration of [^{3}H]-BK (4nM). Incubation pH remained between 6.8 and 7.4 upon aspirin addition. Reactions were allowed to proceed for 20 min at 25 °C. Cells were then washed twice with 0.5 ml of ice-cold HBSS to remove unbound [^{3}H]-BK [9].

In association kinetic experiments, cells were incubated for various times up to 30 min with 4 nM [^{3}H]-BK in the absence or presence of aspirin (10 mM). In saturation analysis of [^{3}H]-BK binding, cells were incubated for 20 min in the absence or presence of aspirin (10 mM) with varying concentrations (0.1–20 nM) of [^{3}H]-BK.

In reversibility experiments, cells exposed 30 min to 10 mM aspirin were washed twice with 0.5 ml HBSS. They were then incubated for up to 60 min in 0.1 ml of aspirin-free solution A. Binding of [^{3}H]-BK (4 nM) was then performed for 20 min in the absence of aspirin. Control cells were exposed or not to