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α_1-Adrenoceptor and serotonin 5-HT_{1A} receptor affinity of homobivalent 4-aminoquinoline compounds: An investigation of the effect of linker length

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ABSTRACT

α_1-adrenoceptor (α_1-AR) subtype-selective ligands lacking off-target affinity for the 5-HT_{1A} receptor (5-HT_{1A}-R) will provide therapeutic benefits in the treatment of urogenital conditions such as benign prostatic hyperplasia. In this study we determined the affinity of 4-aminoquinoline and eleven homobivalent 4-aminoquinoline ligands (diquinolines) with alkane linkers of 2–12 atoms (C2–C12) for α_{1A}, α_{1B} and α_{1D} -ARs and the 5-HT_{1A}-R. These ligands are α_{1A}-AR antagonists with nanomolar affinity for α_{1A} and α_{1B} -ARs. They display linker-length dependent selectivity for $\alpha_{1A/B}$ -ARs over α_{1D}-AR and the 5-HT_{1A}-R. The C2 diquinoline has the highest affinity for α_{1A}-AR (pK_i 7.60 ± 0.26) and greater than 30-fold and 600-fold selectivity for α_{1A}-AR over α_{1D}-AR and 5-HT_{1A}-R respectively. A decrease in affinity for α_1-ARs is observed as the linker length increases, reaching a nadir at 5 ($\alpha_{1A/1B}$-ARs) or 6 (α_{1D}-AR) atoms; after which affinity increases as the linker is lengthened, peaking at 9 ($\alpha_{1A/1B/1D}$-ARs) or 8 (5-HT_{1A}-R) atoms. Docking studies suggest that 4-aminoquinoline and C2 bind within the orthosteric binding site, while for C9 one end is situated within the orthosteric binding pocket, while the other 4-aminoquinoline moiety interacts with the extracellular surface. The limited α_{1D}-AR and 5-HT_{1A}-R affinity of these compounds makes them promising leads for future drug development of α_{1A}-AR selective ligands without α_{1D}-AR and the 5-HT_{1A}-R off-target activity.

1. Introduction

α_1-adrenoceptors (α_1-ARs) are members of the seven-transmembrane-spanning G-protein-coupled receptor (GPCR) superfamily, and exist as three distinct subtypes: α_{1A}, α_{1B} and α_{1D}. α_1-ARs respond to the endogenous catecholamines, norepinephrine and epinephrine, and play a vital role in numerous physiological functions predominantly involving smooth muscle contraction, which makes them a therapeutic target for several urogenital conditions, such as benign prostatic hyperplasia (BPH) and stress urinary retention [1,2]. BPH is a widespread condition in males over 60 with the incidence of this condition increasing as men age. One of the most effective treatments for BPH is therapy with α_1-AR antagonists (ideally α_{1A}-AR antagonists, as this is the predominant subtype in the urogenital tract) [1]. Antagonism of the α_1-AR prevents the contraction of the smooth muscle of the prostate gland and the bladder neck, thus decreasing lower urinary tract symptoms of BPH [1]. The α_1-AR antagonists that are used currently have similar therapeutic outcomes but have different side effect profiles due to the lack of subtype selectivity, and off-target affinity. For example, alfuzosin and terazosin have equal affinity for each of the α_1-AR subtypes: this can result in cardiovascular side effects such as hypotension mediated by the α_{1B}-AR, the predominant subtype in the blood vessels of older men [3]. In contrast, tamsulosin and naftopidil have higher affinity for α_{1A} and α_{1D}-ARs than for α_{1B}-AR and hence reduced cardiovascular side effects. However, they also have nanomolar affinity for the serotonin 5-HT_{1A} receptor (5-HT_{1A}-R), and tamsulosin has nanomolar affinity for the dopamine D_3 receptor (D_3-R) [4,5]. The high off-target affinity of tamsulosin and naftopidil has been implicated in floppy iris syndrome and ejaculatory dysfunction side effects [4,6].

Previously, a series of homobivalent 4-aminoquinoline compounds (diquinolines) was shown to have high affinity for rat α_1-adrenoceptors [7,8], with tissue-specific differences. However,

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at that time the α_1-AR had not been classified into the current three subtypes, therefore the subtype selectivity of these compounds was not determined. In this study, we find that the diquinolines show nano- to micro-molar affinity and a similar linker length-affinity relationship for each α_1-AR subtype. Five of the compounds (C2, C3, C5, C6 and C12) have significantly higher affinity for α_{1A}-AR over α_{1D}-AR and all compounds, with the exception of 4-aminoquinoline, C7, and C3 amine, display significant selectivity for α_{1A}-AR over 5-HT_{1A}-R.

2. Materials and methods

The diquinolines were prepared and isolated as their dihydrochloride salts as previously described [7,8]. C2, C3, C5 and C6 diquinoline were dissolved in ultra-pure water (Millipore, Billerica, MA, USA) at 10 mM, and C7, C8, C9, C10, C11 and C12 were solubilised at 10 mM in 90% dimethyl sulphoxide/water (DMSO). These stock solutions were stored at −80 °C. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine, penicillin and streptomycin were purchased from Thermo Fisher Scientific (Scoresby, VIC, Australia). [^{3}H] prazosin (85 Ci mmol^{-1}) and [^{3}H]-myo-inositol (20 Ci mmol^{-1}) were purchased from PerkinElmer (Waltham, MA, USA) and [^{3}H]-OH-DPAT (226 Ci mmol^{-1}) from GE Healthcare (Uppsala, Sweden). Phentolamine hydrochloride, serotonin hydrochloride, norepinephrine hydrochloride, diethylaminoethyl-dextran (DEAE-dextran), lithium chloride (LiCl), formic acid, ammonium formate and chemicals used in buffered solutions (HEPES, EGTA and $MgCl_2$) were bought from Sigma–Aldrich (St. Louis, MO, USA), and Tris, $CaCl_2$, NaCl, KCl, Na_2HPO_4, and KH_2PO_4 were purchased from Ajax Finechem (Taren Point, NSW, Australia).

2.1. Cell culture and transient transfection

COS-1 cells (American Type Culture Collection, Manassas, VA, USA) were cultured in DMEM supplemented with 10% (v/v) heat-inactivated FBS, 100 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. COS-1 cells were maintained and passaged upon reaching confluence using standard cell culture techniques and replaced by lower passage number cells upon nearing 50 passage cycles. Transient transfection of human α_{1A} and α_{1B} -ARs and 5-HT_{1A}-R was performed using pcDNA 3.1+ vectors containing inserted cDNA for each of the receptors (Missouri S&T cDNA resource center, MO, USA), and for the human α_{1D}-AR via pMV6-XL5 vector (Origene, Rockville, MO, USA) containing inserted α_{1D}-AR cDNA. The DEAE-dextran methodology was used to perform the transfection as previously described [9].

2.2. Membrane preparation

Membrane suspensions were formed from COS-1 cells transiently transfected with the cDNA of interest, as previously described [10]. Cells were scraped from the surface of culture plates, suspended in phosphate-buffered saline, and then centrifuged at 1000 × *g* for 5 min. The pellet was resuspended in 10 mL 0.25 M sucrose containing protease inhibitors, disrupted, and homogenized by 10–15 strokes with a tight-fitting pestle in a Dounce homogenizer. Nuclear debris was removed by centrifugation at 1260 g for 5 min. The membrane pellet was resuspended in HEM buffer (20 mM HEPES, 1.4 mM EGTA and 12.5 mM $MgCl_2$, pH 7.4) and 10% (v/v) glycerol for α_{1D}-AR; and TME buffer (50 mM Tris, 12.5 mM $MgCl_2$, 5 mM EGTA) and 10% (v/v) glycerol for α_{1A} and α_{1B}-ARs and the 5-HT_{1A}-R. All membranes were stored at −80 °C. Protein concentration was determined by using the Bradford reagent (Sigma, St Louis, MO, USA).

2.3. Radioligand binding assay

The reaction mixtures for all binding experiments were incubated at room temperature for 1 h, the reaction was terminated by the addition of PBS (4 °C) and vacuum filtration through GF/B filters (Whatman, Maidstone, UK). Radioactivity was measured by liquid scintillation counting.

2.3.1. α_1-AR binding

All ligands and membranes were suspended in HEM buffer. In saturation binding experiments, membranes containing each α_1-AR subtype were incubated with various concentrations of [^{3}H] prazosin (0.125–16 nM) in a total volume of 200 μL. For competition binding experiments, 1–4 μg of α_{1A} and α_{1B} -ARs and 10–32 μg of α_{1D} -AR membranes were incubated with 200 pM of [^{3}H] prazosin and increasing concentrations of test compounds in a total volume of 200 μL. Non-specific binding was defined as binding in the presence of 100 μM phentolamine.

2.3.2. 5-HT_{1A}-R binding

All ligands and membranes were suspended in 50 mM Tris-HCl and 4 mM $CaCl_2$, pH 7.4. In saturation binding experiments, membranes containing 5-HT_{1A}-R were incubated with varying concentrations of [^{3}H]-OH-DPAT (0.5–16 nM) in a total volume of 200 μL. In competition binding experiments, 6 μg of 5-HT_{1A}-R-containing membranes were incubated with 1 nM of [^{3}H]-OH-DPAT and increasing concentrations of test compounds in a total volume of 200 μL. Non-specific binding was defined as binding in the presence of 10 μM serotonin.

2.4. Inositol phosphate accumulation assays

Accumulation of total [^{3}H] inositol phosphates (IPs) was determined as described previously [11]. Briefly, 1 × 10^5 cells ml^{-1} of transiently transfected COS-1 cells were seeded into 96 well plates and cultured overnight with DMEM supplemented with 10% FBS. Cells were then washed with warmed PBS and labelled overnight with 10 μCi ml^{-1} [^{3}H] myo-inositol in inositol-free DMEM supplemented with 5% FBS. Cells were washed twice with 100 μL warmed PBS, and were treated for 45 min with fully-supplemented inositol-free DMEM containing 20 mM LiCl in the presence or absence of test compounds. An EC_{75} concentration of norepinephrine (10 μM) was then added for 30 min, and the reaction was terminated by addition of 0.4 M formic acid. Cells were lysed by freeze-thawing twice and were applied to AG 1-X8 columns. Total IPs were eluted with 1 M ammonium formate in 0.1 M formic acid. 200 μL of eluted sample was diluted into 1 mL ultra-pure water (Millipore, Billerica, MA, USA) and 4 mL Ultima-flow™ scintillation fluid (PerkinElmer, Waltham, MA, USA), and counted in a liquid scintillation counter.

2.5. Data analysis

Nonlinear regression analysis of saturation, competition binding, and inositol phosphate accumulation assay data was performed using the curve fitting program GraphPad Prism (San Diego, CA, USA). Inhibition constants (K_i) for each tested compound were determined by transformation of the program-calculated IC_{50} (concentration of ligand resulting in 50% inhibition of [^{3}H] prazosin or [^{3}H]-OH-DPAT) value using the Cheng–Prusoff equation, whereby $K_i = IC_{50}/1 + (L/K_D)$, where [*L*] is 200 pM [^{3}H] prazosin (α_1-ARs) or 1 nM [^{3}H]-OH-DPAT (5-HT_{1A}-R) and K_D is the dissociation constant. The competitive binding data for each ligand was tested for both one and two-site binding. A one-site binding model was determined as the appropriate form of analysis for all binding data. Statistically significant differences ($p < 0.05$)