α₁-Adrenoceptor subtype affinities of drugs for the treatment of prostatic hypertrophy

Evidence for heterogeneity of chloroethylclonidine-resistant rat renal α₁-adrenoceptor

Martin C. Michel, Rainer Büscher, Jens Kerker, Henner Kraneis, Wilhelm Erdbrügger, and Otto-Erich Brodde

Department of Medicine, University of Essen, D-45122 Essen, Germany

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Summary. We have used radioligand binding and inositol phosphate accumulation studies to determine the affinity at mixed α₁A- and α₁B-adrenoceptors (rat cerebral cortex and kidney), α₁A-adrenoceptors (rat cerebral cortex and kidney following inactivation of α₁B-adrenoceptors by chloroethylclonidine treatment) and α₁B-adrenoceptors (rat spleen) for drugs currently under investigation for the treatment of benign prostatic hypertrophy, alfuzosin, naftopidil and (-)- and (+)-tamsulosin. Alfuzosin and naftopidil had similar affinities in all model systems (approximately 10 nM and 130 nM, respectively) and lacked relevant selectivity for α₁-adrenoceptor subtypes. Their potency to inhibit noradrenaline-stimulated inositol phosphate formation in cerebral cortex matched their affinities as determined in the binding studies. Tamsulosin had higher affinity at α₁A- than at α₁B-adrenoceptors, and was slightly more potent than alfuzosin and naftopidil at α₁B- and considerably more potent at α₁A-adrenoceptors. However, the interaction of the tamsulosin isomers with chloroethylclonidine-insensitive (α₁A-like) adrenoceptors was complex. A detailed analysis of the tamsulosin data and those obtained with other drugs, most notably noradrenaline and oxymetazoline, suggested that chloroethylclonidine-insensitive α₁-adrenoceptors may be heterogenous and that this heterogeneity may differ between cerebral cortex and kidney of the rat.

Key words: α₁-Adrenoceptor subtypes - Kidney - Alfuzosin - Naftopidil - Tamsulosin

Introduction

Benign prostatic hypertrophy is a frequent disease state in elderly men, but few pharmacological approaches for its rational treatment are available. One treatment option currently under investigation is the use of α₁-adrenoceptor antagonists. This approach is based on three findings: Firstly, prostatic tissue of e.g. cows (Maruyama et al. 1992), dogs (Lepor et al. 1992) and humans (Yamada et al. 1987, 1992; Morita and Kondo 1992) contains α₁-adrenoceptors which have been characterized by radioligand binding and autoradiographic studies; dopamine-β-hydroxylase has also been identified immunohistochemically in the human prostate (Chapple et al. 1991). Secondly, catecholamines elicit contraction of human prostatic tissue via α₁-adrenoceptors (Hieble et al. 1985) which might contribute to a phasic component of urinary obstructions. Thirdly, α₁-adrenoceptors have been implicated in the development of hypertrophy in non-prostatic tissues including heart (Knowlton et al. 1993) and vasculature (van Kleef et al. 1992); if they play a similar role in the development of prostatic hypertrophy this might participate in the tonic component of urinary obstruction.

Several studies have demonstrated enhanced urin flow following treatment with prazosin (Chapple et al. 1989; Yamaguchi et al. 1990; 1992) or terazosin (Fabricius et al. 1990) or with the recently developed α₁-adrenoceptor antagonists alfuzosin (Jardin et al. 1991; Teillac et al. 1992), naftopidil (Yamanaka et al. 1991; Yamaguchi et al. 1992) or tamsulosin (Kawabe et al. 1990; Yoshida et al. 1991). Such studies have also shown that the more recently developed α₁-adrenoceptor antagonists are well tolerated whereas the use of classical α₁-adrenoceptor antagonists such as prazosin is frequently limited by side effects such as orthostatic hypotension, fatigue, and dizziness which occur in up to 20% of patients (Khoury and Kaplan 1991).

One hypothesis to explain the good tolerability of the newer α₁-adrenoceptor antagonists would be selectivity for an α₁-adrenoceptor subtype which mediates increased resistance towards urinary flow but is not involved in side effects such as orthostasis. Although neither subtype has been sufficiently defined until now, this possibility is based on the identification of at least three subtypes of α₁-adrenoceptors by pharmacological
and receptor cloning studies (Lomasney et al. 1991a; Bylund 1992). Therefore, we have used radioligand binding experiments in several rat tissues as well as inositol phosphate formation inhibition experiments in rat kidney and cerebral cortex to determine α1-adrenoceptor subtype affinities for alfuzosin, naftopidil and tamsulosin. Since our data with tamsulosin were not readily explained by the existing α1-adrenoceptor classification we have also re-evaluated some established drugs (methoxamine, 5-methyl-urapidil, noradrenaline, oxymetazoline, phentolamine) in our model systems for comparison.

Methods

Rat tissue preparation for radioligand binding experiments. All experiments were performed on male Wistar rats (150–250 g) obtained from Lippische Versuchstierzucht (Extertal, Germany). Tissues were treated as follows:

Cerebral cortical membranes were prepared by homogenization with 10 strokes in a motor-driven glass homogenizer with a teflon pestle in ice-cold 20 mmol/l NaHCO3. Renal and splenic membranes were prepared by homogenization in the same buffer using an Ultra-Turrax (Janke & Kunkel, Stauffen, Germany; 10 s full speed, followed twice by 20 s at 2/3 speed). The homogenates were centrifuged twice for 20 min each at 50000 g at 4°C, and the final pellets were resuspended in binding buffer at protein concentrations of ~2 mg/ml (cerebral cortex), 3–5 mg/ml (kidney and spleen).

Purified rat liver membranes were prepared using sucrose gradient centrifugation as described (Clarke et al. 1978). The membrane preparation was used at a protein concentration of 200–400 μg/ml.

Cardiac membranes were prepared by homogenization with the Ultra-Turrax in ice-cold 1 mmol/l KHCO3. The homogenates were centrifuged for 20 min at 50000 g and resuspended in KHCO3 solution. Following addition of an equal volume of 1 mmol/l KCI the membranes were incubated for 20 min on ice in order to strip contractile proteins, and subsequently centrifuged for 20 min at 40000 g. The final pellet was resuspended in binding buffer (composition given below) at a protein concentration of 1–2 mg/ml.

In some experiments with cerebral cortical or renal membranes the first centrifugation pellet was resuspended in binding buffer and incubated for 30 min at 37°C in the absence or presence of 10 μmol/l chloroethylclonidine in order to inactivate α1B-adrenoceptors and subsequently washed twice by centrifugation for 20 min at 50000 g and 4°C. Final pellets were resuspended in binding buffer.

Radioligand binding. Aliquots of the membrane suspensions (100 μl) were incubated in a total volume of 1000 μl of binding buffer (50 mmol/l Tris, 0.5 mmol/l EDTA at pH 7.5) for 45 min at 25°C. The incubation was terminated by rapid vacuum filtration over Whatman GF/C filters, and each filter was washed twice with 10 ml binding buffer. Following drying of the filters for 1 h at 60°C, 4 ml scintillator (Quickszint 1, Zinsser, Frankfurt, Germany) was added to each filter, and after vigorous shaking of each sample radioactivity on the filters was quantified in a scintillation counter at 42% efficiency. Non-specific binding was defined as binding in the presence of 10 μmol/l phenolamine. Protein content was determined by the method of Bradford (1976) using bovine IgG as the standard.

Inositol phosphate experiments. Accumulation of [3H]inositol phosphates was determined in renal and cerebral cortical slices as previously described (Michel et al. 1993). Briefly, tissues were chopped into 350 × 350 μm slices which were resuspended in Krebs Henseleit buffer of the following composition (mmol/l): NaCl (108), KCl (4.7), CaCl2 (1.3), MgSO4 (1.2), KH2PO4 (1.2), NaHCO3 (24.9), glucose (11), EDTA (0.001). The buffer was supplemented with 10 mmol/l LiCl to block inositol phosphate degradation, 2 μmol/l adenosine deaminase to remove adenosine from the assay which may have been liberated during tissue chopping, and 20 μmol/l cocaine to block neuronal catecholamine uptake. After two washes into fresh buffer the incubations were continued for another 60 min following the addition of 100 μCi of [3H]myoinositol/11 ml of suspension containing 150–300 μg of slices. Then, 300 μl aliquots of the suspension were pipetted into flat bottom polystyrene tubes under gentle swirling and agonists and antagonists were added to yield a final volume of 330 μl. After 45 min in the absence or presence of agonists and/or antagonists, the incubation was stopped by addition of 330 μl ice-cold methanol and 660 μl chloroform. The mixture was vigorously vortexed twice and thereafter the phases were separated by centrifugation at 820 g for 10 min at 4°C. Aliquots (450 μl) of the upper phase were placed on Dowex AG 1-X8 columns (200 mg/column). Free inositol was eluted twice each with 5 ml H2O and 5 ml of 60 mmol/l ammonium formate. Total inositol phosphates were eluted by addition of twice 1 ml of 1 mol/l ammonium formate dissolved in 100 mmol/l formic acid. Eight ml of scintillator were given to each sample.

Chemicals. Dye reagent for the protein assay was purchased from BioRad (Munich, Germany), oxymetazoline, methoxamine and (−)-noradrenaline from Sigma (Munich, Germany), [3H]prazosin (specific activity ~80 Ci/mmol) from New England Nuclear (Dreieich, Germany), and [3H]naftopidil phosphate (specific activity 80–120 Ci/mmol, pre-purified with PT-6-271) from Amersham (Braunschweig, Germany). The following drugs were gifts of the respective companies: alfuzosin (formerly known as SL 77.0499-10, Synthelabo, Paris Cedex, France), naftopidil (formerly known as BM 15275, Boehringer Mannheim, Mannheim, Germany), stereoisomers of tamsulosine HCl (formerly known as (−)- and (+)-YM 2617, Yamancouchi Pharmaceutical Co., Tokyo, Japan), phentolamine (Ciba Geigy, Basel, Switzerland), 5-methyl-urapidil (Byk Gulden, Konstanz, Germany). Naftopidil was kept as a 10 mmol/l stock solution in dimethylsulfoxide and was diluted into diluted HCl (10 mmol/l), the other drugs were dissolved and kept as 10 mmol/l stock solutions in diluted HCl.

Data analysis. Data are shown as means±SEM of n experiments. Saturation binding experiments were analyzed by fitting the experimental data to a rectangular hyperbolic function. Competition binding and inositol phosphate inhibition experiments were analyzed by fitting the experimental data to monoo-, bi- and triphasic sigmoidal functions; a two-or three-site fit was accepted only when it resulted in a significant improvement of the fit compared to a one- or two-site fit, respectively, as assessed by an F-test. Kd values were calculated from the IC50 values in the binding and functional experiments according to the equation

\[ K_d = IC_{50} / \left[ 1 + \left( L / K_a \right) \right] \]

where L is the concentration of agonist or radioligand and Kd is its EC50 or affinity. All curve fitting procedures were performed using the InPlot program (GraphPAD Software, San Diego, Calif., USA). Statistical significance of differences was assessed by two-tailed t-tests using the Instat program (GraphPAD Software); a P<0.05 was considered significant.

Results

Parameters of [3H]prazosin binding under standard conditions and following chloroethylclonidine treatment

[3H]Prazosin bound with high affinity to membranes from spleen, liver, cerebral cortex, kidney and heart; the Kd values were similar in all tissues and ranged between 40 and 240 pmol/l (Table 1). Depending on the expression density in the tissues and the degree of purification of the membrane preparation receptor densities varied considerably among tissues and ranged from 28 (spleen) to almost 1600 fmol/mg protein (liver).

Since rat cerebral cortex and kidney are known to co-express α1A and α1B-adrenoceptors (Hanf and Gross 1989; Han and Minneman 1991; Michel et al. 1992), we