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Human cloned α_{1A}-adrenoceptor isoforms display α_{1L}-adrenoceptor pharmacology in functional studies

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Abstract

The recombinant α_{1A}-adrenoceptor displays a distinct pharmacological profile ('classical α_{1A}-adrenoceptor') in homogenate binding assays, but displays the properties of the so-called α_{1L}-adrenoceptor in functional studies in whole cells at 37°C. As three splice variants of the human α_{1A}-adrenoceptor have been described previously ($\alpha_{1A\text{-}1}$, $\alpha_{1A\text{-}2}$ and $\alpha_{1A\text{-}3}$), we have compared their functional pharmacological profiles, when expressed stably in Chinese hamster ovary (CHO-K1) cells (antagonist inhibition of noradrenaline-stimulated [^{3}H]inositol phosphates accumulation). A fourth, novel isoform ($\alpha_{1A\text{-}4}$) has also been studied: $\alpha_{1A\text{-}4}$ mRNA predominates in several human tissues including prostate, liver, heart and bladder. In homogenate binding studies, all four isoforms displayed essentially identical affinity profiles, with prazosin (1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furoyl)piperazine), tamsulosin (5-[2-[[2-(2-ethoxyphenoxy)ethyl]-amino]propyl]-2-methoxybenzenesulfonamide), RS-17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1H-indole-3-ethanamine hydrochloride), WB 4101 ((2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) and 5-Me-urapidil (5-methyl-6[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]amino]-1,3-dimethyuracil) all displaying subnanomolar affinities. In functional studies, noradrenaline accelerated [^{3}H]inositol phosphates production with potencies (p[A]$_{50}$) of between 5.8 and 6.6. The affinities of prazosin, RS-17053, WB 4101 and 5-Me-urapidil, at antagonizing responses to noradrenaline, were reduced by approximately 10-fold (cf. binding data), while those for tamsulosin and indoramin (*N*-[1-[2-(1H-indol-3-yl)ethyl]-4-piperidinyl]benzamide) remained constant or increased, consistent with the previously described α_{1L}-adrenoceptor. Thus, all four human recombinant α_{1A}-adrenoceptor isoforms display the pharmacology of the α_{1L}-adrenoceptor when studied in functional assays, consistent with the hypothesis that the putative α_{1L}-adrenoceptor represents a functional phenotype of the α_{1A}-adrenoceptor.

Keywords: α_{1A}-Adrenoceptor; Isoform; Noradrenaline; Prazosin; Inositol phosphate; RS-17053; Tamsulosin

1. Introduction

Current classification recognizes three α_1-adrenoceptor subtypes (α_{1A}, α_{1B} and α_{1D}). Distinct protein sequences and pharmacology are documented (i.e., sequence, second messenger and pharmacological profile established; Hieble et al., 1995). A fourth α_1-adrenoceptor, the 'putative α_{1L}-adrenoceptor', has been proposed (Holck et al., 1983; Flavahan and Vanhoutte, 1986; Muramatsu, 1992) based on functional data (e.g., contraction of human lower urinary tract; Ford et al., 1996). However, distinct cDNA encoding the α_{1L}-adrenoceptor has not been described.

Recent data from our laboratory suggest that the α_{1L}-adrenoceptor, despite presenting a pharmacological profile distinct from the α_{1A}-adrenoceptor, may be encoded by the same human gene (α_{1a}-adrenoceptor; Ford et al., 1997). Radioligand binding studies of the human cloned α_{1A}-adrenoceptor expressed in Chinese hamster ovary (CHO-K1) cells gave estimates of antagonist affinity typical of the 'classically' defined α_{1A}-adrenoceptor (Hieble et al., 1995). However, functional data, inositol phosphates (InsPs) accumulation, revealed a profile consistent with the

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α_{1L}-adrenoceptor. Accordingly, affinity estimates obtained for some antagonists (RS-17053, prazosin, 5-methylurapidil, WB 4101, *S*-niguldipine), but not others (tamsulosin, indoramin and REC 15/2739) differed contingent upon the method of investigation employed.

Three splice variants of the human α_{1A}-adrenoceptor have been described previously ($\alpha_{1A\text{-}1}$, $\alpha_{1A\text{-}2}$ and $\alpha_{1A\text{-}3}$; Hirasawa et al., 1995). A fourth, novel isoform ($\alpha_{1A\text{-}4}$) has also been identified, which predominates in human prostate, liver, heart and bladder (Chang et al., 1997, 1998) based upon mRNA determinations. All four variants have a common sequence up to and including transmembrane domain 7, but vary considerably in length and sequence within the C-terminal tail (Chang et al., 1998). In this study, we have compared the functional pharmacological profile of each isoform, when expressed stably in CHO-K1 cells, using antagonist inhibition of noradrenaline-stimulated [^{3}H]InsPs accumulation. In this manner, the question is addressed whether the explanation for the α_{1A} vs. α_{1L} pharmacology of the recombinant α_{1A}-adrenoceptor gene resides in differences between recognition properties of any of these four splicing variants.

2. Materials and methods

2.1. Cloning and expression of human α_1-adrenoceptor isoforms

Human α_{1A}-adrenoceptor isoforms were cloned by polymerase chain reaction (PCR) amplification using subtype-specific primers on cDNAs from human prostate (Chang et al., 1997, 1998). The amplified products were cloned into the eukaryotic expression vector, pSW104, a derivative of pCD-SRα (Takebe et al., 1988). CHO-K1 cells stably expressing the α_1-adrenoceptor isoforms were obtained by co-transfection of receptor cDNAs with the plasmid pSV2neo using lipofection (Flegner et al., 1987). Stable clones were initially selected for resistance to geneticin (G418, 500 mg/ml), and then maintained in Ham's F-12 nutrient medium supplemented with 10% foetal bovine serum, G418 (150 μg/ml) and penicillin/streptomycin (30 u/ml, 30 μg/ml) at 37°C in 7% CO_2.

2.2. Inositol phosphates accumulation

The method used was a modification of well-established procedures (Brown et al., 1984; Ford et al., 1997). Briefly, cells were washed with phosphate buffered saline (PBS) and incubated in 15 ml inositol-free Ham's F-12 containing 10% dialyzed foetal bovine serum and [^{3}H]*myo*-inositol (2 μCi/ml) overnight. Following the incorporation of inositol, medium was aspirated and the cells washed with PBS to remove unincorporated [^{3}H]*myo*-inositol. PBS containing EDTA (ethylene diamine tetraacetic acid; 30 μM for 5–10 min at 37°C) was used to dissociate cells from the flask. The cell suspension was spun for 5 min at $500 \times g$ at 37°C and washed 3 times in PBS. The cells were resuspended in inositol-free Ham's F-12 to $\sim 5 \times 10^6$ cells/ml. Reactions were performed in triplicate tubes containing 300 μl final reaction volume. Cell suspension (240 μl) was added for pre-equilibration to 30 μl antagonist or vehicle at 37°C for 20 min. The reaction was initiated with the addition of 30 μl agonist or vehicle, containing LiCl (final concentration 10 mM). Tubes were then gently mixed and placed in a 37°C bath for 10 min. Reactions were terminated by the addition of 50 μl ice-cold perchloric acid (20%). Tubes were allowed to sit in an ice–water bath for 20 min, samples were then neutralized with 160 μl 1 M KOH, vortex-mixed and then diluted with the addition of 2 ml Tris–HCl (50 mM, pH 7.5). Neutralized samples were decanted onto disposable columns containing 1 ml Dowex 1X8, chloride form (1:1, w/v) slurry which had been washed with 5 ml distilled H_2O. Columns were then washed with 20 ml distilled H_2O and the eluate discarded. [^{3}H]InsPs were eluted with 3 ml HCl (1 M) into scintillation vials containing 15 ml Ready-Safe liquid scintillation cocktail.

Accumulated [^{3}H]InsPs were measured by liquid scintillation spectroscopy using a Packard 1900TR and expressed as disintegrations per minute (dpm) using standard [^{3}H] window settings. The dpm values were imported into Microsoft Excel for determination of mean and standard deviation values for each triplicate. Iterative nonlinear curve-fitting methods using Kaleidagraph software were used to fit data to the general logistic functions: $E = \text{basal} + E_m \cdot A^{n_H}/(A^{n_H} + [A]_{50}^{n_H})$ for agonist stimulation curves; $E = \text{basal} + E_m - (E_m \cdot B^{n_H}/(B^{n_H} + [B]_{50}^{n_H}))$ for antagonist inhibition curves. $[A]_{50}$ or $[B]_{50}$ values, maxima (E_m) and Hill slopes (n_H) for each curve were estimated by use of this software. Affinity values of test substances (pK_b) were calculated according to Leff and Dougall (1993), such that $K_b = [B]_{50}/((2 + ([A]/[A]_{50})^{n_H})^{1/n_H} - 1)$. $\Sigma(y - x)^2$ is the sum of squares of differences in affinity estimates for each plot, and describes, in relative terms, to what degree two data sets differ. Pharmacological terms and definitions used throughout this manuscript are in accordance with recently published IUPHAR guidelines (Jenkinson et al., 1995).

2.3. Radioligand binding

Homogenate membrane saturation binding studies were conducted using [^{3}H]prazosin as previously described (Ford et al., 1997) for the determination of receptor expression levels.

2.4. Materials

Ham's F-12 nutrient medium, PBS, geneticin (G418), foetal bovine serum (qualified and dialyzed), penicillin/

Table 1
Fold stimulations above basal [^{3}H]InsPs levels and Noradrenaline potency ($p[A]_{50}$) in human cloned α_{1A}-adrenoceptor isoforms and functional studies (noradrenaline-stimulated InsPs accumulation)

Isoform	Fold above basal[a]	n	$p[A]^a_{50}$	n^a_H
$\alpha_{1A\text{-}1}$	10.00 ± 1.60	4	6.63 ± 0.05	0.96 ± 0.05
$\alpha_{1A\text{-}2}$	8.21 ± 0.57	6	6.01 ± 0.13	1.02 ± 0.11
$\alpha_{1A\text{-}3'}$	6.50 ± 1.25	4	6.24 ± 0.04	0.98 ± 0.06
$\alpha_{1A\text{-}4}$	5.84 ± 0.32	5	5.72 ± 0.05	1.01 ± 0.03
$\alpha_{1A\text{-}1}$ (binding)[b]			5.5[c]	
α_{1L} (hLUT)[d]			6.4[e]	

[a]Values are means ± S.E.M. from numbers of determinations shown (n), [b]CHO-K1 (Jasper et al., 1997), [c]Timothy J. Williams, unpublished data, [d]Functional tissue bath assay, human lower urinary tract, [e](Ford et al., 1996).

streptomycin and versene (EDTA) were obtained from Gibco (Gaithersburg, MD). *Myo*-[2-^{3}H]Inositol (10–20 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). Disposable plastic columns (CC-09-M) were obtained from E&K Scientific Products (Saratoga, CA). Prazosin (1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furoyl)piperazine), WB 4101 ((2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride), and 5-methylurapidil (5-methyl-6[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]amino]-1,3-dimethyuracil) were obtained from Research Biochemicals International (Natick, MA). Noradrenaline and bulk chemicals and reagents were obtained from Sigma (St. Louis, MO). RS-17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1H-indole-3-ethanamine hydrochloride), tamsulosin (5-[2-[[2-(2-ethoxyphenoxy)ethyl]-amino]-propyl]-2-methoxybenzenesulfonamide) and indoramin (*N*-[1-[2-(1H-indol-3-yl)ethyl]-4-piperidinyl]benzamide) were synthesized in the Department of Chemistry, Neurobiology Unit, Roche Bioscience (Palo Alto, CA). Kaleidagraph Software was purchased from Synergy Software (Reading, PA). Ready-Safe liquid scintillation cocktail was purchased from Baxter Scientific (McGraw Park, IL).

3. Results

Second messenger studies, measuring [^{3}H]InsPs accumulation, were performed in intact CHO-K1 cells expressing human cloned α_{1A}-adrenoceptor isoforms. Noradrenaline (1 nM to 100 μM) elicited robust concentration-dependent increases in [^{3}H]InsPs accumulation yielding

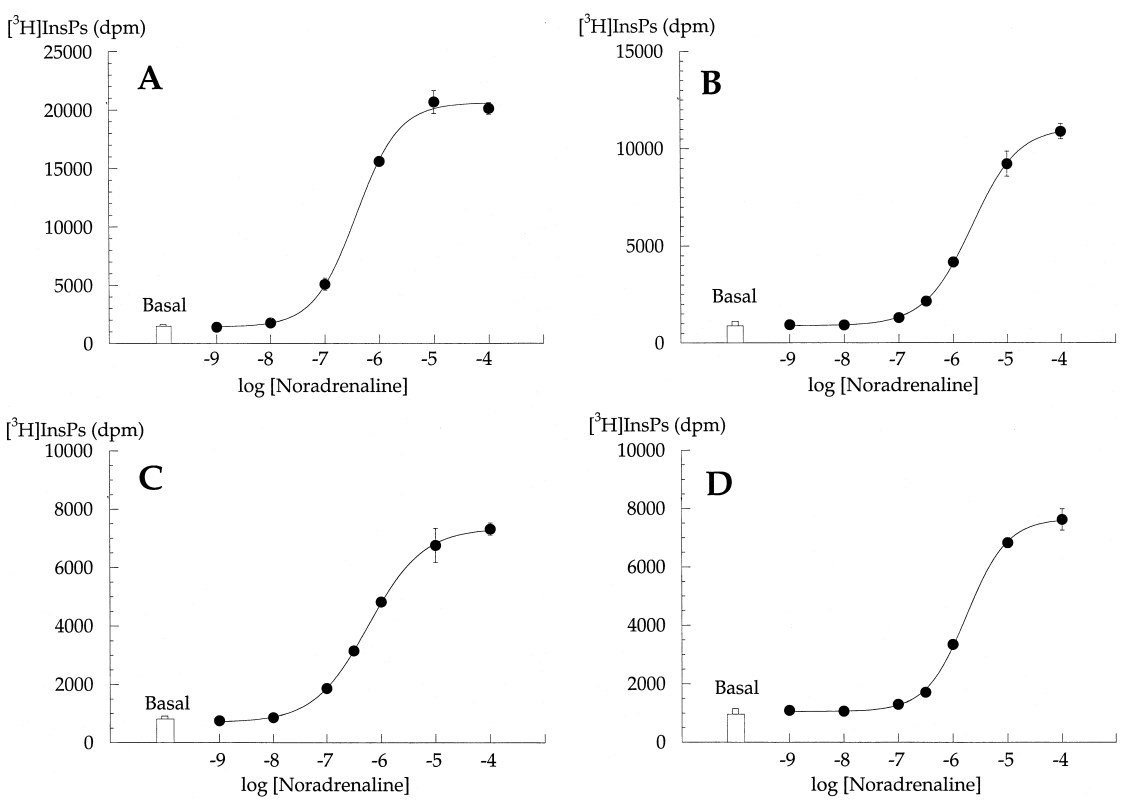

Fig. 1. Noradrenaline-stimulated [^{3}H]InsPs accumulation in CHO-K1 cells expressing human cloned (A) $\alpha_{1A\text{-}1}$-, (B) $\alpha_{1A\text{-}2}$-, (C) $\alpha_{1A\text{-}3}$-, and (D) $\alpha_{1A\text{-}4}$-adrenoceptor isoforms. Data shown are from single experiments, which were performed in triplicate. Within-experiment means and S.E.M. are shown through which non-linear regressions were constructed. Each experiment was repeated, with number of experiments shown in Table 1 (n = 4–6). Basal levels of [^{3}H]InsPs accumulation were similar in the four cell populations.

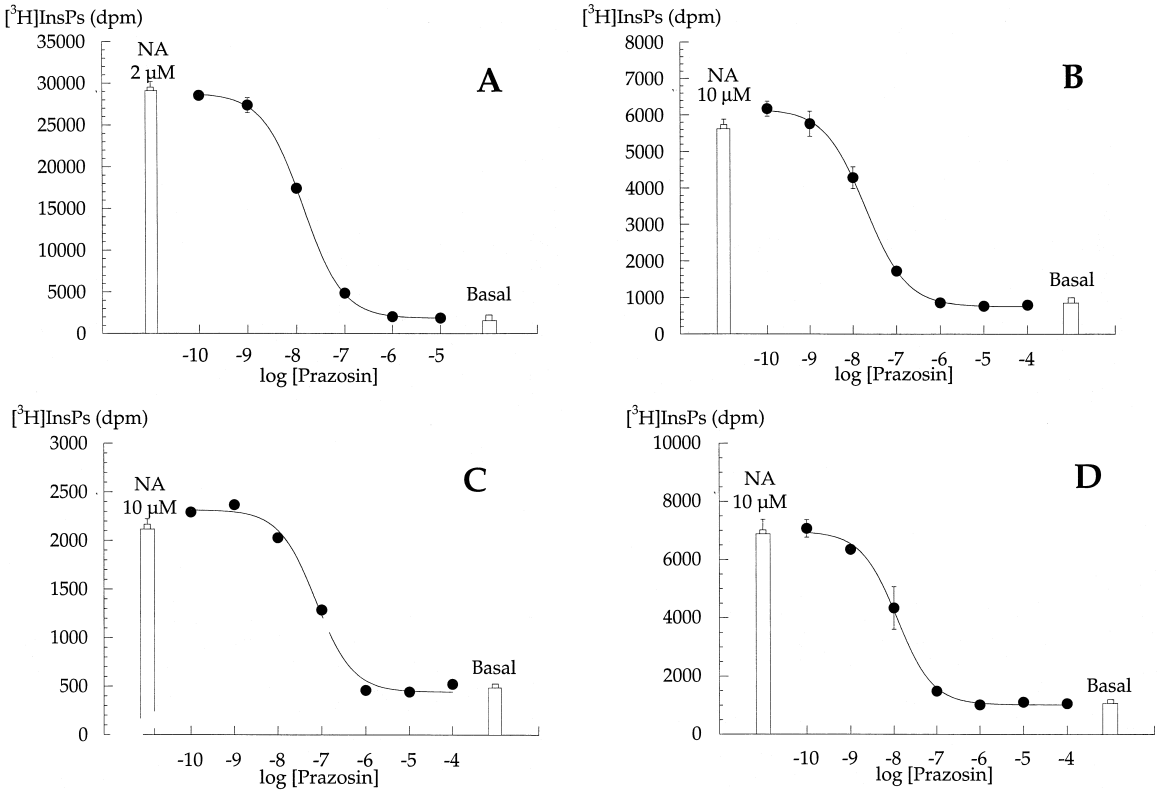

Fig. 2. Inhibition curves for prazosin vs. noradrenaline-stimulated [^{3}H]InsPs accumulation in CHO-K1 cells expressing human cloned (A) $\alpha_{1A\text{-}1}$-, (B) $\alpha_{1A\text{-}2}$-, (C) $\alpha_{1A\text{-}3}$-, and (D) $\alpha_{1A\text{-}4}$-adrenoceptor isoforms. Responses were obtained to a single concentration (2–10 μM) of noradrenaline (NA) alone or after equilibration with increasing concentrations of antagonist (20-min equilibration, 10-min agonist stimulation). Data shown are from representative single experiments performed in triplicate. Within-experiment means and standard deviations are shown through which non-linear regressions were constructed.

maximal stimulation above basal [^{3}H]InsPs levels of between 5.84 and 10.0 fold (Table 1 and Fig. 1).

Potencies for noradrenaline (as $p[A]_{50}$) are displayed in Table 1 for the four isoforms and human lower urinary tract (α_{1L}-adrenoceptor; Ford et al., 1996). The order of potency to noradrenaline for the isoforms is $\alpha_{1A\text{-}1}$-adrenoceptor $(6.63 \pm 0.05) > \alpha_{1A\text{-}3}$-adrenoceptor $(6.24 \pm 0.04) > \alpha_{1A\text{-}2}$-adrenoceptor $(6.01 \pm 0.13) > \alpha_{1A\text{-}4}$-adrenoceptor (5.72 ± 0.05). Fig. 2 shows the effect of prazosin on the generation of [^{3}H]InsPs in CHO-K1 cells expressing the cloned human α_{1A}-adrenoceptor isoforms. Concentration-dependent inhibitions of the response to noradrenaline (2 or 10 μM) were obtained without any effect upon basal levels. Thus, evidence for endogenous agonist stimulation

Table 2
Antagonist affinity estimates at α_{1A}-adrenoceptor isoforms from functional studies (noradrenaline-stimulated InsPs accumulation)

Receptor	$\alpha_{1A\text{-}1}$ (CHO-K1, InsPs)		$\alpha_{1A\text{-}2}$ (CHO-K1, InsPs)		$\alpha_{1A\text{-}3}$ (CHO-K1, InsPs)		$\alpha_{1A\text{-}4}$ (CHO-K1, InsPs)		α_{1L} (hLUT, FTB)[a]	$\alpha_{1A\text{-}1}$ (CHO-K1, Binding)
Antagonist	pK_b (sem)	n_H (sem)	pK_b (sem)	n_H (sem)	pK_b (sem)	n_H (sem)	pK_b (sem)	n_H (sem)	pA_2	pK_i
Prazosin	8.73 (0.02)	1.2 (0.1)	8.70 (0.11)	0.92 (0.08)	8.52 (0.12)	0.88 (0.09)	8.69 (0.08)	1.03 (0.10)	8.7	9.9
RS-17053	8.24 (0.06)	1.3 (0.1)	8.21 (0.21)	1.25 (0.23)	8.49 (0.03)	1.09 (0.05)	8.37 (0.06)	0.91 (0.11)	7.3	9.3
WB-4101	8.87 (0.13)	1.3 (0.1)	9.21 (0.08)	0.89 (0.06)	8.95 (0.11)	0.96 (0.06)	9.05 (0.08)	1.06 (0.09)	8.9	9.8
5-me-urapidil	8.17 (0.03)	1.2 (0.1)	8.20 (0.06)	0.89 (0.05)	8.05 (0.13)	0.93 (0.07)	8.26 (0.06)	0.85 (0.10)	8.2	9.2
Tamsulosin	10.53 (0.08)	1.0 (0.1)	10.70 (0.10)	0.76 (0.06)	10.53 (0.09)	0.70 (0.04)	11.15 (0.07)	0.70[b](0.07)	10.4	10.4
Indoramin	8.39 (0.04)	1.1 (0.2)	8.24 (0.09)	0.90 (0.07)	8.13 (0.14)	1.18 (0.04)	8.33 (0.05)	0.95 (0.12)	8.5	8.4
$\Sigma(y-x)^2$ vs. $\alpha_{1A\text{-}1}$	–		0.17		0.20		0.42		0.92	4.44

[a] FTB = Functional Tissue Bath Assay, [b] Hill slope (n_H) significantly ($P < 0.05$) different from agonist n_H.

or antagonist 'negative efficacy' was not apparent. In each case, the profile of inhibition was apparently monophasic, inhibiting the response to noradrenaline by 100%. Affinity estimates made from these studies are summarized in Table 2. In each individual experiment, an $E/[A]$ curve to noradrenaline was constructed to estimate a $p[A]_{50}$, which was then used with the Hill slope (n_H) and $p[B]_{50}$ to estimate antagonist affinity (pK_b). All computations were made using the modification of Leff and Dougall (1993) of the equation of Cheng and Prusoff (1973). In most cases, the value of n_H for the antagonist inhibition curve did not differ significantly from that for the agonist $E/[A]$ curve. Such a result would be expected for a competitive, reversible antagonist interacting under equilibrium conditions. Table 2 also contains pK_i values (membrane binding; Ford et al., 1997) for α_{1A-1}-AR and functional pA_2 values in human lower urinary tract tissues (Ford et al., 1996).

Figs. 3 and 4 illustrate and compare the relationships found between affinity estimate profiles displayed in Table 2 for the human α_{1A}-adrenoceptor isoforms. In each panel, the line of identity ($y = x$) is drawn (dotted line). In Fig. 3, equivalence of functional affinity profiles for human α_{1A-2}-, α_{1A-3}- and α_{1A-4}-adrenoceptor isoforms each vs. the α_{1A-1}-adrenoceptor isoform, respectively, is shown (with all points in each panel lying close to the line of identity), with low sum of squares of affinity differences ($\Sigma(y-x)^2 = 0.17$, 0.20 and 0.42, respectively). Comparison of affinity estimates from functional studies in the human α_{1A}-adrenoceptor isoforms with functional pA_2 values from hLUT data for the α_{1L}-adrenoceptor (Ford et al., 1997) are shown in Fig. 4 with sum of squares of affinity differences of 0.92, 1.08, 1.63 and 1.76 for α_{1A-1}, α_{1A-2}, α_{1A-3} and α_{1A-4}, respectively.

Receptor expression levels ($\alpha_{1A-1} = 10.4$ pmole/mg protein; $\alpha_{1A-3} = 2.49$; $\alpha_{1A-2} = 1.47$; $\alpha_{1A-4} = 1.20$) were determined with membrane homogenate radioligand saturation binding studies using [^{3}H]prazosin.

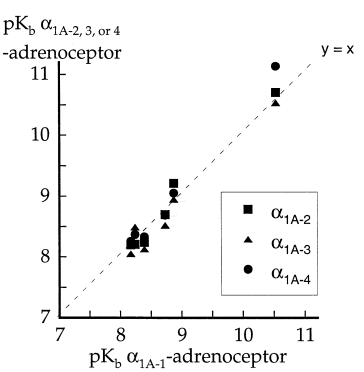

Fig. 3. Correlation plot showing relationship of affinity estimates from [^{3}H]InsPs accumulation studies for selected antagonists in CHO-K1 cells expressing human cloned α_{1A}-adrenoceptor isoforms: (■) α_{1A-1} vs. α_{1A-2}, (▲) α_{1A-1} vs. α_{1A-3}, (●) α_{1A-1} vs. α_{1A-4}. The dotted line indicates the line of identity ($y = x$). Data are from Table 2.

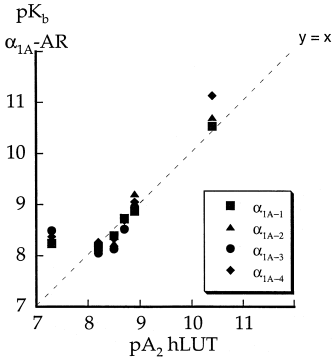

Fig. 4. Correlation plot showing relationship of affinity estimates from [^{3}H]InsPs accumulation studies for selected antagonists in CHO-K1 cells expressing human cloned α_{1A}-adrenoceptor isoforms compared to pA_2 estimates determined in functional tissue bath assays with human lower urinary tract (hLUT): (■) α_{1A-1} vs. hLUT, (▲) α_{1A-2} vs. hLUT, (●) α_{1A-3} vs. hLUT (♦) α_{1A-4} vs. hLUT. The dotted line indicates the line of identity ($y = x$). Data are from Table 2.

4. Discussion

The α_{1A}-adrenoceptor is the most thoroughly characterized of the α_1-adrenoceptor family. The original definition, based on high affinity for WB 4101, 5-methylurapidil and *S*-niguldipine has been strengthened considerably by the addition of further α_{1A}-adrenoceptor-selective antagonists, from diverse chemical series, such as SNAP 5089 (Wetzel et al., 1995), Rec 15/2739 (SB 216469; Testa et al., 1995), KMD 3213 (Shibata et al., 1995), and RS 17053 (Ford et al., 1996). The high, subnanomolar affinity displayed by all of these antagonists, along with subnanomolar affinities of prazosin and tamsulosin, and relatively high affinity of indoramin, provides a pharmacological fingerprint that is unique for the 'classical' α_{1A}-adrenoceptor (Hieble et al., 1995).

In contrast, the definition of the α_{1L}-adrenoceptor has been less rigorously documented, although some recent reports have established characteristics in addition to the low affinity for prazosin (Holck et al., 1983; Flavahan and Vanhoutte, 1986), including relatively low affinities (compared with α_{1A}-adrenoceptor) for WB 4101, 5-methylurapidil (Muramatsu, 1992), RS-17053 and the dihydropyridines, *S*-niguldipine and SNAP 5089 (Ford et al., 1996). Important as these α_{1A}-/α_{1L}-adrenoceptor discriminating antagonists are, equally critical to the definition of the α_{1L}-adrenoceptor are the non-discriminating antagonists, tamsulosin, Rec 15/2739 and indoramin. The latter three antagonists offer internal validation that an assay system is not merely underestimating affinities of antagonists as a result of methodological inadequacies. It is only with the use of this broad range of high affinity probes that a clear

characterization of the α_{1L}-adrenoceptor, and its separation from other subtypes, can be achieved.

Noradrenaline stimulates InsPs production in all four identified human cloned α_{1A}-adrenoceptor isoforms when examined in the CHO-K1 expression system, indicative of coupling through a similar G-protein, Gq. The differences found in noradrenaline potency (i.e., $\alpha_{1A\text{-}1}$-adrenoceptor > $\alpha_{1A\text{-}3}$-adrenoceptor > $\alpha_{1A\text{-}2}$-adrenoceptor > $\alpha_{1A\text{-}4}$-adrenoceptor) are consistent with the differences in receptor expression levels ($\alpha_{1A\text{-}1} = 10.4$ pmole/mg protein; $\alpha_{1A\text{-}3} = 2.49$; $\alpha_{1A\text{-}2} = 1.47$; $\alpha_{1A\text{-}4} = 1.20$) of the four clones. Profiles of antagonist inhibition of noradrenaline-stimulated InsPs production indicate a very high degree of pharmacological similarity between the human cloned α_{1A}-adrenoceptor isoforms. There is evidence that these antagonist inhibitions are under equilibrium conditions and are competitive, with pK_b values comparing well with estimates from Schild regression (Ford et al., 1997). Although prazosin, RS-17053, WB 4101 and 5-methylurapidil failed to display the subnanomolar affinities characteristic of the 'classical α_{1A}-adrenoceptor', high affinity was still displayed by tamsulosin and indoramin (relative to its known α_{1A}-adrenoceptor affinity). However, pK_b estimates for tamsulosin may be considered 'less robust' due to low Hill slopes. These antagonist affinity profiles were consistent with those from functional studies of lower urinary tract of man (α_{1L}-adrenoceptor) and differed distinctly from that obtained in membrane homogenate binding ($\alpha_{1A\text{-}1}$-adrenoceptor). The greater $\Sigma(y-x)^2$ values illustrate clearly the lack of pharmacological identity between the α_{1L}- and α_{1A}-adrenoceptor. The apparent divergence of the values obtained for RS-17053 in hLUT and the α_{1A}-adrenoceptor isoforms is most likely due to the physicochemical characteristics of the compound. RS-17053, while being selective for the α_{1A}-adrenoceptor, also possesses a high degree of 'stickiness'. We have optimized the conditions in the functional clone experiments to limit the loss of compound to the glass or plastic. However, in the previously published hLUT tissue bath experiments, the large surface area of both the tissue and the glass tissue bath, may have contributed to an underestimate of the compound's affinity. More recent experiments in our lab with the rabbit bladder neck, also an α_{1L}-adrenoceptor preparation, using smaller tissue pieces and optimized compound dilution conditions, resulted in a pA_2 of 7.8 (unpublished results). This value is more closely aligned with the pK_b values, 8.21–8.49 obtained in the clones where tissue size and glass surface area are much less than the tissue bath experiments.

Given the sequence identity of these four isoforms up to transmembrane domain 7, the lack of pharmacological diversity between these isoforms is consistent with studies directed at examining sites in receptor protein sequence responsible for ligand recognition. The next question which remains to be addressed is the extent to which C-terminal tail differences between these four isoforms confer altered phosphorylation/desensitization properties upon sustained agonist exposure.

Despite the existence of four distinct isoforms of the human α_{1A}-adrenoceptor, all known isoforms display apparently identical pharmacological properties on functional analysis. This pharmacology most closely correlates with that of the α_{1L}-adrenoceptor as displayed by both the hLUT and rabbit bladder neck. Thus, no individual isoform represents the distinct pharmacological phenotype of α_{1A}- or α_{1L}-adrenoceptor. Factors which determine the pharmacological pleiotropism of the α_{1A}-adrenoceptor gene product are currently being investigated.

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