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Pharmacological Characteristics of α_2-Adrenergic Receptors: Comparison of Pharmacologically Defined Subtypes with Subtypes Identified by Molecular Cloning

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SUMMARY

On the basis of extensive radioligand data and more limited functional data, three pharmacological subtypes of α_2-adrenergic receptors have been identified. More recently, three human genes or cDNAs for α_2-adrenergic receptors have been identified by molecular cloning. The relationship, however, among the pharmacologically defined subtypes and those identified by molecular cloning has not been clear. In order to resolve this issue, we have compared the pharmacological characteristics of the receptors identified by molecular cloning and expressed in COS-7 cells with the characteristics of the pharmacologically defined receptors in their respective prototypic tissue or cell line. The affinities (K_i values) of 12 subtype-selective α_2-adrenergic antagonists were determined for the α_2 receptor in the six preparations, by radioligand binding. Correlation analyses of the pK_i values indicate that the α_{2A} subtype, as defined in the HT29 cell line, the α_{2B} receptor of the neonatal rat lung, and the α_{2C} subtype, as defined in an opossum kidney cell line, correspond to the cloned human α_2-C10, α_2-C2, and α_2-C4 receptor subtypes, respectively.

Three major types or classes of adrenergic receptors have been identified, as α_1-adrenergic, α_2-adrenergic, and β-adrenergic (1). Each of these three major types can be divided into three or more subtypes (1-4). Among the early indications of α_2-adrenergic receptor heterogeneity were radioligand binding studies showing a 40-100-fold difference in the affinities of drugs, such as prazosin and oxymetazoline, for the human platelet α_2-adrenergic receptor, compared with receptors from neonatal rat lung (5) and rat brain (6). Additional studies resulted in the identification of the α_2-adrenergic receptors in the human platelet and neonatal rat lung as being of the α_{2A} and α_{2B} subtypes, respectively (7). Cell lines were identified that contained only one of the α_2-adrenergic receptor subtypes, namely, the HT29 cell line for the α_{2A} and the NG108 cell line for the α_{2B} (8). The validity of this definition, which was based on radioligand binding studies, was supported by functional studies demonstrating the attenuation of cAMP production in these cell lines (9). In addition to these pharmacological differences, biochemical differences were also noted, with the observation that, in contrast to the α_{2A} receptor, the α_{2B} receptor of the neonatal rat lung (10) and the NG108 cell line (11) was not glycosylated. The characteristics of the α_2-adrenergic receptor in the OK cell line indicated a profile that was intermediate between the α_{2A} and α_{2B} profiles (12). Because the characteristics of this receptor appeared to be closer to those of the α_{2B} than the α_{2A} receptor, it was tentatively classified as an α_{2B}-like receptor. Subsequent work, however, led to the conclusion that this was yet a third subtype, which was called the α_{2C}-adrenergic receptor (1, 13).

The gene coding for the α_2-adrenergic receptor of the human platelet has been cloned, sequenced, and expressed (14). As expected, the pharmacological characteristics of the expressed receptor indicated that it was of the α_{2A} subtype. Somatic cell hybrid analysis revealed that this gene resided on human chromosome 10 and was, therefore, designated α_2-C10. Southern blot analyses of human genomic DNA suggested the possible existence of two additional α_2-adrenergic receptors, residing on chromosomes 4 and 2 (14). Subsequently, these additional but related genes were isolated. The expression of these genes showed that they encoded distinct α_2-adrenergic receptor subtypes, which were designated α_2-C4 and α_2-C2 (15-17). The

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ABBREVIATIONS: OK, opossum kidney; RNG, rat nonglycosylated.

homologous gene/cDNA for the human α_2-C10 receptor has been cloned in the pig (18) and in the rat (19, 20). Similarly, the homologous rat genes/cDNA for the human α_2-C2 (21) and the α_2-C4 (19, 22, 23) have also been cloned.

The relationship among the pharmacologically defined α_2-adrenergic receptor subtypes and those identified by molecular cloning has not been firmly established, except for the identification of α_2-C10 as α_{2A}. The lack of a clear correlation between the molecular and pharmacological classifications has led to some confusion in the literature. For example, the human α_2-C4 and its rat homologues have been classified as α_{2B}, α_{2C}, and non-α_{2A}, -α_{2B}, or -α_{2C} receptors. Thus, the goal of the present study was to compare the pharmacological characteristics (by radioligand binding) of the pharmacologically defined α_2-adrenergic receptor subtypes (as identified in the prototypic tissues of the HT29 cell line, the neonatal rat lung, and the OK cell line) with those of the α_2-adrenergic receptor subtypes identified by molecular cloning and expressed in COS-7 cells.

Materials and Methods

Drugs and chemicals. [^{3}H]Rauwolscine (76–85 Ci/mmol) was obtained from Du Pont NEN (Boston, MA) and stored at −20°. Yohimbine and chlorpromazine were obtained from Sigma (St. Louis, MO), WB 4101 and spiroxatrine from Research Biochemicals Inc. (Wayland, MA), and rauwolscine from Accurate Chemical (Westbury, NY). The following drugs were kindly donated by the indicated companies: prazosin, Pfizer Chemical Division (New York, NY); oxymetazoline, Schering (Kenilworth, NJ); phentolamine, CIBA-Geigy (Summit, NJ); raubasine and aukammigine, Pierre Fabre Labs (Castres, France); ARC-239, Thomae GmbH (Biberach, West Germany); and BAM 1303, Maruko (Nagoya, Japan). Yohimbine and prazosin were prepared as 1 mM stock solutions in ethanol and were then diluted with 5 mM HCl. All other drugs were prepared and diluted in 5 mM HCl.

Radioligand binding assays. The preparation of membranes for the radioligand binding assays, from HT29 human colonic adenocarcinoma cells, from lungs from 1-day old rats (Sprague Dawley, of either sex), and from the OK cell line, was as previously described (8, 12). The cloned human α_2-C10-, α_2-C2-, and α_2-C4-adrenergic receptor-encoding cDNAs were subcloned into the mammalian expression vector pBC12BI, as previously described (15, 16). These constructs were transfected into COS-7 cells by the DEAE-dextran method (24). Membranes from these cells were prepared and shipped frozen, on dry ice, from Duke University to the University of Nebraska Medical Center.

Competition and saturation binding experiments were performed as described previously (8, 12). For competition experiments, membrane pellets were thawed and resuspended in 25 mM glycylglycine buffer, pH 7.6. Ten microliters of unlabeled competing drug, 970 μl of membrane suspension, and 20 μl of [^{3}H]rauwolscine (at a concentration near its K_d for that tissue) were added to duplicate tubes. After a 30-min incubation at 23°, the suspension was filtered rapidly through GF/B glass fiber filter strips (Whatman, Clifton, NJ), using a 48-sample manifold (Brandel cell harvester; Biomedical Research and Development, Gaithersburg, MD). Tubes and filters were washed twice with 50 mM Tris·HCl (pH 8.0) at 4°, and radioactivity retained on the filters was determined by liquid scintillation counting. Inhibition experiments, routinely consisting of 11 concentrations of the unlabeled ligand, were analyzed using nonlinear least-squares curve-fitting programs (GraphPAD Software, San Diego, CA), to obtain IC_{50} values. IC_{50} values were converted to K_i values (25) and are presented as geometric means. Saturation experiments used six concentrations of [^{3}H]rauwolscine (final concentration ranged from approximately 20 pM to 2.5 nM). Nonspecific binding was determined using 100 μM (−)-norepinephrine. K_d and B_{max} values were calculated by nonlinear regression of the data and are given as geometric and arithmetic means, respectively.

Results

As shown in Table 1, the density of cloned receptors expressed in COS-7 cells was significantly higher than the density of receptors in the prototypic tissues. The protein concentration in the assay tubes was adjusted so that approximately equivalent amounts of receptor were used in the various assays.

The affinities of [^{3}H]rauwolscine for the HT29 cell and COS-C10 receptors were similar (0.4 nM). The affinity for the rat lung and COS-C2 receptors were slightly lower (0.6 nM), whereas the affinity of [^{3}H]rauwolscine for the OK cell and the COS-C4 receptors was higher (0.2 nM; Table 1). The K_d values for the three prototypic tissues were similar to those previously reported (8, 13). The K_d values for [^{3}H]rauwolscine in the COS-C10, COS-C4, and COS-C2 cells were 3–7-fold lower (higher affinity) than previously reported, presumably due to the use of glycylglycine buffer in the current studies, as opposed to Tris magnesium buffer in the previous studies (15, 16).

In order to compare the pharmacological characteristics of the human cloned receptors expressed in COS-7 cells with those of the pharmacologically defined subtypes, K_i values were determined, by radioligand competition studies, for 12 α_2-adrenergic antagonists that have been previously shown to have selectivity for the various subtypes (Table 2). The K_i for rauwolscine in inhibiting [^{3}H]rauwolscine binding was lowest (highest affinity) in the OK cell and the COS-C4 membrane preparations. For all six tissues, the K_i values agreed well with the K_d calculated from saturation analyses. BAM 1303 had lower affinity for the rat lung and COS-C2 receptors than for the other four preparations. ARC-239 had low affinity for the HT29 cell and the COS-C10 membranes, whereas it had much higher affinity for the other four preparations. Oxymetazoline was the best single drug for differentiating the three subtypes. It had highest affinity for the HT29 cell and COS-C10, approximately 10–20-fold lower affinity for the OK cell and COS-C4, and another 10–20-fold lower affinity for the rat lung and COS-C2 receptors.

The ratio of the K_i values for two drugs is often a sensitive indicator of pharmacological differences among receptor subtypes. The K_i ratio of prazosin to oxymetazoline has been previously shown to differentiate the α_{2A} and α_{2B} subtypes. As shown in Table 3, this ratio was 3000–5000-fold greater in the HT29 and COS-C10 cells, compared with the rat lung and COS-C2 cells. The K_i ratio of oxymetazoline to spiroxatrine was 6000-fold greater in the rat lung and COS-C2 cells, compared with HT29 and COS-C10 cells. This ratio also differentiated HT29 and COS-C10 cells from OK and COS-C4 cells

TABLE 1

Parameters from membrane saturation binding experiments with prototypic tissues and cloned receptors expressed in COS-7 cells

The affinity (K_D) and receptor density (B_{max}) for [^{3}H]rauwolscine binding are given as means ± standard errors for n experiments.

Receptor preparation	K_d	B_{max}	n
	nM	fmol/mg of protein	
HT29 cell	0.46 ± 0.03	71 ± 5	3
COS-C10	0.40 ± 0.02	3860 ± 290	9
Rat lung	0.58 ± 0.04	76 ± 12	4
COS-C2	0.71 ± 0.05	2190 ± 710[a]	5
OK cell	0.18 ± 0.02	66 ± 7	5
COS-C4	0.15 ± 0.01	630 ± 180	6

[a] The high standard error results from several different preparations that had various levels of receptor expression.

TABLE 2

Affinities of adrenergic antagonists for α_2-adrenergic receptors from various sources

The affinity (K_i) (mean ± standard error) and the pseudo-Hill coefficient (slope) are given for n determinations. In cases where only the K_i value is given, the value is from Blaxall *et al.* (13).

Antagonist	HT29 cell			COS-C10			Rat lung			COS-C2			OK cell			COS-C4		
	K_i	Slope	n	K_i	Slope	n	K_i	Slope	n	K_i	Slope	n	K_i	Slope	n	K_i	Slope	n
	nM			nM			nM			nM			nM			nM		
1 Rauwolscine	0.44			0.32 ± 0.02	1.07	5	0.45			0.37 ± 0.05	1.14	3	0.12 ± 0.03	0.99	3	0.13 ± 0.01	1.02	2
2 Yohimbine	0.65			0.50 ± 0.02	1.38	4	1.00			1.18 ± 0.06	1.00	3	0.19			0.30 ± 0.02	0.98	2
3 WB 4101	1.27 ± 0.03	1.23	2	0.91 ± 0.03	1.01	3	7.89 ± 0.33	1.00	2	4.34 ± 0.32	1.00	3	0.75 ± 0.06	0.98	2	0.40 ± 0.04	1.06	3
4 BAM 1303	1.34 ± 0.08	1.03	5	0.39 ± 0.03	1.16	8	6.94 ± 0.36	1.04	4	5.28 ± 0.66	1.10	5	0.38			0.39 ± 0.05	0.91	2
5 Oxymetazoline	1.45 ± 0.08	1.00	2	3.3 ± 0.3	0.98	4	308 ± 49	0.70	2	754 ± 23	1.06	4	31			31 ± 1	0.98	2
6 Phentolamine	4.22 ± 0.64	0.91	2	4.1 ± 0.3	1.07	3	5.2 ± 0.8	0.91	2	6.8 ± 0.9	0.97	3	29 ± 14	1.19	2	12.7 ± 0.6	1.00	3
7 Spiroxatrine	8.3 ± 1.6	0.94	2	5.5 ± 0.8	1.21	4	0.29 ± 0.02	1.08	2	0.19 ± 0.02	1.38	4	0.26 ± 0.02	1.10	2	0.13 ± 0.01	1.11	4
8 Raubasine	8.9			6.4 ± 0.3	1.10	2	31.2 ± 5.3	0.95	2	14.5 ± 0.4	0.91	3	1.71 ± 0.2	1.01	3	5.0 ± 0.7	1.16	3
9 Aukammigine	104 ± 10	1.14	2	71 ± 7	0.91	3	159 ± 19	0.93	2	116 ± 6	0.99	3	20 ± 5	1.07	2	28 ± 3	0.99	3
10 ARC-239	171			131 ± 16	0.98	4	1.60			2.67 ± 0.01	0.90	2	13			3.82 ± 0.45	0.75	2
11 Chlorpromazine	253 ± 22	1.05	3	78 ± 10	1.08	4	25 ± 2	1.13	3	4.80 ± 0.94	1.09	4	238 ± 25	1.03	4	41 ± 7	1.02	4
12 Prazosin	316			302 ± 18	1.14	3	11.6 ± 0.5	1.01	3	30.6 ± 3.2	1.06	6	15 ± 83			10.7 ± 0.4	1.17	2

TABLE 3

Comparison of K_i value ratios for α_2-adrenergic receptor subtypes

Data are derived from Table 2.

Receptor preparation	K_i ratios			
	Prazosin/oxymetazoline	Oxymetazoline/spiroxatrine	Chlorpromazine/BAM 1303	Chlorpromazine/WB 4101
HT29 cell	218	0.18	188	200
COS-C10	91	0.60	202	85
Rat lung	0.04	1060	3.53	3.11
COS-C2	0.04	4060	0.91	1.11
OK cell	0.48	119	627	319
COS-C4	0.35	237	107	103

moderately well (500-fold). The K_i ratios of chlorpromazine to BAM 1303 and chlorpromazine to WB 4101 were the best at differentiating rat lung and COS-C2 cells from OK and COS-C4 cells (100–200-fold).

In order to compare the affinities of these 12 drugs at the various receptors in a more systematic manner, correlation analyses were performed by plotting the negative logarithm of the K_i values (pK_i values) of the various tissue preparations against each other (Fig. 1). The correlation coefficients and slopes of the correlation lines are given in Table 4. Table 4 includes the nine correlations represented in Fig. 1 plus an additional six correlations. The correlations between HT29 cell and COS-C10, rat lung and COS-C2, and OK cell and COS-C4 receptors were all in the range of 0.95–0.98, indicating that the 12 drugs have very similar K_i values in each pair of preparations. These results support the identification of the COS-C10 as the α_{2A}, the COS-C2 as the α_{2B}, and the COS-C4 as the α_{2C} receptor.

All of the other correlations were much poorer. Rat lung and HT29 cells had a very low correlation ($r = 0.27$), whereas the correlations of OK cells with both HT29 cells and rat lung were higher ($r = 0.70$). Similarly, COS-C2 correlated poorly with COS-C10 ($r = 0.35$), whereas COS-C4 correlated better with both COS-C10 and COS-C2 ($r = 0.7$–0.8). The slopes of the correlation lines for both HT29/COS-C10 and rat lung/COS-C2 were close to unity (0.96 and 0.99, respectively), whereas the slope for OK cells and COS-C4 was lower (0.85).

Discussion

The major conclusion of this study is that the cloned human α_2-adrenergic receptor subtypes designated as α_2-C10, α_2-C2, and α_2-C4 have pharmacological characteristics identical or very similar to those of the pharmacologically defined α_{2A}, α_{2B}, and α_{2C} receptor subtypes, respectively. This conclusion is based on 12 adrenergic antagonists that have been shown previously to be subtype selective.

The extent to which the pharmacological characteristics of receptors are conserved among species is an important factor in receptor classification and the definition of subtypes. The correlation of the affinities of the rat lung receptor (α_{2B}) with those of the human COS-C2 receptor (α_{2B}) gives a high correlation coefficient (0.95), and the slope of the correlation line is close to unity (0.99). The correlation of the affinities of the OK cell receptor (α_{2C}) with those of the human COS-4 receptor (α_{2C}) also gives a high correlation coefficient (0.95); however, the slope of the correlation line (0.85) is significantly less than unity. This suggests that it may be more likely that drugs will be found that differentiate these two α_{2C} receptors than the two α_{2B} receptors. Pharmacological differences among species homologues are not unexpected, because amino acid substitutions may slightly alter the ligand binding site.

One might expect receptor subtypes that have very similar amino acid sequences also to have very similar pharmacological characteristics. The cloned rat homologue of the human α_2-C2 is the α_2-RNG receptor (21). When corresponding amino acid residues of the deduced primary sequences are compared, these two receptors have an 82% overall amino acid sequence identity. They have an even greater amino acid sequence identity, of 98%, in the seven membrane-spanning domains, which are the regions of the receptor protein thought to be most important in determining the specificity of the ligand/receptor interaction. Among adrenergic receptors, amino acid identities range from 36 to 73%. Among members of the same subtype group (α_2-C10, α_2-C2, and α_2-C4) the transmembrane domain identity is usually 70–80%, whereas among different subtype groups (α_1 versus α_2, α_2 versus β, etc.) the transmembrane domain identities are characteristically lower (30–40%). As expected from the highly conserved primary sequences of human α_2-C2 and rat α_2-RNG, the ligand-binding characteristics are nearly identical. Although these two receptors have not been directly compared in the same ligand binding studies, they both have pK_i values for various antagonists that are highly correlated with those determined for the rat neonatal lung α_{2B}-adrenergic receptor (0.95 and 0.97, respectively). Thus, on the basis of the

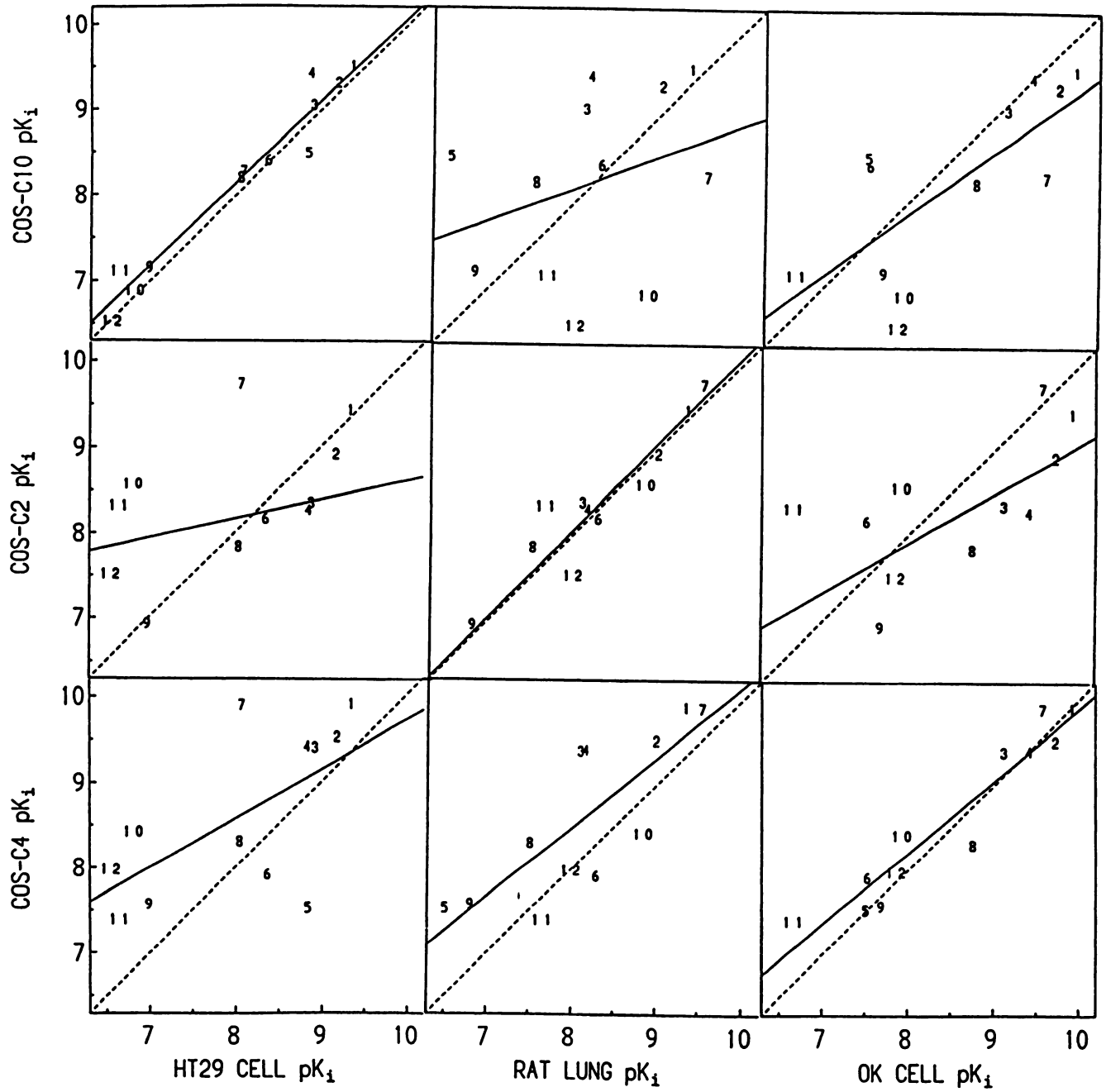

Fig. 1. Correlation plots of pK$_i$ values for α_2-adrenergic receptors from various sources. The drugs are numbered as in Table 2. —, Correlation line; – – –, line of identity. Correlation coefficients and slopes are summarized in Table 3.

highly conserved primary structures and the nearly identical ligand-binding characteristics, the human α_2-C2 and the rat α_2-RNG are clearly species homologues.

An example of species homologues with very conserved primary sequences but demonstrated differences in ligand binding can be found with the presumed rat homologue of the α_2-C10 adrenergic receptor, which has been designated as RG20 (19). RG20 has an 89% overall amino acid sequence identity, and a 98% amino acid sequence identity in the transmembrane domains, to the human α_2-C10 receptor. Therefore, based on the highly conversed primary sequences, these two receptors are structural species homologues. Although RG20 has a ligand-binding profile similar to that of α_2-C10, there are at least four compounds that can discriminate the two receptors, rauwolscine, yohimbine, BAM 1303, and SKF 104078. This distinct pharmacology has led to the classification of RG20 as a pharmacological α_{2D} receptor subtype (19, 26–28).

The affinities of the 12 selective drugs for the α_{2C} receptor (OK cell or COS-C4) correlate better with those for either the α_{2A} (HT29 cell or COS-C10) or α_{2B} (rat lung or COS-C2) receptors than the affinities of the α_{2A} and α_{2B} receptors correlate with each other. This could possibly be an artifact of the drugs used, because many of these drugs were selected on the basis of their selectivity for the α_{2A} versus the α_{2B} receptor. If this were the case, then it should be possible, using a different set of drugs, to find that the correlation between α_{2A} and α_{2B} is better than that between α_{2A} and α_{2C}, for example. Alternatively it is possible that, once the nature of the binding site is known,

TABLE 4

Correlation of pK_i values for pharmacologically defined subtypes with those for cloned human subtypes of α_2-adrenergic receptors

The correlation coefficient (r) and the slope of the correlation line are given.

	HT29 cell	Rat lung	OK cell	COS-C10	COS-C2	COS-C4
HT29 cell		0.24[a]	0.71[a]	0.96[a]	0.23[a]	0.57[a]
Rat lung	0.27[b]		0.76[a]	0.37[a]	0.99[a]	0.82[a]
OK cell	0.70[b]	0.66[b]		0.72[a]	0.58[a]	0.85[a]
COS-C10	0.98[b]	0.33[b]	0.74[b]		0.33[a]	0.64[a]
COS-C2	0.24[b]	0.95[b]	0.63[b]	0.35[b]		0.75[a]
COS-C4	0.63[b]	0.80[b]	0.95[b]	0.69[b]	0.78[b]	

[a] Slope value.
[b] Correlation coefficient.

the α_{2B} and α_{2C} subtypes will turn out to have structurally more similar binding sites, compared with the α_{2A}.

The original identification of prazosin as a drug that differentiates the α_{2A} and α_{2B} receptor subtypes (7) has led to the concept of the α_{2B} receptor being prazosin sensitive and the α_{2A} receptor being prazosin insensitive. Because the α_{2C} receptor also has a high affinity for prazosin, this has led some authors to consider α_{2C} to be α_{2B} or α_{2B}-like. The inappropriateness of using a single drug to define a receptor subtype is emphasized by the results of this paper.

In conclusion, radioligand binding studies clearly assign each of the cloned human α_2-adrenergic receptors to a pharmacologically defined subtype present in various prototypic tissues or cell lines. The availability of genes/cDNA encoding three distinct human α_2-adrenergic receptors has led to a more extensive characterization of these physiologically important mediators. Now that there is a clear correlation between the molecular and pharmacological classifications, there is hope that, as the information gained from the study of these receptors in transfected cell lines, nontransfected cell lines, and animal tissues is combined, more selective and specific therapeutic agents may be developed.

References

1. Bylund, D. B. Subtypes of α_2-adrenoceptors: pharmacological and molecular biological evidence converge. *Trends Pharmacol. Sci.* **9**:356–361 (1988).
2. Lomasney, J. W., S. Cotecchia, R. J. Lefkowitz, and M. G. Caron. Molecular biology of α-adrenergic receptors: implications for receptor classification and for structure-function relationships. *Biochim. Biophys. Acta* **1095**:127–139 (1991).
3. Harrison, J. K., W. R. Pearson, and K. R. Lynch. Molecular characterization of α_1- and α_2-adrenoceptors. *Trends Pharmacol. Sci.* **12**:62–67 (1991).
4. Bylund, D. B. Subtypes of *alpha*-1 and *alpha*-2 adrenergic receptors. *FASEB J.* **6**:832–839 (1992).
5. Latifpour, J., S. B. Jones, and D. B. Bylund. Characterization of [^{3}H] yohimbine binding to putative *alpha*-2 adrenergic receptors in neonatal rat lung. *J. Pharmacol. Exp. Ther.* **223**:606–611 (1982).
6. Cheung, Y., D. B. Barnett, and S. R. Nahorski. [^{3}H]Rauwolscine and [^{3}H] yohimbine binding to rat cerebral cortex and human platelet membranes: possible heterogeneity of *alpha*-2 adrenoceptors. *Eur. J. Pharmacol.* **84**:79–85 (1982).
7. Bylund, D. B. Heterogeneity of *alpha*-2 adrenergic receptors. *Pharmacol. Biochem. Behav.* **22**:835–843 (1985).
8. Bylund, D. B., C. Ray-Prenger, and T. J. Murphy. *Alpha*-2A and *alpha*-2B adrenergic receptor subtypes: antagonist binding in tissues and cell lines containing only one subtype. *J. Pharmacol. Exp. Ther.* **245**:600–607 (1988).
9. Bylund, D. B., and C. Ray-Prenger. *Alpha*-2A and *alpha*-2B adrenergic receptor subtypes: attenuation of cyclic AMP production in cell lines containing only one receptor subtype. *J. Pharmacol. Exp. Ther.* **251**:640–644 (1989).
10. Lanier, S. M., C. J. Homcy, C. Patenaude, and R. M. Graham. Identification of structurally distinct α_2-adrenergic receptors. *J. Biol. Chem.* **263**:14491–14496 (1988).
11. Wilson, A. L., K. Seibert, S. Brandon, E. J. Cragoe, Jr., and L. E. Limbird. Monovalent cation and amiloride analog modulation of adrenergic ligand binding to the unglycosylated α_{2B}-adrenergic receptor subtype. *Mol. Pharmacol.* **39**:481–486 (1991).
12. Murphy, T. J., and D. B. Bylund. Characterization of *alpha*-2 adrenergic receptors in the OK cell, an opossum kidney cell line. *J. Pharmacol. Exp. Ther.* **244**:571–578 (1988).
13. Blaxall, H. S., T. J. Murphy, J. C. Baker, C. Ray, and D. B. Bylund. Characterization of the *alpha*-2C adrenergic receptor subtype in the opossum kidney and in the OK cell line. *J. Pharmacol. Exp. Ther.* **259**:323–329 (1991).
14. Kobilka, B. K., H. Matsui, T. S. Kobilka, T. L. Yang-Feng, U. Francke, M. G. Caron, R. J. Lefkowitz, and J. W. Regan. Cloning, sequencing, and expression of the gene coding for the human platelet α_2-adrenergic receptor. *Science (Washington D. C.)* **238**:650–656 (1987).
15. Regan, J. W., T. S. Kobilka, T. L. Yang-Feng, M. G. Caron, R. J. Lefkowitz, and B. K. Kobilka. Cloning and expression of a human kidney cDNA for an α_2-adrenergic receptor subtype. *Proc. Natl. Acad. Sci. USA* **85**:6301–6305 (1988).
16. Lomasney, J. W., W. Lorenz, L. F. Allen, K. King, J. W. Regan, T. L. Yang-Feng, M. G. Caron, and R. J. Lefkowitz. Expansion of the α_2-adrenergic receptor family: cloning and characterization of a human α_2-adrenergic receptor subtype, the gene for which is located on chromosome 2. *Proc. Natl. Acad. Sci. USA* **87**:5094–5098 (1990).
17. Weinshank, R. L., J. M. Zgombick, M. Macchi, N. Adham, H. Lichtblau, T. A. Branchek, and P. R. Hartig. Cloning, expression, and pharmacological characterization of a human α_{2B}-adrenergic receptor. *Mol. Pharmacol.* **38**:681–688 (1990).
18. Guyer, C. A., D. A. Horstman, A. L. Wilson, J. D. Clark, E. J. Cragoe, Jr., and L. E. Limbird. Cloning, sequencing, and expression of the gene encoding the porcine α_2-adrenergic receptor: allosteric modulation by Na^+, H^+, and amiloride analogs. *J. Biol. Chem.* **265**:17307–17317 (1990).
19. Lanier, S. M., S. Downing, E. Duzic, and C. J. Homcy. Isolation of rat genomic clones encoding subtypes of the α_2-adrenergic receptor: identification of a unique receptor subtype. *J. Biol. Chem.* **266**:10470–10478 (1991).
20. Chalberg, S. C., T. Duda, J. A. Rhine, and R. K. Sharma. Molecular cloning, sequencing and expression of an α_2-adrenergic receptor complementary DNA from rat brain. *Mol. Cell. Biochem.* **97**:161–172 (1990).
21. Zeng, D., J. K. Harrison, D. D. D'Angelo, C. M. Barber, A. L. Tucker, Z. Lu, and K. R. Lynch. Molecular characterization of a rat α_{2B}-adrenergic receptor. *Proc. Natl. Acad. Sci. USA* **87**:3102–3106 (1990).
22. Voigt, M. M., S. K. McCune, R. Y. Kanterman, and C. C. Felder. The rat α_2-C4 adrenergic receptor gene encodes a novel pharmacological subtype. *FEBS Lett.* **278**:45–50 (1991).
23. Flordellis, C. S., D. E. Handy, M. R. Bresnahan, V. I. Zannis, and H. Gavras. Cloning and expression of a rat brain α_{2B}-adrenergic receptor. *Proc. Natl. Acad. Sci. USA* **88**:1019–1023 (1991).
24. Cullen, B. R. Use of eukaryotic expression technology in the functional analysis of cloned genes. *Methods Enzymol.* **152**:684–704 (1987).
25. Cheng, Y., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzyme reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
26. Harrison, J. K., D. D. D'Angelo, D. Zeng, and K. R. Lynch. Pharmacological characterization of rat α_2-adrenergic receptors. *Mol. Pharmacol.* **40**:407–412 (1991).
27. Simonneaux, V., M. Ebadi, and D. B. Bylund. Identification and characterization of α_{2D}-adrenergic receptors in bovine pineal gland. *Mol. Pharmacol.* **40**:235–241 (1991).
28. Michel, A. D., D. N. Loury, and R. L. Whiting. Differences between the α_2-adrenoceptor in rat submaxillary gland and the α_{2A}- and α_{2B}-adrenergic receptor subtypes. *Br. J. Pharmacol.* **98**:890–897 (1989).

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