Quantitative Relationships between α-Adrenergic Activity and Binding Affinity of α-Adrenoceptor Agonists and Antagonists

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Quantitative relationships between in vitro affinity for α_1- and α_2-adrenoceptors (specific binding sites in rat brain membranes of [^{3}H]prazosin and [^{3}H]clonidine, respectively) and in vitro and in vivo α_1/α_2-adrenoceptor agonist/antagonist activities were derived for a series of 11 α-adrenergic antagonists and 35 agonists of dissimilar chemical structure. For the antagonists, the α_1/α_2-binding selectivity ratio most significantly correlated with the functional α_1/α_2-blocking selectivity ratios assessed in vitro (rabbit isolated pulmonary artery: antagonism of α_1-adrenoceptor-induced vasoconstriction and α_2-adrenoceptor-evoked facilitation of transmitter release) and in vivo (antagonism of α_1- and α_2-adrenoceptor-mediated vasoconstriction in pithed normotensive rats). These results show that the in vitro α_1- and α_2-adrenoceptor binding affinities of the antagonists provide adequate information concerning their functional α_1- and α_2-adrenoceptor blocking potencies against agonists. For the agonists, the central, α_2-adrenoceptor-elicited, hypotensive activity was not correlated with α_1-adrenoceptor binding affinity but was most significantly described in terms of affinity for α_2-adrenoceptors and a parabolic dependence on log P' (octanol/buffer; pH 7.4; 37 °C). The relevance of log P' in the regression is explained by the difference in accessibility to the membrane-bound α_2-adrenoceptors in the radioligand displacement experiments and the central medullary (hypotensive) α_2-adrenoceptors in the intact animal. In contrast, the affinity parameters for α_1- and α_2-adrenoceptors were found to be poor descriptors of the hypertensive potency of the agonists in which α_1- and α_2-adrenoceptors are known to play a role. The correlations in which the individual binding parameters and the combination of both variables were included reached only a moderate significance level. This could be ascribed to the failure to correlate α_1-adrenoceptor-induced hypertensive potency with α_1-adrenoceptor affinity. Our results indicate that only the agonistic effects at central medullary (hypotensive) and peripheral vascular α_2-adrenoceptors can be predicted by affinity, whereas this cannot be accomplished for (vascular) α_1-adrenoceptors. It is discussed whether large differences in the variations in efficacy (intrinsic activity) at α_1- and α_2-adrenoceptors within the series of agonists used in this study can account for the present findings.

Based on the pharmacological and functional dissimilarity among pre- and postsynaptically located α-adrenoceptors, the prefixes α_1 and α_2 have originally been proposed to denote the classical postsynaptic α-adrenoceptor on vascular smooth muscle and the neuronal (presynaptic) α-adrenoceptor on noradrenergic nerve terminals, respectively.[1] The presynaptic α-adrenoceptors differ from the more classical postsynaptic α-adrenoceptors with respect to the relative activity and affinity of agonists and antagonists, respectively.[2-9] However, the existence of α_2-adrenoceptors outside noradrenergic terminal axons, on some organelles lacking synapses and even at postsynaptic sites possessing the general characteristics of those identified presynaptically, made it necessary to apply the prefixed α_1 and α_2 exclusively on the basis of their specificity toward drugs, irrespective of their location or function.[10-15]

Hand in hand with the pharmacological approach, the direct identification of α-adrenoceptors with the aid of radioligand binding studies has also led to the hypothesis of a subdivision of α-adrenoceptors into distinct α_1- and α_2-receptor types.[16-19] Labeling of α-adrenoceptors has been performed in the central nervous system and in numerous peripheral tissues by means of a variety of radioligands of high specific radioactivity. At present, the α_1-adrenoceptor antagonists [^{3}H]WB 4101[20-23] and [^{3}H]-prazosin[23-25] are suitable ligands for labeling α_1-adrenoceptors. Recently, [^{125}I]BE 2254 has been introduced as a new high-affinity radioligand for α_1-adrenoceptors.[26,27] The α_2-adrenoceptor agonists [^{3}H]clonidine[20,21,28] and *p*-

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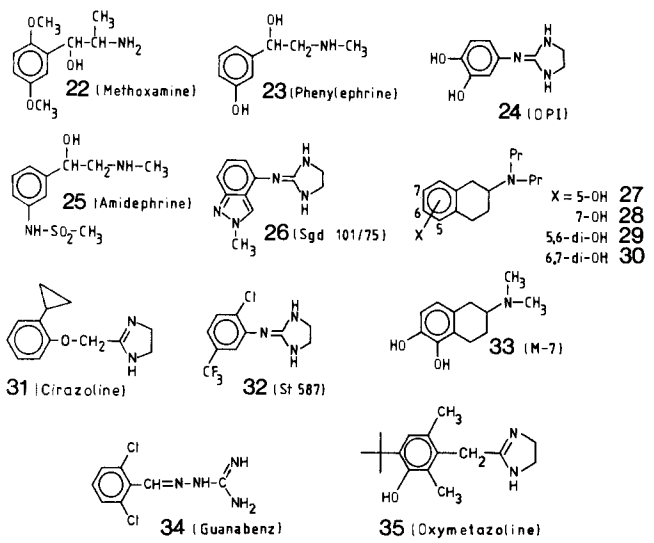
Chart I. Structural Formulas of the α-Adrenoceptor Agonists 22–35 Used in This Study



amino[^{3}H]clonidine,[29] as well as the α_2-adrenoceptor blocking drugs [^{3}H]yohimbine[30,31] and [^{3}H]rauwolscine, are currently being used for the characterization of α_2-adrenoceptor-like binding sites.

Both functional (pharmacological) studies and radioligand binding experiments therefore offer the possibility to identify and characterize α_1- and α_2-adrenoceptors.[9,11,15] In vitro receptor affinity, determined by the displacement of radioligands from their specific binding sites, is generally considered a parameter indicative for the selectivity and functional activity of drugs. Relationships between binding affinity and pharmacological activity have been reported for α-adrenoceptor agonists and antagonists. In general, the orders of affinity in vitro of α-adrenoceptor agonists and antagonists especially correspond qualitatively well with their orders of potency in functional tests in vitro[33–38] and in vivo,[39–41] although marked discrepancies have also been noticed in particular for some α-adrenoceptor agonists.[9–11,41] Furthermore, the hypotensive activity of clonidine-like derivatives and some related compounds has been correlated in a more or less quantitative manner with the binding affinity for [^{3}H]clonidine binding sites in brain tissue with various degrees of success.[42–46]

Table I. In Vivo and in Vitro Binding Affinities of Various α-Adrenoceptor Antagonists for α_1- and α_2-Adrenoceptors[a]

α-adrenoceptor antagonist	$pA_{2\ post}(\alpha_1)$[b] (in vivo)	$pA_{2\ post}(\alpha_2)$[c] (in vivo)	$\log [K_{B\ post}(\alpha_2)/K_{B\ post}(\alpha_1)]$[d] (in vivo)	$pA_{2\ post}(\alpha_1)$[e] (in vitro)	$pC_{30\ pre}(\alpha_2)$[f] (in vitro)	$\log [EC_{30\ pre}(\alpha_2)/K_{B\ post}(\alpha_1)]$[g] (in vitro)	$IC_{50}(\alpha_1)$[h]	$IC_{50}(\alpha_2)$[i]	$\log [IC_{50}(\alpha_2)/IC_{50}(\alpha_1)]$[j]
prazosin	8.33	5.91	2.42	8.70			5.8×10^{-10}	5.0×10^{-6}	3.94
phentolamine	6.95	6.47	0.48	7.49	7.07	0.43	3.9×10^{-8}	2.0×10^{-8}	−0.29
dihydroergotamine	6.68	7.25	−0.57	7.55	8.40	−0.85	2.7×10^{-8}	3.7×10^{-9}	−0.85
clozapine	6.29	4.69	1.60	7.43	6.05	1.38	1.25×10^{-7}	1.4×10^{-6}	1.06
corynanthine	6.25	5.03	1.22	6.60	5.00	1.60	2.2×10^{-7}	1.75×10^{-5}	1.90
azapetine	6.09	4.88	1.21	6.96	5.89	1.08	2.05×10^{-7}	1.3×10^{-6}	0.81
yohimbine	5.72	6.83	−1.11	6.40	8.10	−1.70	1.3×10^{-6}	1.65×10^{-7}	−0.89
piperoxane	5.71	6.23	−0.52	6.06	6.22	−0.16	7.2×10^{-7}	1.4×10^{-7}	−0.70
tolazoline	5.66	5.82	−0.16	5.41	6.15	−0.74	2.1×10^{-6}	2.8×10^{-7}	−0.89
mianserin	5.41	5.67	−0.26	6.60	6.26	0.34	3.8×10^{-7}	2.1×10^{-7}	−0.26
rauwolscine	5.12	7.06	−1.96	5.89	8.30	−2.41	6.3×10^{-6}	1.65×10^{-7}	−1.59

[a] For experimental details, see Experimental Section. [b] Antagonism against (−)-phenylephrine-induced α_1-adrenoceptor-mediated vasoconstriction in pithed normotensive rats (from ref 39). [c] Antagonism against B-HT 933-induced α_2-adrenoceptor-mediated vasoconstriction in pithed normotensive rats (from ref 53). [d] Index of α_1/α_2-adrenoceptor antagonist selectivity at postjunctional vascular sites in vivo. [e] Antagonism against (−)-phenylephrine- or (−)-norepinephrine-induced α_1-adrenoceptor-mediated constriction of rabbit isolated pulmonary artery (from ref 57 and 58). [f] Negative log concentration (molar) facilitating [^{3}H]norepinephrine release from rabbit isolated pulmonary artery by 30% (from ref 57 and 58). [g] Index of α_1/α_2-adrenoceptor antagonist selectivity at pre- and postsynaptic sites in vitro. [h] Concentration (molar) inhibiting the specific [^{3}H]prazosin binding (0.2 nM) to rat brain membranes by 50% (from ref 39). [i] Concentration (molar) inhibiting the specific [^{3}H]clonidine binding (0.4 nM) to rat brain membranes by 50%. [j] Index of α_1/α_2-adrenoceptor binding selectivity.

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In the present study, relationships were searched for between in vitro affinity for α_1-adrenoceptors (specific [^{3}H]prazosin binding sites) and α_2-adrenoceptors (specific [^{3}H]clonidine binding sites) and in vitro and in vivo α_1/α_2-adrenoceptor agonistic/antagonistic activities for a series of 35 agonists and 11 antagonists of diverse chemical structure (see Tables I and II and Chart I). The quantitative comparisons between functional variables and binding data were performed in order to test the relevance and predictive value of the in vitro binding to α_1- and α_2-adrenoceptors for cardiovascular activity and selectivity of α-adrenergic drugs.

Parameters. The following biological variables and binding data were employed (for details see Experimental Section). Hypotensive activity has been determined for the agonists **1–21** after intravenous (iv) administration to anesthetized normotensive rats.[47] This action is most likely mediated by the stimulation of central α_2-adrenoceptors.[12,15,48–50] Hypotensive potency was quantified by means of log $1/C_{25}$ (pC_{25}), calculated from log dose–depressor response curves; C_{25} equals the dose, in micromoles per kilogram, required to induce a 25% decrease in mean arterial pressure (data from ref 47; see Table II).

Hypertensive activity has previously been measured for the agonists **1–21**[47] and was determined for the stimulants **22–35** in pithed rats following iv injections. There is ample evidence at present that vascular α_1- and α_2-adrenoceptors participate in this drug-induced vasoconstrictor response.[11,14,15,51,52] Hypertensive activity was expressed as pC_{60}; C_{60} equals the dose, in micromoles per kilogram, needed to elevate mean arterial pressure to 60 mmHg (see Table II).

The antagonists have been tested for antagonism against (–)-phenylephrine (**23**) or B-HT 933 (**18**) induced increases in diastolic pressure in pithed normotensive rats.[39,53] The pressor effects of (–)-phenylephrine (**23**) in pithed rats are solely mediated by postjunctional α_1-adrenoceptors, whereas postsynaptic α_2-adrenoceptors induce the vasoconstriction to B-HT 933 (**18**).[54–56] The $pA_{2\,post}(\alpha_1)$ and $pA_{2\,post}(\alpha_2)$ values are listed in Table I.

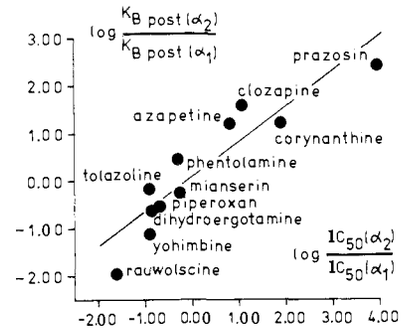

Figure 1. Relationship between the blocking selectivity of the α-adrenoceptor antagonists for postjunctional α_1- and α_2-adrenoceptors in vivo (vascular smooth muscle of the pithed rat) and the selectivity of these drugs to bind to α_1- and α_2-adrenoceptors (inhibition of [^{3}H]prazosin and [^{3}H]clonidine binding to rat brain membranes).

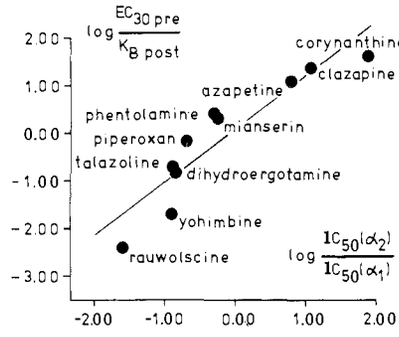

Figure 2. Relationship between the blocking selectivity of the α-adrenoceptor antagonists for pre (α_2) and post (α_1) adrenoceptors in vitro (rabbit isolated pulmonary artery) and the selectivity of these agents to bind to α_1- and α_2-adrenoceptors (inhibition of [^{3}H]prazosin and [^{3}H]clonidine binding to rat brain membranes).

Antagonism in vitro has been assessed quantitatively against (–)-norepinephrine- or (–)-phenylephrine-induced contractions of the rabbit isolated pulmonary artery.[57,58] α_1-Adrenoceptors only mediate this response.[59] In vitro blocking potency for presynaptic α_2-adrenoceptors has been quantified as the molar concentration facilitating [^{3}H]norepinephrine release from the same tissue by 30%.[57,58] The $pA_{2\,post}(\alpha_1)$ and $pC_{30\,pre}(\alpha_2)$ values are reported in Table I.

In vitro binding affinity of α-adrenoceptor agonists and antagonists for α_1- or α_2-adrenoceptors was measured as the molar concentration inhibiting the specific [^{3}H]prazosin (0.2 nM) or [^{3}H]clonidine (0.4 nM) binding, respectively, to rat isolated brain membranes by 50% (IC_{50}). Displacement data of the antagonists against [^{3}H]prazosin binding were taken from Timmermans et al.[39] The IC_{50} values are listed in Tables I (antagonists) and II (agonists).

To account for possible differences in accessibility of the drugs to the membrane-bound binding sites in vitro and the central α-adrenoceptors in vivo, we employed the apparent octanol/aqueous buffer (pH 7.4, 37 °C) partition coefficient (log P') (data from ref 47; see Table II).

Results

Linear regression equations were calculated for the antagonists in order for us to judge the quantitative rela-

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Table II. Hypotensive (pC_{25}) and Hypertensive (pC_{60}) Activities, Apparent Partition Coefficients (Log P', pH 7.4, 37 °C), and Binding Affinities (IC_{50}) for α_1- and α_2-Adrenoceptors of 35 Structurally Dissimilar α-Adrenoceptor Agonists[a]

no.	compd	pC_{25} obsd[b]	pC_{25} calcd[c]	$\lvert\Delta pC_{25}\rvert$	pC_{60}[d]	log P'[d]	$IC_{50}(\alpha_1)$[e]	log $1/IC_{50}(\alpha_1)$[f]	$IC_{50}(\alpha_2)$[g]	log $1/IC_{50}(\alpha_2)$[f]
1	44-549	2.77	2.27	0.50	2.40	2.02	6.0×10^{-8}	1.22	1.6×10^{-9}	2.80
2	Bay-a 6781	2.32	1.87	0.45	2.11	1.39	1.35×10^{-6}	−0.13	6.0×10^{-9}	2.22
3	lofexidine	2.09	2.24	0.15	1.99	0.73	6.6×10^{-7}	0.18	2.5×10^{-9}	2.60
4	clonidine	2.04	2.17	0.13	1.78	0.85	1.2×10^{-6}	−0.08	3.1×10^{-9}	2.51
5	Bay-c 6014	1.96	1.63	0.33	1.51	1.28	3.7×10^{-6}	−0.57	9.9×10^{-9}	2.00
6	UK-14,304	1.55	1.86	0.31	1.56	0.31	2.4×10^{-6}	−0.38	3.6×10^{-9}	2.44
7	B-HT 920	1.43	1.18	0.25	0.69	1.09	5.0×10^{-4}	−2.70	2.5×10^{-8}	1.60
8	naphazoline	0.95	0.94	0.01	1.83	−0.52	4.1×10^{-7}	0.39	4.8×10^{-9}	2.32
9	St-1967	0.88	0.93	0.05	1.24	1.36	1.0×10^{-6}	0.00	4.15×10^{-8}	1.38
10	St-871	0.84	1.37	0.53	1.22	2.31	1.25×10^{-6}	−0.10	6.5×10^{-9}	2.19
11	tiamenidine	0.69	1.02	0.33	1.20	−0.17	4.85×10^{-6}	−0.69	9.1×10^{-9}	2.04
12	St-1913	0.68	0.76	0.08	1.17	−0.53	5.4×10^{-7}	0.27	6.8×10^{-9}	2.17
13	KUM 32	0.63	0.54	0.09	0.23	2.12	3.4×10^{-6}	−0.53	4.8×10^{-8}	1.32
14	xylazine	0.62	0.33	0.29	−0.02	1.34	6.1×10^{-5}	−1.79	1.4×10^{-7}	0.85
15	tramazoline	0.55	0.23	0.32	1.80	−0.62	1.1×10^{-6}	−0.04	1.6×10^{-8}	1.80
16	xylometazoline	0.26	0.01	0.25	1.12	0.40	5.8×10^{-7}	0.24	2.3×10^{-8}	1.64
17	St-739	−0.02	0.07	0.09	0.65	2.51	1.4×10^{-6}	−0.15	6.5×10^{-8}	1.19
18	B-HT 933	−0.14	−0.26	0.12	−0.41	0.05	9.4×10^{-4}	−2.97	1.85×10^{-7}	0.73
19	tetryzoline	−0.16	−0.49	0.33	0.90	−0.90	1.6×10^{-6}	−0.20	3.0×10^{-8}	1.52
20	St-889	−1.02	−0.86	0.16	−0.26	2.80	1.1×10^{-5}	−1.04	2.25×10^{-7}	0.65
21	St-404	−1.31	−1.20	0.11	−0.79	−0.34	5.3×10^{-5}	−1.72	5.9×10^{-7}	0.23
22	methoxamine				0.75		1.7×10^{-4}	−2.23	3.6×10^{-6}	−0.56
23	phenylephrine				1.55		2.0×10^{-6}	−0.30	5.2×10^{-7}	0.28
24	DPI				2.44		5.1×10^{-6}	−0.71	1.4×10^{-8}	1.85
25	amidephrine				1.36		4.0×10^{-5}	−1.60	1.9×10^{-6}	−0.28
26	Sgd 101/75				−0.24		6.5×10^{-5}	−1.81	1.0×10^{-6}	0.00
27	DP-5-ADTN				−0.13		1.8×10^{-5}	−1.26	8.5×10^{-7}	0.07
28	DP-7-ADTN				−0.09		1.8×10^{-5}	−1.26	1.2×10^{-6}	−0.08
29	DP-5,6-ADTN				0.54		1.5×10^{-5}	−1.18	8.0×10^{-8}	1.10
30	DP-6,7-ADTN				0.36		2.4×10^{-5}	−1.38	8.7×10^{-8}	1.06
31	cirazoline				2.36	0.53	8.9×10^{-7}	0.05	5.9×10^{-8}	1.23
32	St-587				1.21	1.54	4.7×10^{-7}	0.33	1.5×10^{-7}	0.82
33	M-7				1.20		3.9×10^{-5}	−1.59	1.7×10^{-8}	1.77
34	guanabenz				0.93		4.5×10^{-7}	0.35	1.7×10^{-9}	2.77
35	oxymetazoline				2.24	−0.32	3.0×10^{-7}	0.52	5.0×10^{-9}	2.30

[a] For experimental details, see Experimental Section. The structural formulas of compounds 1–21 have been published elsewhere,[47] and those of 22–35 have been depicted in Figure 1. [b] From ref 47; C_{25} in micromoles per kilogram. [c] Calculated by eq 10. [d] Data for compounds 1–21, taken from ref 47; C_{60} in micromoles per kilogram. [e] Concentration (molar) inhibiting the specific [^{3}H]prazosin binding (0.2 nM) to rat brain membranes by 50%. [f] IC_{50} in micromolar concentration. [g] Concentration (molar) inhibiting the specific [^{3}H]clonidine binding (0.4 nM) to rat brain membranes by 50%.

tionships between their α-adrenoceptor binding affinity determined in vitro in radioligand displacement studies and their antagonism of α-adrenergic effects detected in functional tests in vitro or in vivo. Equations 1 and 2 were

$$\log \frac{K_{B\,post}(\alpha_2)}{K_{B\,post}(\alpha_1)} = 0.735\ (\pm 0.11) \log \frac{IC_{50}(\alpha_2)}{IC_{50}(\alpha_1)} + 0.064 \quad (1)$$

$$n = 11;\ r = 0.912;\ s = 0.561;\ F = 44.6$$

$$\log \frac{EC_{30\,pre}}{K_{B\,post}} = 1.118\ (\pm 0.18) \log \frac{IC_{50}(\alpha_2)}{IC_{50}(\alpha_1)} + 0.087 \quad (2)$$

$$n = 10;\ r = 0.914;\ s = 0.569;\ F = 40.8$$

derived. Equations 1 (see Figure 1) and 2 (see Figure 2) establish linear relationships between the selectivity ratios of the antagonists to block α_1- and α_2-adrenoceptors in vivo and in vitro, on the one hand, and their in vitro α_1/α_2-binding selectivity, on the other hand. The selectivity ratios obtained by radioligand displacement experiments account for 83 (r^2) (eq 1) and 84% (eq 2) of the variance in the pharmacological data.

The following linear correlation between the binding affinities for α_1- and α_2-adrenoceptors was generated for the α-adrenoceptor agonists used in this study (eq 3).

$$\log 1/IC_{50}(\alpha_1) = 0.681\ (\pm 0.28) \log 1/IC_{50}(\alpha_2) - 1.597 \quad (3)$$

$$n = 35;\ r = 0.650;\ s = 0.753;\ F = 24.2$$

Equation 3 indicates that within this series of 35 structurally dissimilar α-adrenoceptor agonists, the vectors describing the binding affinities for α_1- and α_2-adrenoceptors are quite orthogonal.

The relationships given by eq 4–10 were calculated between the α_2-adrenoceptor-mediated central hypotensive activity of the agonists (pC_{25}) and their binding affinity for α_1- or α_2-adrenoceptors ($\log 1/IC_{50}$), with or without inclusion of lipophilicity ($\log P'$). The linear correlation between pC_{25} and $\log P'$, given by eq 4, is statistically meaningless but gains some relevance upon incorporation of a squared term in $\log P'$ ($F_{1,18} = 10.64$; $F_{1,18;p=0.005} = 10.22$), yielding eq 5. This equation shows that 39% of the variance in central hypotensive activity is already explained by the penetration abilities of the compounds which parabolically depend on their lipophilities.

$$pC_{25} = 0.148\ (\pm 0.45) \log P' + 0.716 \quad (4)$$

$$n = 21;\ r = 0.157;\ s = 1.072;\ F = 0.5$$

$$pC_{25} = -0.565\ (\pm 0.36)\ (\log P')^2 + 1.163\ (\pm 0.75) \log P' + 0.943 \quad (5)$$

$$n = 21;\ r = 0.622;\ s = 0.873;\ F = 5.7$$

$$pC_{25} = 0.436\ (\pm 0.45) \log 1/IC_{50}(\alpha_1) + 1.063 \quad (6)$$

$$n = 21;\ r = 0.422;\ s = 0.894;\ F = 4.1$$

$$pC_{25} = 0.131\ (\pm 0.42) \log P' + 0.430\ (\pm 0.46) \log 1/IC_{50}(\alpha_1) + 0.950 \quad (7)$$

$$n = 21;\ r = 0.444;\ s = 0.999;\ F = 2.2$$

$$pC_{25} = 1.265\ (\pm 0.38) \log 1/IC_{50}(\alpha_2) - 1.342 \quad (8)$$

$$n = 21;\ r = 0.846;\ s = 0.578;\ F = 48.0$$

$$pC_{25} = 0.209\ (\pm 0.23) \log P' + 1.290\ (\pm 0.36) \log 1/IC_{50}(\alpha_2) - 1.558 \quad (9)$$

$$n = 21;\ r = 0.875;\ s = 0.540;\ F = 29.3$$

$$pC_{25} = -0.368\ (\pm 0.15)\ (\log P')^2 + 0.863\ (\pm 0.31) \log P' + 1.125\ (\pm 0.24) \log 1/IC_{50}(\alpha_2) - 1.120 \quad (10)$$

$$n = 21;\ r = 0.952;\ s = 0.350;\ F = 55.3$$

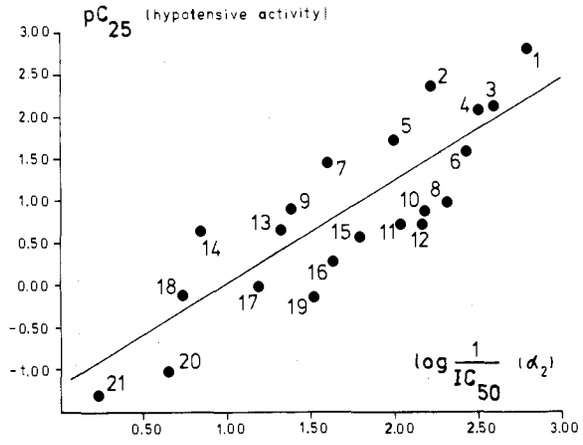

Figure 3. Relationship between central hypotensive activity (iv administration to anesthetized normotensive rats) and binding affinity to α_2-adrenoceptors (displacement of [^{3}H]clonidine binding from rat brain membranes) for 21 structurally dissimilar α-adrenoceptor agonists. The numbering refers to Table II.

The linear correlation between pC_{25} and $\log 1/IC_{50}(\alpha_1)$ (eq 6) does not satisfy at all, indicating that the affinity of the agonists for α_1-adrenoceptor-like binding sites does not correspond to their hypotensive activity. Equation 6 was not significantly improved upon adding $\log P'$ (eq 7), and the inclusion of a parabolic dependency on $\log P'$ was not a significant improvement over eq 5. In contrast, eq 8 and Figure 3 show an appreciable correlation between pC_{25} and $\log 1/IC_{50}(\alpha_2)$, which was only slightly improved upon inducing $\log P'$ (eq 9). Relationship 10, which is derived for an additional $(\log P')^2$, was, however, a great improvement over eq 9, containing a linear term in $\log P'$ ($F_{1,17} = 25.91$; $F_{1,17;p=0.001} = 15.72$).

To summarize, the central hypotensive activity of the α-adrenoceptor agonists bears no relationship with the binding affinity for α_1-adrenoceptors but can be correlated with the binding affinity for α_2-adrenoceptors. This linear relationship is improved to a highly significant level by adding $\log P'$ in a parabolic function.

Equations 11–15 were calculated by relating the peripherally induced hypertensive potency (pC_{60}) of the α-adrenoceptor stimulants to the binding properties and/or lipophilicity. The correlation between pC_{25} and $\log P'$ (eq 11) is statistically irrelevant and is also inap-

$$pC_{60} = -0.112\ (\pm 0.36) \log P' + 1.225 \quad (11)$$

$$n = 24;\ r = 0.136;\ s = 0.905;\ F = 0.4$$

$$pC_{60} = -0.229\ (\pm 0.36)\ (\log P')^2 + 0.294\ (\pm 0.72) \log P' + 1.326 \quad (12)$$

$$n = 24;\ r = 0.310;\ s = 0.889;\ F = 1.1$$

$$pC_{60} = 0.607\ (\pm 0.23) \log 1/IC_{50}(\alpha_1) + 1.437 \quad (13)$$

$$n = 35;\ r = 0.677;\ s = 0.655;\ F = 27.9$$

$$pC_{60} = 0.602\ (\pm 0.26) \log 1/IC_{50}(\alpha_2) + 0.206 \quad (14)$$

$$n = 35;\ r = 0.641;\ s = 0.683;\ F = 23.0$$

$$pC_{60} = 0.405\ (\pm 0.29) \log 1/IC_{50}(\alpha_1) + 0.326\ (\pm 0.31) \log 1/IC_{50}(\alpha_2) + 0.852 \quad (15)$$

$$n = 35;\ r = 0.727;\ s = 0.621;\ F = 17.9$$

propriate when derived for an additional squared term in log P' (eq 12). These results stress that overall lipophilic behavior of the agonists is of no importance in determining hypertensive activity. This is in contrast to their hypotensive potency, in which lipophilicity plays a significant role (see eq 5).

As described by the eq 13 and 14, the affinity for α_1-adrenoceptors (eq 13) and α_2-adrenoceptors (eq 14) can be significantly correlated with hypertensive activity, although the relationships explain but 46 and 41%, respectively, of the variance in this biological parameter. The combination of both binding variables predicted the hypertensive activity only slightly better (eq 15). The statistical quality of eq 15 is far less than that found for the relationship between central hypotensive activity and binding to α_2-adrenoceptors (eq 10).

It is obvious that the experimental observations showing that both α_1- and α_2-adrenoceptors are involved in drug-induced increases in arterial pressure (see above) are only weakly reflected by the significance of eq 15 over eq 13 and 14, which correlate pC_{60} with binding to the two types of receptors. In order for us to test for which meaningful relationships between hypertensive activity and binding affinities of both vascular (vasoconstrictor) α-adrenoceptors could be derived, separate correlations were generated in which only selective agonists were included.

Equation 16 formulates the linear relationship between

$$pC_{60} = 0.532\ (\pm 0.83)\ \log 1/IC_{50}(\alpha_1) + 1.658 \quad (16)$$

$$n = 6;\ r = 0.664;\ s = 0.726;\ F = 3.1$$

the hypertensive activity and α_1-adrenoceptor binding affinity for the highly selective α_1-adrenoceptor agonists methoxamine (**22**),[56,60] phenylephrine (**23**),[56,61-63] amidephrine (**25**),[64,65] Sgd 101/75 (**26**),[66] cirazoline (**31**),[56,67] and St 587 (**32**).[68] As is apparent from this relationship, which is visualized in Figure 4, there is hardly an appropriate linear correlation between both variables. In contrast, the linear relationship between hypertensive activity and binding affinity for α_2-adrenoceptors derived for the highly selective α_2-adrenoceptor agonists UK-14,304 (**6**),[56,67] B-HT 920 (**7**),[56,60] xylazine (**14**),[62] B-HT 933 (**18**),[54-56], DP-5,6-ADTN (**29**),[69] DP-6,7-ADTN (**30**),[69] and M-7 (**33**)[63,70]

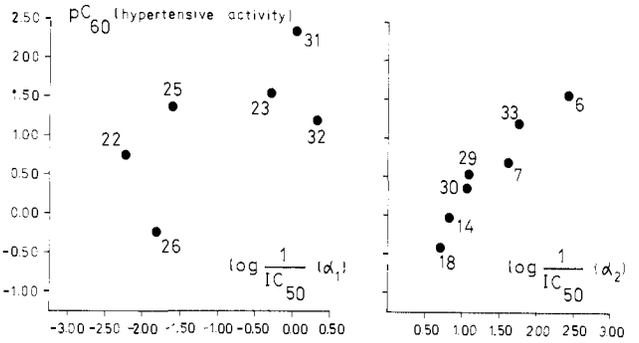

Figure 4. Relationship between peripherally induced hypertensive activity (iv administration to pithed normotensive rats) and binding affinity to α_1-adrenoceptors (left) and α_2-adrenoceptors (right) for highly selective α_1-adrenoceptor (left) and α_2-adrenoceptor (right) agonists. The numbering refers to Table II.

identifies a close correlation between both parameters (eq 17, Figure 4).

$$pC_{60} = 1.063\ (\pm 0.39)\ \log 1/IC_{50}(\alpha_2) - 0.890 \quad (17)$$

$$n = 7;\ r = 0.953;\ s = 0.224;\ F = 49.8$$

In conclusion, relationships between binding affinity and hypertensive activity, which are peripherally induced and mediated by α_1- and α_2-adrenoceptors, reach a but moderate significance level. This is due to the failure to correlate α_1-adrenoceptor-induced hypertensive potency with the α_1-adrenoceptor affinity of the drugs.

Discussion

It is conceivable to consider the effect of a biologically active agent in many, if not in most, cases as the result of an interaction between the molecules of this active agent, the drug, and the particular molecular sites of action, the receptors, sometimes enzymes, in the biological object. According to current concepts of receptor theory, two essential parameters should be distinguished in drug–receptor interactions, viz., the affinity of the drug to its receptor and the efficacy (intrinsic activity) of the drug, which is related to its capacity to induce an effect after binding to the receptor (also see ref 71–75 for reviews). Compounds that can only bind or attach to the receptor without efficacy (intrinsic activity) will behave as blocking agents. These are the so-called competitive antagonists of the agonists, the agents possessing affinity as well as efficacy (intrinsic activity). Determination of the affinity of drugs for receptors by radioligand displacement techniques is much easier to perform than the methods that use the pharmacological approach. Since in radioligand binding experiments only affinity for the particular receptor site is measured, it should be evident from the foregoing that for antagonists this binding affinity can be expected to correspond with functional antagonism determined against agonists. The present study verifies this assumption by showing that for 11 commonly known α-adrenoceptor antagonists, their α_1/α_2-binding selectivity ratio correlates very satisfactorily with their functional α_1/α_2-blocking selectivity ratios assessed in vitro as well as in vivo.

Due to the inability of the radioligand binding assay to provide information on intrinsic activity (or efficacy) of agonists, a successful generation of meaningful relationships between binding affinity and biological activity may be more exception than rule. However, within the present series of 21 structurally dissimilar α-adrenoceptor agonists,

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the central α_2-adrenoceptor-mediated hypotensive activity was highly significantly correlated with the affinity for α_2-adrenoceptors, provided that log P' was present as a parabolic function in the equation. It is very likely that log P' accounts for the difference in accessibility to the membrane-bound α_2-adrenoceptors, which are easily accessible in the radioligand binding assay, and the central (hypotensive) α_2-adrenoceptors, which are located behind the blood–brain barrier in the intact animal. The importance of log P' as a transport parameter for brain penetration is further emphasized by our previous observations that for clonidine-like imidazolidines a parabolic equation in log P' describes the ability of these compounds to enter the rat brain[76] and that for the same series of compounds used in this study a parabolic description in log P' is also required to correlate central hypotensive activity with peripheral hypertensive potency.[47] In the latter study, the log P' value for optimal brain penetration (log P_0') is found to be 1.16. It is intriguing to see that by using eq 10 derived in this study a similar value of 1.17 results for log P_0'. It should be remarked that in the reports of Titeler and Seeman[46] and of De Jong and Soudijn[45] lipophilicity was either completely ignored or was found not to improve the correlation between hypotensive activity and α_2-adrenoceptor affinity. The relevance of the correlation presented in the former study is, however, seriously hampered by the inhomogeneity of the data set, whereas the data set employed in the latter was poorly designed and too small in number. It therefore seems safe to conclude that lipophilicity accounts for the difference in accessibility to the α_2-adrenoceptor populations encountered in the binding assay and in the experiments quantifying hypotensive activity. Within the present series of α-adrenoceptor agonists, the agonist activity at central medullary α_2-adrenoceptors, giving rise to hypotension, parallels their affinity for α_2-adrenoceptors.

In attempts to generate similar relationships between hypertensive activity, which is mediated by α_1- as well as α_2-adrenoceptors, and α_1- and/or α_2-adrenoceptor binding affinity, the results were much less impressive. The individual α_1- and α_2-binding parameters, as well as the combination of both, were found to be poor descriptors of peripheral pressor activity of the α-adrenoceptor agonists. After selection of the selective α_1- and α_2-adrenoceptor agonists it could be established that only for the group of α_2-agonists did α_2-adrenoceptor binding affinity correlate with the hypertensive activity.

Our results show that apparently only the effects of the agonists initiated at central medullary and peripheral vascular α_2-adrenoceptors can be predicted by affinity, whereas binding affinity for the α_1-adrenoceptor provides but very limited information on functional agonistic activity at vascular α_1-adrenoceptors. A discrepancy between affinity for α_1-adrenoceptors and agonist activity at vasoconstrictor α_1-adrenoceptors has also been noted by us within a series of 22 meta-substituted phenylimino-imidazolidines.[41] Within pairs of substitution isomers, the decrement in affinity for α_1-adrenoceptors due to substitution at the 5-position of the phenyl nucleus contrasted with a generally observed increment in agonist activity.[41] On the other hand, our observations conflict with those of Schmitz et al.,[36] showing that the relative potencies of adrenergic agonists in the isolated perfused rat kidney correlated with the inhibitory potency to displace [^{3}H]-prazosin from rat renal membrane binding sites. In addition, Hieble et al.[38] have presented a linear relationship between α_1-adrenoceptor-induced vasoconstrictor potency (perfused rabbit ear artery) and [^{3}H]WB 4101 displacement for α-adrenergic 2-aminotetralins, provided that the partial agonists were excluded, indicating that such a linear relationship only holds when there is little variation in efficacy (intrinsic activity).

As discussed above, only when both efficacy and affinity are known can the agonist activity be predicted with reasonable certainty. Consequently, the successful calculation of a significant relationship between α_2-adrenergic agonist activity and α_2-affinity and the failure to accomplish this for α_1-adrenoceptors within the same series of α-adrenoceptor agonists, as encountered in the present study, may be due to a large variation in efficacies of the agonists used at the α_1-adrenoceptors, whereas this variation is much less pronounced at the α_2-adrenoceptors. It has been discussed by Starke[11] that the stimulus–response relationship of α-adrenoceptor agonists at vascular α_1-adrenoceptors is a hyperbolic function (also see ref 75). For agonists that stimulate these α_1-adrenoceptors in a manner similar to phenethylamines, a modest affinity is translated into high activity because of this particular kind of stimulus–response relationship. However, this mechanism does not hold for many imidazoli(di)nes, which have a low efficacy and, consequently, their occupancy–response relationships are approximately linear. It has been mentioned by Starke[11] that published data show no analogous difference between imidazoli(di)nes and phenethylamines at α_2-adrenoceptors, indicating that for the α_2-adrenergic responses studied so far, the occupancy–response function is nearly linear. The present study would suggest that this characteristic can also be applied to central medullary (hypotensive) and vascular postjunctional (hypertensive) α_2-adrenoceptors. It should, however, be added that no phenethylamine-like α-adrenoceptor agonists were or could be included into the data sets showing significant relationships between α_2-affinity and α_2-potency. Furthermore, it is not correct to conclude that differences in intrinsic activity (efficacy) can be completely ignored in the relationships between α_2-adrenoceptor activity and affinity. In a recent study, we could show that for a series of 18 α-adrenergic imidazolidines, the linear correlation between the agonist activity at peripheral presynaptic (cardiac) α_2-adrenoceptors and α_2-affinity was greatly improved upon inclusion of a substituent parameter that can be related to intrinsic activity.[41,77]

In view of the foregoing, the statistical quality of the relationships between α_1-adrenergic activity and α_1-affinity should greatly improve upon incorporation of one or more properties that are related to the efficacies (intrinsic activities) of the agonists. However, we were not able to accomplish this in the relationship between α_1-adrenoceptor-induced vasopressor potency and affinity for [^{3}H]prazosin occupied binding sites in cerebral membranes for 22 structurally related imidazolidines.[41] This has led us to speculate that the postjunctional vascular α_1-adrenoceptors stimulated by these imidazolidines differ from the central α_1-adrenoceptors labeled by [^{3}H]prazosin.

Experimental Section

Central Hypotensive Activity. Hypotensive activity originating from a stimulation of central α_2-adrenoceptors has been measured following iv administration of the α-adrenoceptor agonists to anesthetized male normotensive Wistar rats.[47] The pC_{25} values reported in Table I are taken from this study.

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Peripheral Hypertensive Activity. Hypertensive activity reflecting peripherally induced vasoconstriction of the α-adrenoceptor agonists has been quantified following iv injections into pithed normotensive rats.[47] The pC_{60} values of compounds 1–21 have been taken from this study. Hypertensive activity of the remaining compounds, **22–35**, was evaluated as described previously.[47]

In brief, male normotensive Wistar rats (190–220 g) were anesthetized with hexobarbitone sodium [150 mg/kg, administered intraperitoneally (ip)]. The trachea was cannulated, and, subsequently, the animals were pithed by means of a blunt needle introduced into the spinal canal via the orbit. Thereafter, ventilation was maintained artificially via a Braun–Melsungen respiration pump with positive pressure. Rectal temperature was kept at about 37 °C. A catheter was placed into a jugular vein for the intravenous injection of drugs, and heparin (about 1000 IU/kg) was administered. Arterial pressure was taken from a common carotid artery and recorded continuously via a Statham P23 Db pressure transducer on a Hellige-HE 19 device. After a 20-min period of equilibration, increases in mean arterial pressure (mmHg) were determined after intravenous injections of single doses of the drugs. Substances, as well as doses, were administered in random order in a volume of 0.05 mL/100 g of body weight. No more than three or four separate measurements were made per animal. Recovery from the pressor effects was ensured between the subsequent doses. The peripheral hypertensive activity of the agonists was quantified by means of log dose–response curves, from which the log of the reciprocal dose (in micromoles per kilogram) required to cause an increase in mean arterial pressure of 60 mmHg (pC_{60}) was calculated.

Octanol/Buffer (pH 7.4, 37 °C) Partition Coefficient. Lipophilicity of most of the α-adrenoceptor agonists has been measured as the log partition coefficient (log P') between octanol and 0.1 M phosphate buffer at physiological conditions (pH 7.4, 37 °C) according to the procedure outlined in detail elsewhere.[47] The log P' values reported in Table II have been taken from this study.

Antagonism of α_1- and α_2-Adrenoceptor-Mediated Vasoconstriction in Vivo. The ability of (−)-phenylephrine (**23**) and B-HT 933 (**18**) to selectively stimulate postjunctional α_1- and α_2-adrenoceptors, respectively, in vascular smooth muscle of the pithed rat has been used to quantify the antagonism of α_1- and α_2-adrenoceptor-mediated vasoconstriction in vivo for various α-adrenoceptor-blocking drugs.[39,53] Accordingly, the blocking activity of these agents has been established against the increase in diastolic pressure of (−)-phenylephrine (**23**) and B-HT 933 (**18**) following iv application (for details see ref 39 and 53).

pA_2 values were obtained from the equation pA_2 = log (dose ratio − 1) − log (antagonist concentration), or they were calculated according to Arunlakshana and Schild.[78] The $pA_{2\,post}(\alpha_1)$ and $pA_{2\,post}(\alpha_2)$ values reported in Table I result from these studies.

Antagonism of α_1- and α_2-Adrenoceptor-Mediated Effects in Vitro. The antagonism of various α-adrenoceptor blocking drugs against (−)-norepinephrine or (−)-phenylephrine (**23**) evoked α_1-adrenoceptor-mediated constrictor effects of the rabbit isolated pulmonary artery has been determined by Borowski et al.[57] and Weitzell et al.[58] In addition, these authors also measured the concentration of the antagonists needed to facilitate [^{3}H]norepinephrine release from this tissue by 30%, reflecting the in vitro blocking activity of these drugs at presynaptically located α_2-adrenoceptors. The $pA_{2\,post}(\alpha_1)$ and $pC_{30\,pre}(\alpha_2)$ values listed in Table I are taken from these publications or were kindly provided by Professor K. Starke (Freiburg, FRG).

Binding Affinity for α_1- and α_2-Adrenoceptors. Male Wistar normotensive rats (200–250 g) were decapitated, and their brains (minus cerebellum) were quickly removed and homogenized in 20 vol (w/v) of ice-cold Tris-HCl (50 mmol/L) buffer (pH 7.7, 25 °C). The homogenate was centrifuged at 50000g for 10 min at 4 °C. The pellet was rehomogenized in fresh cold Tris-HCl buffer and again centrifuged (see above). The final crude brain membranes were suspended in Tris-HCl buffer. Protein concentration, as assayed by the method of Lowry et al.,[79] amounted to 1 and 4 mg/mL for the [^{3}H]prazosin and [^{3}H]clonidine displacement studies, respectively.

Routine [^{3}H]prazosin and [^{3}H]clonidine binding assays were run by incubating 500 μL of crude rat brain membrane suspensions (see above) at 25 °C for 45–60 min with [^{3}H]prazosin (specific activity 33 Ci/mmol, 0.2 nM) or [^{3}H]clonidine (specific activity 26.7 Ci/mmol, 0.4 nM) with shaking in a total volume of 1 mL of incubation buffer (50 mmol/L of Tris-HCl). The inhibition of the specific binding of both radioligands was determined in the presence of various concentrations of unlabeled competing drugs. Incubations were terminated by rapid vacuum filtration through Whatman GF/B glass-fiber filters. Filters were washed with three 5-mL portions of ice-cold Tris-HCl buffer, solubilized in 10 mL of Instagel (Packard-Becker) for 24 h, and counted for radioactivity at 35–40% efficiency. The specific binding of [^{3}H]clonidine was determined as the excess over blanks containing 10 μM (−)-norepinephrine, whereas 2 μM phentolamine was used to define the specific binding of [^{3}H]prazosin.

The affinity of drugs for the specific binding sites labeled by [^{3}H]clonidine and [^{3}H]prazosin was expressed as the molar concentration inhibiting the specific binding by 50% (IC_{50}). These values were calculated from the displacement curves by log probit analysis.

Correlations. Linear relationships between α_1- or α_2-adrenergic agonistic and antagonistic activities, on the one hand, and binding affinity for α_1- and/or α_2-adrenoceptors, on the other hand, with or without inclusion of lipophilicity (log P') were calculated by the method of least-squares by means of a Wang 700B computer. The correlation coefficient (r), the standard deviation (s), and the significance of the regression (F) are given. The figures in parentheses are the 95% confidence limits. Inclusion of parameters was judged by application of the F test.

Drugs. Drugs used in this study and their sources were as follows: amidephrine mesylate (**25**), Mead Johnson; azapetine phosphate, Hoffman-La Roche; Bay-a 6781 (**2**), Bay-c 6014 (**5**), and xylazine hydrochloride (**14**), Bayer; B-HT 920 dihydrochloride (**7**) and B-HT 933 dihydrochloride (**18**), Thomae; cirazoline hydrochloride (**31**), Sythélabo; clonidine hydrochloride (**4**), [^{3}H]-clonidine hydrochloride (sp act. 26.7 Ci/mmol), DPI hydrobromide (**24**), KUM 32 hydriodide (**13**), St-404 nitrate (**21**), St-587 nitrate (**32**), St-889 hydrobromide (**20**), St-1913 hydrochloride (**12**), St-1967 hydrochloride (**9**), and tramazoline hydrochloride (**15**), Boehringer Ingelheim; clozapine, Wander; compound 44-549 fumarate (**1**), Sandoz Wander; corynanthine hydrochloride and rauwolscine hydrochloride, Roth; dihydroergotamine methanesulfonate, Sandoz; DP-5- (**27**), DP-7- (**28**), DP-5,6- (**29**), and DP-6,7-ADTN (**30**) hydrobromide, ref 69; quanabenz acetate (**34**), Wyeth; lofexidine hydrochloride (**3**), Nattermann; M-7 (**33**), Research Biochem. Inc.; (±)-*erythro*-methoxamine hydrochloride (**22**), Burroughs Wellcome; mianserin hydrochloride, Organon; naphazoline hydrochloride (**8**), oxymetazoline hydrochloride (**35**), phentolamine hydrochloride, tolazoline hydrochloride, and xylometazoline hydrochloride (**16**), Ciba-Geigy; (−)-phenylephrine hydrochloride (**23**) and yohimbine hydrochloride, Sigma; piperoxane hydrochloride, Janssen Pharmaceutica; prazosin hydrochloride, [^{3}H]prazosin hydrochloride (sp act. 33 Ci/mmol), tetryzoline hydrochloride (**19**), and UK-14,304 tartrate (**6**), Pfizer; Sgd 101/75 hydrochloride (**26**), Siegfried; St-739 hydrochloride (**17**) and St-871 hydrochloride (**10**), ref 80; tiamenidine hydrochloride (**11**), Hoechst.

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Registry No. 1, 56987-45-2; **2**, 24248-22-4; **3**, 31036-80-3; **4**, 4205-90-7; **5**, 31235-50-4; **6**, 59803-98-4; 7, 36085-73-1; **8**, 835-31-4; **9**, 76833-40-4; **10**, 40065-09-6; 11, 31428-61-2; **12**, 52115-81-8; **13**,

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β_1-Selective Adrenoceptor Antagonists. 3. 4-Azolyl-Linked Phenoxypropanolamines

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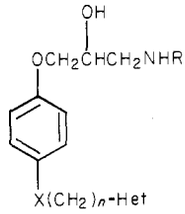
A series of 4-substituted phenoxypropanolamines has been prepared and examined for β-adrenoceptor activity. The 4-substituents, di- and triazole ring systems connected to the phenoxy ring by different length chains, were chosen as a means of introducing cardioselectivity. This has been achieved, especially in the 1-[4-[(4-chloropyrazol-1-yl)methoxy]phenoxy]-3-(isopropylamino)-2-propanol (**11**), the 4-[(2*H*-1,2,3-triazol-2-yl)methoxy] analogue (**21**), and the 4-[2-(2*H*-1,2,3-triazol-2-yl)ethoxy] analogue (**22**), which show potent β_1-blockade with selectivity ratios in excess of 100:1. Structure–activity relationships are discussed, and the optimum position of the heteroatom in the 4-substituent is defined.

The preceding papers[1,2] in this series describe the synthesis and β-blocking activities of variously substituted (aryloxy)propanolamines and support the contention[2,3] that a heteroatom suitably positioned in a 4-substituent is necessary to produce both potent and cardioselective β-blockade.

Although, in general, the more potent agents are those with oxygen functions in the 4-substituent, we wished to find an alternative functionality that could achieve the same interaction with the β_1-receptor. Since this interaction is likely to involve the lone pairs of electrons on the oxygen atom, a situation was sought in which a nitrogen atom lone pair was available in similar fashion. Having excluded simple amines because of protonation at physiological pH and amides because of delocalization over the carbonyl system, we considered the possibility that the pyridinic nitrogen atoms in diazoles and triazoles closely fulfilled the requirement. Consequently, we have synthesized a series of phenoxypropanolamines (**1–26**)[4] sub-

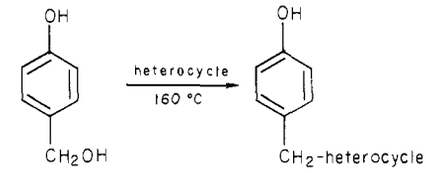


1–26, R = *i*-Pr, *t*-Bu; X = O, S; *n* = 1–3; Het = pyrazoles, triazoles, benzoazoles

stituted in the 4-position by groups incorporating di- and triazole rings.[5] The use of one of the ring nitrogen atoms as the point of attachment to the chain afforded easy synthetic access to molecules with variable heteroatom position and basicity. This paper describes their synthesis and evaluation as β-adrenoceptor antagonists.

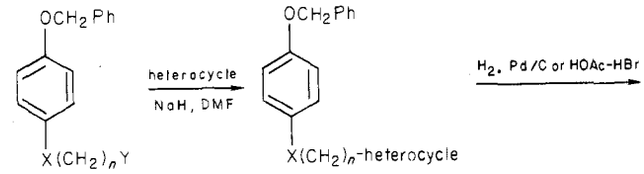
Chemistry. All but two of the oxypropanolamines listed in Table I were obtained by the classical phenol–epoxide–amino alcohol sequence using the conditions described previously.[2] The phenol starting materials were prepared by the following general methods (A–E).

Scheme I. Method A



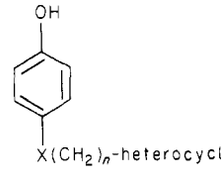
27

Scheme II. Method B



28, Y = OMs; *n* = 2
29, X = O; Y = Br; *n* = 2
30, X = O; Y = Br; *n* = 3

31, *n* = 2
32, X = O; *n* = 2
33, X = O; *n* = 3



34, *n* = 2
35, X = O; *n* = 2
36, X = O; *n* = 3

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