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Mydriasis elicited by imidazol(in)e α_2-adrenomimetics in comparison with other adrenoceptor-mediated effects and hydrophobicity

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Abstract

α_2-Adrenoceptor agonists cause both mydriasis and platelet aggregation. This work is aimed at identifying the factors accompanying and affecting mydriatic activity. For eight imidazol(in)e drugs mydriatic, hypotensive and bradycardic activities were determined in rats. The lipophilicity of the agents was determined chromatographically and calculated theoretically. A correlation was found between the hypotensive and the bradycardic potency and between the mydriatic activity and both the hypotensive and bradycardic activity. Mydriatic activity depended on the lipophilicity of the agents studied. The human platelet antiaggregatory activity of the drugs did not correlate with either the mydriatic or cardiovascular activity and it was independent of lipophilicity. The dependence of the centrally induced effects on lipophilicity and the lack of such a dependence in the case of the in vitro α_2-adrenoceptor-mediated platelet aggregation may be interpreted as resulting from heterogeneity of the rat cerebral and the human platelet α_2-adrenoceptors. The α_2-adrenergic activity of drugs in the model of mydriasis in rats cannot be predicted from their activity in causing human platelet aggregation in vitro.

Keywords: α_2-Adrenoceptor; Bradycardic activity; Hypotensive activity; Imidazole derivative; Imidazoline derivative; Lipophilicity; Mydriatic activity

1. Introduction

Since Walland and Kobinger (1971) noted that clonidine induced mydriasis in some species, a number of imidazoline derivatives have been investigated. It was found that pupillary dilation was produced by close analogues of clonidine (Koss, 1986; Pitts and Marwah, 1989) as well as by other imidazoline drugs classified as α_2-adrenoceptor agonists. Among the latter group of agents were xylazine (Hsu et al., 1981; Virtanen and MacDonald, 1985), UK-14,304 (Berridge et al., 1983; Virtanen et al., 1988) and the imidazole derivatives medetomidine (Virtanen et al., 1988) and detomidine (Virtanen and MacDonald, 1985; Hanson and Hsu, 1987). Walland and Kobinger (1971) and Kobinger (1978) ascribed the mechanism of the clonidine-induced mydriasis in rats to a direct sympathomimetic action on the iris. Koss (1986) suggested the mechanism of action to be the α_2-adrenergic stimulation of the sciatic nerve, which produced reflex mydriasis by inhibition of parasympathetic tone to the iris (Koss, 1986). There is reasonable agreement that mydriasis is mediated via α_2-adrenoceptors (Koss and Christiansen, 1979; Heal et al., 1989).

Mydriasis appears to be a simple and reliable model for assessing central α_2-adrenoceptor activity. Pupillary responses in rats to five azole drugs were compared by Virtanen et al. (1988). The potency order found by those authors was: medetomidine > clonidine = detomidine > UK14,304 > xylazine.

With pharmacological and structural data for a longer series of congeneric drugs one can attempt to derive quantitative structure-activity relationships. Statistically significant and physically meaningful quantitative structure-activity relationships allow identification of physicochemical features of drugs and/or receptors which determine their mutual fitting and interactions. QSAR studies within a group of imidazoline deriva-

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tives have been undertaken only occasionally. Struyker Boudier et al. (1976) reported weak correlations between affinities of a series of 11 imidazolines towards peripheral adrenoceptors and physicochemical properties like chloroform-water partition coefficients and pK_a. There was no significant correlation between the hypotensive activity of the agents and the two physicochemical parameters considered. This observation led Struyker Boudier et al. (1976) to the conclusion that structural requirements for central activity of imidazolines were different from those for peripheral vascular activity. The lack of correlation between hydrophobicity and hypotensive activity was also reported by Rouot et al. (1976) for a larger set of newly synthesized clonidine analogues. Timmermans et al. (1984) reported that, for agonists, the central α_2-adrenoceptor-elicited hypotensive activity could be described in terms of the parabolic dependence of the logarithm of octanol-buffer (pH 7.4; 37°C) partition coefficient.

The logarithm of the octanol-water partition coefficient (log P) is a standard reference measure of the hydrophobicity (lipophilicity) of chemical compounds. Instead of log P the approximate measure of hydrophobicity (calculated log P) can be obtained from the structural formula of a drug by summation of the structural fragment contributions (Hansch and Leo, 1979). Experimental lipophilicity measures can readily be obtained by high-performance liquid chromatographic (HPLC) methods (Kaliszan, 1993). Gami-Yilinkou and Kaliszan (1992) subjected to multivariate chemometric analysis a set of hydrophobicity parameters determined chromatographically in several reversed-phase HPLC systems for a series of imidazole and imidazoline drugs. The resulting clustering of the drugs was in a good agreement with the accepted classification of the agents according to their affinity towards α_1-, α_1/α_2- and α_2-adrenoceptors. A general observation was that the hydrophobicity of imidazol(in)es increased when moving from pure α_2-adrenoceptor agonists to α_1-agonists, with typical imidazoline α-adrenoceptor antagonists (tolazoline, phentolamine) being in the middle.

Mydriasis in rats and aggregation of human blood platelets are recognized as reliable pharmacological models to study α_2-adrenoceptors. Known α_2-adrenoceptor agonists cause both mydriasis and aggregation. However, the relative activities of individual drugs need not be similar in the two tests: one in vivo, the other in vitro. Previously we determined the inhibition of the adrenaline-induced human platelet aggregation in vitro by several imidazol(in)e drugs (Petrusewicz and Kaliszan, 1991). Here we attempt to identify the factors accompanying and possibly affecting the mydriatic activity of a representative series of imidazol(in)e drugs classified rather unequivocally as α_2-adrenoceptor agonists but of different potency. Such a study should provide an additional insight into adrenoceptor pharmacology, especially in view of the long-lasting discussion on subtypes of α_2-adrenoceptors (Young et al., 1989; Alberts, 1993; Trendelenburg et al., 1993) and possible involvement of specific imidazoline binding sites (Ernsberger et al., 1987; Lehmann, 1989; Molderings et al., 1993).

2. Materials and methods

2.1. Measurement of drug-induced mydriasis

The basic procedure proposed by Gherezghiher and Koss (1979) was applied. Male Wistar rats weighing 250–300 g were anesthetized with pentobarbital (60 mg/kg i.p.). Pupillary diameter was measured at the point of greatest horizontal diameter, using a calibrated operating microscope with an internal light source. A green filter was utilized in order to reduce the degree of light-induced pupillary constriction. All observations were made under the same lighting conditions in a dark room. Initial pupil diameter was about 0.4 ± 0.1 mm before drug administration. Drugs to be studied were administered via a cannulated femoral vein in cumulative doses at 5-min intervals. Controls were injected with an appropriate volume of 0.9% NaCl. The results were means ($\pm$S.E.M.) of 5–6 experiments. As previously observed by Berridge et al. (1983) and Koss (1986), the dilation was rapid in onset (within the first minute after injection) and of long duration (> 1 h for most doses).

2.2. Effect on the mean arterial blood pressure and heart rate

Male Wistar rats (weight 250–350 g) were anesthetized with urethane (1.5 g/kg i.p.) and the trachea was cannulated to allow respiration. Mean blood pressure was measured in a cannulated carotid artery with a pressure transducer connected to a blood pressure meter (8041, S&W Medico Teknik, Denmark). The blood pressure pulses were used to trigger a heart rate meter (Trendscope 8031, S&W Medico Teknik, Denmark). Both blood pressure and heart rate were recorded on the Trendscope 8031 monitor. A femoral vein was cannulated for i.v. injections. Drugs to be studied were administered at a volume of 1 ml/kg of body weight. A single dose was given to each animal only. Rats having a mean arterial pressure higher than 130 or lower than 90 mm Hg were discarded. Measurements were carried on for 30 min. Maximum changes in blood pressure and the accompanying changes in heart rate were recorded. The results were means ($\pm$S.E.M.) of 5 experiments for each of the 4–6 doses administered.

2.3. Determination of chromatographic measures of hydrophobicity

The column used was of the so-called immobilized artificial membrane (IAM) type (Pidgeon et al., 1992). It was formed from 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-*sn*-3-glycerophosphocholine (lecithin-COOH) bonded to silica-propylamine with the unreacted propylamine moieties end-capped with methylglycolate. A commercially distributed IAM.PC.MG 150 × 4.6 mm i.d. column was purchased from the Regis Chemical Company, Morton Grove, IL, USA.

The chromatographic system consisted of a Model L-6200A pump, a Model L-4250 UV-VIS detector and a Model D-2500 chromato-integrator (all from Merck-Hitachi, Vienna, Austria).

An established procedure of assessing solute hydrophobicity by HPLC (Kaliszan, 1993) was employed. The drugs tested were chromatographed with acetonitrile-0.1 M sodium phosphate buffer of pH 7.0 10:90% (v/v) as eluent. The experiments were carried out isocratically at ambient temperature, using a flow rate of 1 ml/min. Detection wavelength was 254 nm.

Chromatographic retention parameters (k'_{IAM}) were calculated from the standard formula:

$$k'_{IAM} = (t_R - t_0)/t_0$$

where t_R was retention time of the compound studied and t_0 corresponded to migration time of a nonretained marker (methanol).

2.4. Calculation of logarithms of octanol-water partition coefficients

Theoretical parameter of hydrophobicity of nonionized forms of the imidazol(in)e drugs studied (calculated log P = CLOGP) was calculated from their structural formulae by means of the fragmental constants according to the method of Hansch and Leo (1979).

2.5. Statistical procedure

A professional statistical package (Statgraphic Plus, Manugistics, Rockville, MA, USA) was used on a personal computer. The requirements for a meaningful regression analysis (Charton et al., 1985) were observed.

2.6. Drugs

The drugs used and their sources were as follows: clonidine HCl = 2-(2,6-dichlorophenylimino) imidazolidine hydrochloride (gift of Boehringer Ingelheim), lofexidine HCl = 2-[1-(2,6-dichlorophenoxy) ethyl]-2-imidazoline hydrochloride (gift of Dr. H. Betzing, A. Nattermann and Cie., Cologne, Germany), detomidine HCl = 4-(2,3-dimethylbenzyl)-1*H*-imidazole hydrochloride and medetomidine HCl = 4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole hydrochloride (gift of Dr. A. Karjalainen, Farmos-Group, Oulu, Finland), moxonidine = 4-chloro-5-(2′-imidazolin-2′-ylamino)-6-methoxy-2-methyl-pyrimidine (gift of Dr. B.I. Armah, BDF Research Laboratories, Hamburg, Germany), tiamenidine HCl = 2-chloro-4-methyl-3-(2′-imidazolin-2′-ylamino)-thiophene hydrochloride (gift of Hoechst), UK 14,304 tartrate = 5-bromo-6-[2′-imidazolin-2′-ylamino]quinoxaline tartrate (gift of Pfizer), xylazine HCl = 2-(2,6-dimethyl-phenylamino)-4*H*5,6-dihydro-1,3-thiazine hydrochloride (gift of Bayer).

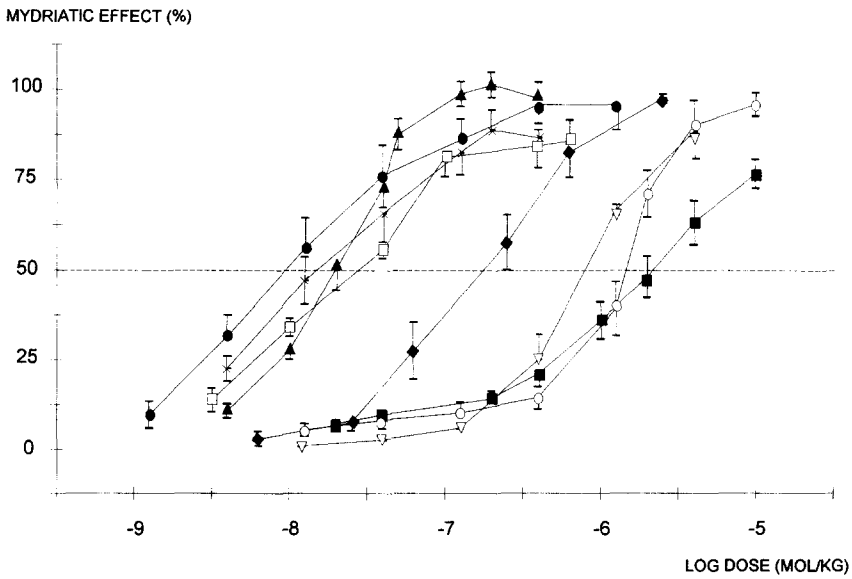

Fig. 1. Plots of mydriatic effect (percent of maximum response to clonidine) against logarithm dose of imidazol(in)e drugs: (▲) clonidine, (●) medetomidine, (×) detomidine, (□) lofexidine, (◆) UK-14,304, (○) moxonidine, (▽) xylazine, (■) tiamenidine. Pupillary responses of rats were measured after cumulative administration of the agents at 5-min intervals. Means ± S.E.M. are presented (n = 5–6).

Table 1
Pharmacological activity and hydrophobicity parameters of imidazol(in)es studied

No.	Drug	Mydriatic effect [a]	Bradycardic effect [a]	Hypotensive effect [a]	Platelet aggregation inhibitory effect [b]	Hydrophobicity parameters [a]	
		ED_{50}^{m} (mol/kg)	ED_{50}^{b} (mol/kg)	ED_{50}^{bp} (mol/kg)	IC_{25} (mol/dm^3)	log k'_{IAM}	CLOGP [c]
1.	Clonidine	1.9×10^{-8}	2.1×10^{-8}	1.3×10^{-8}	8.9×10^{-10}	0.299	0.73
2.	Detomidine	1.6×10^{-8}	3.3×10^{-8}	7.2×10^{-8}	–	0.918	2.90
3.	Lofexidine	2.6×10^{-8}	6.8×10^{-9}	1.2×10^{-8}	1.8×10^{-7}	0.705	2.32
4.	Medetomidine	9.7×10^{-9}	1.1×10^{-8}	3.3×10^{-8}	1.5×10^{-7}	1.083	3.44
5.	Moxonidine	1.5×10^{-6}	7.0×10^{-7}	3.2×10^{-7}	–	−0.244	−1.84
6.	Tiamenidine	2.2×10^{-6}	5.2×10^{-7}	1.4×10^{-6}	2.2×10^{-6}	0.323	−0.05
7.	UK-14,304	1.7×10^{-7}	1.8×10^{-8}	2.3×10^{-8}	–	0.100	0.84
8.	Xylazine	7.8×10^{-7}	1.8×10^{-7}	4.1×10^{-7}	4.2×10^{-7}	0.586	1.35

[a] See: Materials and methods; [b] According to Petrusewicz and Kaliszan (1991); [c] Calculated log P.

3. Results

The i.v. administration of the eight imidazol(in)e drugs studied produced a dose-dependent increase (with regard to control value) of pupillary diameter in anesthetized rats (Fig. 1). The greatest mydriatic effect of the agents studied was observed for clonidine. This effect, corresponding to an average pupil diameter of 4.0 ± 0.4 mm, was assumed as 100% maximum response. A similar maximum effect of about 5 mm was obtained by Virtanen et al. (1988) for female Sprague-Dawley rats. For the individual imidazol(in)es considered, the equieffective doses, ED_{50} (mol/kg), inducing half-maximum pupil dilation, i.e. 2 mm, were determined. Respective numerical data are presented in Table 1.

Dose-effect relationships are also given for the independently measured lowering of arterial blood pressure (Fig. 2) and decrease of the heart rate (Fig. 3). The percent decrease of initial values is plotted against logarithm dose. Among the imidazol(in)es studied, clonidine had the greatest hypotensive and bradycardic effects.

For individual imidazol(in)e derivatives the equieffective doses, ED_{50} (mol/kg), inducing a blood pressure and heart rate decrease of 27 mm Hg and 60 beats/min, respectively (half of the maximum clonidine effect), are shown in Table 1.

For five of the eight agents presently studied we had previously determined their inhibitory activity against the adrenaline-induced human blood platelet aggregation in vitro (Petrusewicz and Kaliszan, 1991). Respective activity data, IC_{25}, are included in Table 1.

In Table 1 are also listed the values of the two hydrophobicity parameters considered: the experimentally determined logarithm of the HPLC capacity fac-

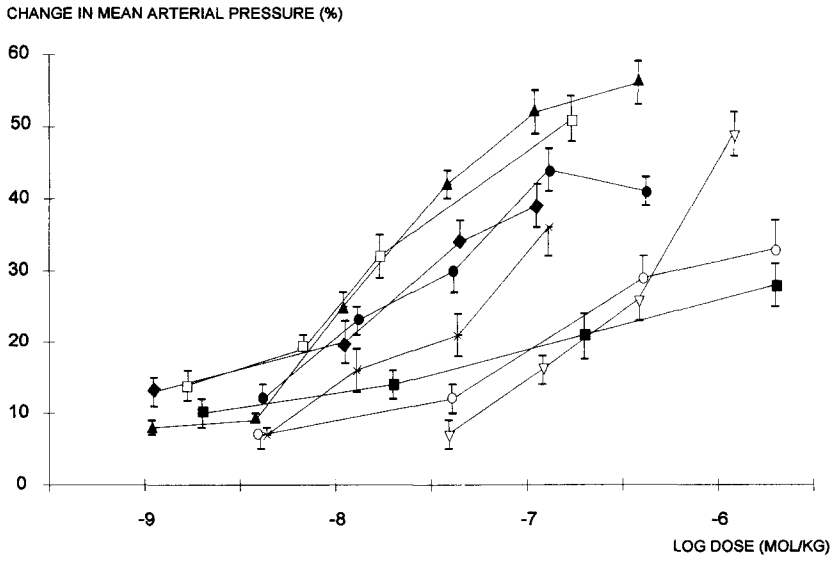

Fig. 2. Plot of the maximum decrease of mean arterial blood pressure (percent of initial value) against logarithm dose of imidazol(in)e drugs after single bolus injections to individual rats: (▲) clonidine, (●) medetomidine, (×) detomidine, (□) lofexidine, (◆) UK-14,304, (○) moxonidine, (▽) xylazine, (■) tiamenidine. Means ± S.E.M. are presented ($n = 5$).

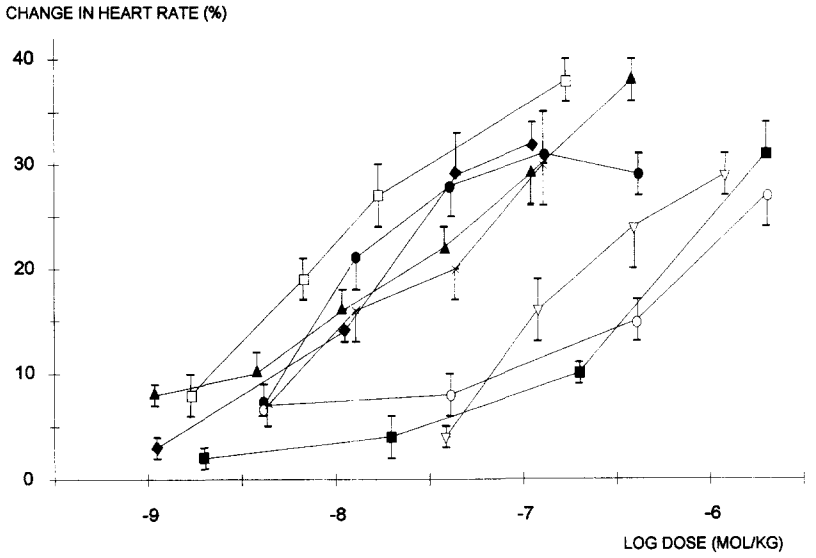

Fig. 3. Plots of the heart rate decrease (percent of initial value) against logarithm dose of imidazol(in)e drugs after single bolus injections to individual rats: (▲) clonidine, (●) medetomidine, (×) detomidine, (□) lofexidine, (◆) UK-14,304, (○) moxonidine, (▽) xylazine, (■) tiamenidine. Means ± S.E.M. are presented ($n = 5$).

tor, log k'_{IAM}, and the theoretically calculated logarithm of octanol-water partition coefficient for nonionized forms of the drugs, CLOGP.

Table 2 gives the results of regression analyses of the relationships between the data given in Table 1. Negative logarithms of doses causing 50% maximum mydriasis, blood pressure decrease and decrease in heart rate were regressed against each other and against both the experimental (log k'_{IAM}) and theoretical hydrophobicity parameters (calculated log P, CLOGP). The correlation between heart rate and blood pressure was significant at 99.9% significance level ($P \leq 0.0008$) with a correlation coefficient $r = 0.930$ for the eight agents studied ($n = 8$). Highly significant (> 99% significance level) correlations were found between mydriasis and both hypotensive and bradycardic effects. Correlations between mydriatic activity and lipophilicity of the agents, expressed either by the experimental or by theoretical parameters, were significant at the level of about 95%.

4. Discussion

In their comparative studies on imidazol(in)e derivatives, Virtanen et al. (1988) reported half-maximum mydriatic effects at doses 4, 9, 10, 60 and 120 μg/kg for medetomidine, clonidine, detomidine, UK 14,304 and xylazine, respectively. In the case of common agents, our results (Table 1) agree quite well with those reported. The other imidazolines studied by us, i.e., lofexidine, moxonidine and tiamenidine, also induced a dose-dependent pupillary dilation.

The α_2-adrenergic potency of moxonidine and tiamenidine as reflected in our mydriasis test is in agreement with the results of Armah (1988) and Lindner

Table 2
Statistical parameters of regression analysis of significant relationships between pharmacological and hydrophobicity data for imidazol(in)e drugs given in Table 1

y	x	a	b	n	r	P
$-\log ED_{50}^{m}$	$-\log ED_{50}^{h}$	−1.02 (±1.67)	1.09 (±0.23)	8	0.890	0.003
$-\log ED_{50}^{m}$	$-\log ED_{50}^{bp}$	−0.18 (±1.87)	1.00 (±0.26)	8	0.842	0.009
$-\log ED_{50}^{h}$	$-\log ED_{50}^{bp}$	0.88 (±1.03)	0.90 (±0.14)	8	0.930	0.0008
$-\log ED_{50}^{m}$	$\log k'_{IAM}$	6.22 (±0.41)	1.51 (±0.66)	8	0.686	0.06
$-\log ED_{50}^{m}$	CLOGP	6.38 (±0.28)	0.45 (±0.14)	8	0.797	0.02

General regression equation was $y = a + bx$; n = number of data points; r = correlation coefficient; P = significance level; in parentheses are given the standard deviations of regression coefficients. Superscripts at activity measures denote: m = mydriasis; h = heart rate; bp = arterial blood pressure.

and Kaiser (1974) obtained in experiments on isolated organs and on blood pressure, respectively. Further proof of the reliability of our pharmacological results is provided by the statistical analysis of the relationships between selected activity data (Table 2). First of all, there was a highly significant relationship between the negative logarithm of the dose causing a 50% decrease in the heart rate, $-\log ED_{50}^{h}$, and an analogous parameter reflecting lowering of arterial blood pressure, $-\log ED_{50}^{bp}$. This relationship was significant at the level $P \leq 0.0008$ and was characterized by a correlation coefficient $r = 0.930$. It should be noted here that Timmermans and Van Zwieten (1977) obtained an even better correlation between the two types of pharmacological activity but they dealt with a series of seven highly congeneric clonidine analogues whereas our imidazole and imidazoline derivatives represent relatively diverse structures.

If the cardiovascular and mydriatic effects are of central origin, then intercorrelations between mydriasis and the lowering of both blood pressure and heart rate could be anticipated. The results of statistical analyses presented in Table 2 confirm this expectation. The negative logarithm of the dose causing 50% mydriasis, $-\log ED_{50}^{m}$, correlated significantly with $-\log ED_{50}^{h}$ ($P < 0.003$; $r = 0.890$) and with $-\log ED_{50}^{bp}$ ($P < 0.009$; $r = 0.842$).

We did not get any significant correlation between the supposedly centrally mediated mydriatic or cardiovascular effects and the peripheral α_2-adrenoceptor-mediated human blood platelet aggregation in vitro. This observation agrees with the statement by Ruffolo et al. (1991): 'although the α_2-adrenoceptors in human cerebral cortex and human platelets appear to be identical with each other, both appear to be different from those in rat cerebral cortex'.

Of the pharmacological data collected in Table 1, only mydriatic activity correlated significantly with hydrophobicity measures. As a matter of fact, the correlation of the $-\log ED_{50}^{m}$ with the chromatographic hydrophobicity parameter, $\log k'_{IAM}$, was rather weak ($P < 0.06$; $r = 0.686$) but the correlation between $-\log ED_{50}^{m}$ and the classical octanol-water hydrophobicity parameter, CLOGP, was significant at 98% significant level ($r = 0.797$). The better correlation obtained with CLOGP might suggest that undissociated forms of the agents determine the mydriatic effect.

Whereas the subtype of α_2-adrenoceptor present in human platelet membranes is rather unanimously classified as α_{2A} (Nahorski et al., 1985; Bylund, 1985; Harrison et al., 1991), individual authors are of the opinion that α_2-adrenoceptors in rat cerebral cortex are of the α_{2A} subtype (Alberts, 1993; Uhlén et al., 1992), a mixture of α_{2A} and α_{2B} (Petrash and Bylund, 1986), an α_{2D} subtype (Trendelenburg et al., 1993), etc. There is also a report (Deckert et al., 1991) that central α_2-adrenoceptors in rats are responsible for hypotension and bradycardia, but these receptors are accompanied by specific imidazoline binding sites which do not contribute to heart rate control but which are engaged in the hypotensive action of the drugs. The high correlation between bradycardic and hypotensive effects of the imidazol(in)es observed here and reported elsewhere (Timmermans and Van Zwieten, 1982) would rather contradict the suggestion by Deckert et al. (1991), unless the input of the postulated imidazoline binding sites to cardiovascular effects is of secondary importance.

It should be mentioned here that we performed several experiments with oxymetazoline, which is reported to stimulate the α_{2A} subtype of adrenoceptor (Petrash and Bylund, 1986). We noted rather weak mydriasis at very high doses of the agent, e.g., with 3000 μg/kg of oxymetazoline $65 \pm 3\%$ of the maximum clonidine response was observed. The doses appear too high for specific responses and we excluded oxymetazoline from our considerations. Its low mydriatic activity supports the view that the receptors determining mydriasis are not pure α_{2A}-adrenoceptors which are typical for human blood platelets.

Our data support the assumption that α_2-adrenoceptors in human blood platelets are different from those responsible for the centrally mediated effects of imidazoles and imidazolines in the rat. This is not only due to the lack of correlation between ED_{50}^{m} and IC_{25} but also because the first parameter depends on hydrophobicity whereas the latter does not.

The hydrophobicity of imidazoline derivatives has been shown (Walland and Hoefke, 1974) to be of importance for the central antihypertensive activity of the drugs when injected intravenously. This was not observed by Struyker Boudier et al. (1976) who administered a set of imidazolines intracerebrally. Attempts to quantitatively describe the hypotensive activity of imidazolines in terms of hydrophobicity were rather unsuccessful (De Jong and Soudijn, 1981; De Jonge et al., 1984). The hydrophobicity parameters used in the regression analysis appeared of secondary importance for activity at best. In our correlation analysis, the parameter CLOGP was correlated at only 80% significance level with $-\log ED_{50}^{bp}$. Thus, hydrophobicity alone cannot be a molecular feature determining the hypotensive activity of the series of imidazol(in)es studied. The same holds true for the bradycardic activity of the agents.

The dependence of the mydriatic effect on the hydrophobicity of imidazol(in)es and the lack of significance of the hydrophobicity parameter in describing cardiovascular effects do not prove unequivocally the heterogeneity of central α-adrenoceptors in rats. The observed increase in mydriatic activity with increasing lipophilicity of the agents may be due to a better

penetration of the drugs to the centrally localized α_2-adrenoceptors and not due to the stronger drug-receptor interactions. Anyway, the α_2-adrenergic activity of drugs in the model of mydriasis in rats is not the same as the α_2-adrenergic activity measures in the model of human platelet aggregation in vitro.

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