



Structure–Activity Relationship at α -Adrenergic Receptors Within a Series of Imidazoline Analogues of Cirazoline

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Abstract—Several analogues of cirazoline (**2**), a selective α_1 -adrenoreceptor agonist, were prepared and their pharmacological profiles studied. Although at the α_1 -adrenoreceptor all the compounds displayed a significant agonist activity, at the α_2 -adrenoreceptor they showed either agonist or antagonist activity depending on the nature of the phenyl substituent. The qualitative structure–activity relationship led us to the conclusion that the oxygen atom in the side-chain is essential for α_1 -agonist activity, while the cyclopropyl ring is not, and may be replaced by several groups. Of the groups studied, isopropoxy appears to be the best. Instead, the same substitution (i.e., isopropoxy for the cyclopropyl ring) at α_2 -adrenoreceptors causes a reversal of activity. On the other hand, the cyclopropyl ring seems to be important for α_1 -selectivity. Compound **20** is the most potent α_1 -agonist of the series, being equiactive with cirazoline on rat vas deferens and in pithed rat. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Since the discovery of α -adrenergic receptor subtypes, an intense effort has been mounted to develop specific agonists and antagonists as potential therapeutic agents and a significant amount of structure–activity data has emerged in pursuit of these goals.^{1–3}

One of the most studied classes of compounds is the imidazolines, which have been shown to be very peculiar in that small structural modifications can alter the balance between agonist and antagonist, as well as the α_1/α_2 selectivity.^{2,4}

Idazoxan (**1**) has been described as a selective α_2 -adrenoreceptor blocking agent.⁵ Extensive structure–activity relationship studies have been carried out on **1** to determine the effect of substituents on both aromatic and imidazoline rings, as well as of structural modification of these moieties.^{6–10} Opening the benzodioxane ring of **1** to give **7** (Chart 1) resulted in a drop in α_2 -adrenoreceptor antagonist potency and, interestingly, disclosure of a selective postsynaptic α_1 -adrenoreceptor

agonist activity.⁶ These results, together with the structural similarity between **7** and cirazoline (**2**), prompt us to investigate further the effects of other substituents in the *ortho*-position of the phenyl ring. In preceding papers^{11,12} we reported on the α -adrenergic activity of compounds **4–7**, **10–18** and **20**.

Instead, here we report on the synthesis of compounds **8**, **9**, **19** and **21–24** and on the α -adrenergic activity obtained, on the rat, of the imidazolines **4–24** (Chart 2).

Chemistry

Compounds **8**, **9**,¹³ **19**¹³ and **21–24** were synthesized as shown in Scheme 1, through the alkylation of appropriate phenol with **25** or **26**.¹⁴ The free bases were transformed into the corresponding hydrochloride (**8**, **9**, **19**) or hydrogen oxalate salts (**21–24**). In the case of compound **24**, the free base was obtained by oxidation of the 2-thiomethyl precursor with H₂O₂/CH₃COOH.

Pharmacology

The compounds in Chart 2, as hydrogen oxalate salts (**4**, **5**, **11**, **16**, **20–24**) or hydrochloride salts (**6–10**, **12–15**,

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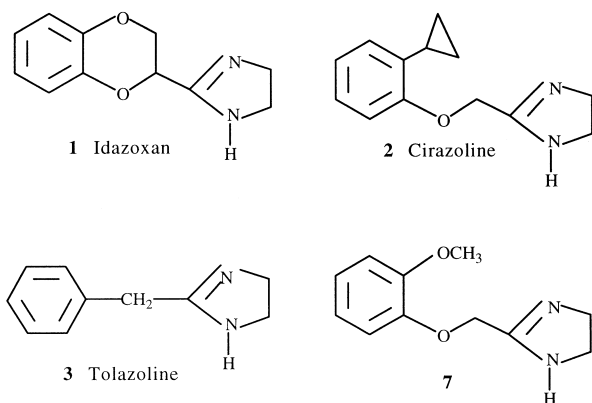
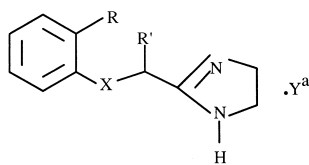


Chart 1.

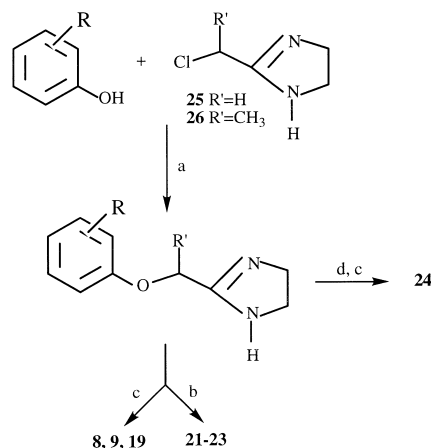


Compd	X	R	R'	Compd.	X	R	R'
4	CH ₂	H	H	15	O	i-C ₃ H ₇	CH ₃
5	S	H	H	16	O	sec-C ₄ H ₉	H
6	O	H	H	17	O	ter-C ₄ H ₉	H
7	O	OCH ₃	H	18	O	CH ₂ CH=CH ₂	H
8	O	3-OCH ₃	H	19	O	OC ₂ H ₅	H
9	O	4-OCH ₃	H	20	O	O <i>i</i> -C ₃ H ₇	H
10	O	CH ₃	H	21	O	O <i>i</i> -C ₃ H ₇	CH ₃
11	O	CH ₃	CH ₃	22	O	SCH ₃	H
12	O	C ₂ H ₅	H	23	O	SCH ₃	CH ₃
13	O	n-C ₃ H ₇	H	24	O	SOCH ₃	CH ₃
14	O	i-C ₃ H ₇	H				

^aY = C₂O₄H₂ for compounds 4, 5, 16, 20–24. Y = HCl for compounds 6–10, 12–15, 17–19.

Chart 2.

17–19), were tested for α_1 - and α_2 -adrenergic activity using epididymal and prostatic portions, respectively, of isolated rat vas deferens, following standard testing procedures.^{15,16} Agonist potency of the drugs was expressed as pD_2 . In the case of antagonists, pA_2 values, calculated according to Arunlakshana and Schild¹⁷ or van Rossum,¹⁸ were used. In order to determine the interaction at α_1 -adrenoreceptor, antagonism with prazosin was studied for a few selected compounds. Prazosin displaced the dose–response curves to the right in parallel manner and to a very similar extent to the displacement of noradrenaline and cirazoline dose–response curves (Table 1). Interaction at α_2 -adrenoreceptor was determined by the ability of idazoxan or yohimbine to reverse the twitch inhibition of electrically stimulated rat vas deferens caused by the agonist under study. Compounds 10, 14–16, 19 and 20 were also studied in pithed rat, and the contribution of α_1 and α_2 vascular adrenoreceptors to the increase of mean arterial pressure was determined by using selective antagonists.



Scheme 1. (a) Na/C₂H₅OH; (b) (CO₂H)₂/(C₂H₅)₂O; (c) HCl concd/C₂H₅OH; (d) H₂O₂/CH₃COOH.

Table 1. Antagonist potency of prazosin against a few selected agonists

Compd	pA_2^a
Noradrenaline	8.85 ± 0.04
2	8.93 ± 0.03
7	9.01 ± 0.07
15	8.94 ± 0.09
16	8.85 ± 0.08
19	8.98 ± 0.07
20	9.15 ± 0.11

^a pA_2 values were calculated according to Arunlakshana and Schild¹⁷ and are the means plus or minus SEM of, in each case, a minimum of six experiments.

Results and Discussion

In the present investigation, the α_1 - and α_2 -adreno-receptor activities of the compounds 4–24 were determined and compared with those of idazoxan (1), cirazoline (2) and tolazoline (3). The results are shown in Tables 2 and 3. It can be seen that opening the benzodioxane ring of idazoxan (1) to give 7 results in a 30-fold reduction of α_2 -antagonist potency while at α_1 site a reversal of activity is seen, since compound 7 is a nearly full agonist. These findings confirm the results previously reported by Chapleo et al.⁶ Compound 7 may be regarded as a very close derivative of cirazoline (2) where the cyclopropyl substituent has been replaced by a methoxy function. The *meta* (8) and *para* (9) isomers are less active than the *ortho* one, which in turn is slightly more active than unsubstituted compound 6, indicating that a substituent in the *ortho* position may positively contribute to drug–receptor interaction.

Tolazoline (3) is a 10-fold selective α_2 -adrenoreceptor antagonist. The introduction of a second methylene group between the phenyl and imidazoline rings (4) causes a 10-fold decrease in affinity at both subtypes. If bridge lengthening involves a heteroatom, such as sulphur or oxygen, as in 5 or in 6 the antagonist activity at α_2 site is maintained, while at α_1 site there is a reversal of activity, since compound 5 is a partial agonist and compound 6 a nearly full agonist. Ruffolo et al.,¹⁹ studying the effect of the bridge length in another series

Table 2. α_1 - And α_2 -adrenoreceptor pD_2 (or pA_2) and intrinsic activity (ia) values in the isolated rat vas deferens

Compd	α_1		α_2	
	pD_2^a	ia ^c	pD_2^b	ia ^c
1 (Idazoxan)	5.99 ± 0.10 ^b	—	8.02 ± 0.08 ^b	—
2 (Cirazoline)	7.33 ± 0.27	1.00	5.50 ± 0.11 ^b	—
3 (Tolazoline)	5.42 ± 0.06 ^b	—	6.55 ± 0.07 ^b	—
4	4.51 ± 0.07 ^b	—	5.60 ± 0.06 ^b	—
5	4.69 ± 0.16	0.60 ± 0.09	6.03 ± 0.05 ^b	—
6	5.26 ± 0.17	0.95 ± 0.06	6.71 ± 0.10 ^b	—
7	5.74 ± 0.33	0.80 ± 0.08	6.51 ± 0.04 ^b	—
8	4.37 ± 0.15	0.57 ± 0.07	5.72 ± 0.07 ^b	—
9	2.78 ± 0.16	0.33 ± 0.05	5.84 ± 0.14 ^b	—
10	3.76 ± 0.08	0.56 ± 0.04	6.67 ± 0.15	0.75 ± 0.08
11	6.01 ± 0.13	0.68 ± 0.04	6.61 ± 0.09	0.96 ± 0.20
12	5.54 ± 0.08	0.81 ± 0.04	6.67 ± 0.24	0.88 ± 0.03
13	6.09 ± 0.09	0.77 ± 0.06	7.06 ± 0.36	0.50 ± 0.13
14	6.62 ± 0.09	1.00 ± 0.04	6.43 ± 0.06 ^b	—
15	6.88 ± 0.12	0.77 ± 0.05	7.31 ± 0.13	0.94 ± 0.03
16	6.55 ± 0.02	0.93 ± 0.07	6.84 ± 0.07	0.92 ± 0.02
17	5.63 ± 0.08	0.85 ± 0.05	6.40 ± 0.08 ^b	—
18	5.84 ± 0.12	0.80 ± 0.07	6.65 ± 0.07 ^b	—
19	6.50 ± 0.05	1.00 ± 0.03	6.34 ± 0.17 ^b	—
20	7.23 ± 0.07	1.00 ± 0.05	7.25 ± 0.08	0.83 ± 0.03
21	6.97 ± 0.18	0.77 ± 0.08	7.44 ± 0.07	1.00 ± 0.04
22	6.41 ± 0.18	0.97 ± 0.06	5.94 ± 0.03	0.88 ± 0.03
23	6.75 ± 0.09	0.79 ± 0.04	7.78 ± 0.07	1.00 ± 0.06
24	5.31 ± 0.11	0.84 ± 0.07	4.58 ± 0.10	0.40 ± 0.11

^a pD_2 is $-\log ED_{50}$.^b pA_2 values, calculated according to van Rossum,¹⁸ using cirazoline (α_1) or clonidine (α_2) as agonist.^cIntrinsic activity (ia) is the maximum effect obtained with the agonist under study, expressed as percentage of those of cirazoline and clonidine, both taken equal to 1. Values are means plus or minus SEM of, in each case, a minimum of six experiments.**Table 3.** Characterization of α -adrenergic receptor-mediated pressor response in pithed rats

Compd	ED_{50}^a (μ g/kg, iv)	Dose ratio ^b	
		Prazosin (0.1 mg/kg, iv)	Yohimbine (1 mg/kg, iv)
2	3.79 ± 1.26	29.5 ± 5.5	1.7 ± 0.6
BHT-920	75.2 ± 0.04	1.9 ± 0.2	12.3 ± 1.2
10	657 ± 204	23.2 ± 7.1	3.6 ± 0.5
14	7.90 ± 0.08	18.0 ± 2.0	2.1 ± 0.5
15	8.06 ± 1.08	10.3 ± 1.2	1.4 ± 0.5
16	20.2 ± 1.3	21.3 ± 5.9	2.2 ± 0.6
19	4.62 ± 0.51	41.0 ± 5.8	2.3 ± 0.4
20	2.03 ± 2.2	21.3 ± 5.9	1.2 ± 0.1

^aDose required to increase diastolic blood pressure by 50 mmHg.^b ED_{50} of agonist following pretreatment with antagonist divided by control ED_{50} .

of tolazoline-like imidazolines, showed a second methylene group to be detrimental for both potency and affinity. The present results, while confirming these findings, indicate the peculiar role of the oxygen in the bridge. Its presence, probably via interaction with an additional binding site, may overcome the decrease of affinity due to the variation of the bridge length, and at α_1 site such an interaction may trigger the biochemical events giving rise to contraction, that is to say the oxygen atom plays a crucial role in determining the efficacy

of the receptor-coupling mechanism. In fact, compound **6** is an α_1 -agonist while its congener tolazoline (**3**) and **4** are α_1 -antagonists.

Cirazoline (**2**) has been known for several years to be a very potent and selective α_1 -adrenoreceptor agonist, possessing also an, albeit modest, α_2 -antagonist activity (ref 20 and present study). Opening the cyclopropyl moiety (**14**) causes a slight decrease of α_1 -agonist activity and a 10-fold increase as α_2 -antagonist. These findings show that the cyclopropyl ring is not an essential structural feature for α_1 -agonist activity, while it appears to be important for α_1 selectivity.

Isosteric replacement of oxygen atom with sulphur was also studied as a substituent in the *ortho* position and compound **22** prepared. Its activity as an α_1 -agonist is about five times higher than the activity of compound **7**. At α_2 -site, the O/S replacement causes a reversal of activity: compound **7** behaves as an antagonist while compound **22** is an α_2 -agonist. Furthermore, oxidation of the sulphur atom to give the sulfoxide **24** decreases both α_1 - and α_2 -activities. This finding is in agreement with our earlier studies with 1,4-benzodioxane-based antagonists where the same isosteric substitutions were investigated.^{10,15}

Compound **20** is the most potent α_1 -agonist ($pD_2 = 7.23$) of the series, equiactive with cirazoline. Although it lacks selectivity, being equiactive as α_2 -agonist ($pD_2 = 7.25$), it shows the role of isopropoxy function, which may replace the cyclopropyl ring. On the other hand, the most potent α_2 -agonist is compound **23** ($pD_2 = 7.78$), while compound **10** shows the highest α_2 -selectivity (about three orders of magnitude). The effect of the insertion of a methyl group in the bridge separating the phenyl and imidazoline rings was not constant: in some cases no variation of the effect was seen, while in some others an increase was observed, together with an interesting change of activity, from antagonist to agonist at α_2 site. It appears that the effect of the methyl group in the bridge depends on the agonist potency of the desmethyl parent compound: the lower the potency of the latter the larger the increase (**10** versus **11** and **22** versus **23**). In an earlier work,¹¹ made on α_1 -adrenoreceptors of the rabbit aorta, where the two parameters that govern agonist potency, affinity and efficacy were separately studied using fewer compounds (**2**, **6–18** and **20**), we showed that the methyl group in the oxymethylene side chain enhances agonist efficacy. Therefore, the increase of agonist potency by a methyl group, found in the present investigation, is most likely related also to an increase of efficacy. The fact that compound **14** is an antagonist (zero efficacy) at α_2 -adrenoreceptor subtype, while its methyl derivative **15** is an agonist, seems to support this explanation.

In addition, although the α_1 -adrenoreceptor activities found in that study, with few exceptions, were higher than those found in the present investigation, the trend is much the same. In both studies, however, with the exception of compound **20**, all the 2-phenoxyethyl-imidazolines were less active α_1 -agonists than cirazoline.

Some selected compounds were also studied in pithed rat. The results are reported in Table 3, together with those obtained with the reference compounds, cirazoline (α_1 -selective agonist) and BHT 920 (α_2 -selective agonist). The most active derivative is again compound **20** with an ED_{50} of 2.03 $\mu\text{g/kg}$ which shows the same potency as cirazoline ($ED_{50} = 3.79$), confirming the peculiar role of isopropoxy group. Since the pressor responses elicited by drugs in pithed rats may be due to a mixed population of post-synaptic vascular α_1 - and α_2 -adren-ergic receptors,^{21–23} the effect of compounds was examined after pretreatment with the α_1 -adrenoreceptor-selective antagonist prazosin (0.1 mg/kg, iv) and the α_2 -adrenoreceptor selective antagonist yohimbine (1 mg/kg, iv) in order to determine the contribution of each subtype to the pressor activity. The dose–ratio values obtained show that the compounds studied produce their effect through α_1 -adrenoreceptors. This is apparently in contrast with the results found in vitro where a high α_2 -adrenoreceptor activity was detected. However, vascular postsynaptic α_2 -adrenoreceptors might be different from the presynaptic ones present in the vas deferens.²⁴

In conclusion we have shown that the oxygen atom in the side-chain of cirazoline is essential for α_1 -agonist activity, while the cyclopropyl ring is not, and may be replaced by several groups. Isopropoxy, among the groups studied, appears to be the best. The same substitution at α_2 -adrenoreceptors causes a reversal of activity. On the other hand, the cyclopropyl ring seems to be important for α_1 -selectivity. These results confirm the peculiarity of this class of compounds since small structural variations change antagonist activity to agonist activity and α_1/α_2 selectivity.

Experimental

Chemistry

Melting points were taken in glass capillary tubes on a Buchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin–Elmer 297 and

Varian EM-390 instruments, respectively. Although the IR and NMR spectra data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. The microanalyses were performed by the Microanalytical Laboratory of our department and the elemental compositions of the compounds agreed to within $\pm 0.4\%$ of the calculated value. The term ‘dried’ refers to the use of anhydrous sodium sulphate.

Synthesis of 23. 2-Thiomethylphenol (1.0 g, 7.1 mmol) was added to a solution of Na (0.3 g, 13.04 mmol) in abs EtOH (15 mL). After 1 h, imidazoline **26**¹⁴ as hydrochloride (1.55 g, 10.0 mmol) was added to the reaction mixture, which was heated to reflux for 6 h under vigorous stirring. The solution was evaporated to dryness to give a residue which was taken up in chloroform (100 mL) and washed with water (25 mL). Removal of the dried solvents gave **23** as the free base which was purified through column chromatography. Eluting with $\text{CHCl}_3:\text{EtOH}:33\% \text{NH}_4\text{OH}$ (13.8:0.2:0.05) gave the free base which was transformed into the hydrogen oxalate salt. Anal. ($\text{C}_{12}\text{H}_{16}\text{N}_2\text{OS}\cdot\text{H}_2\text{C}_2\text{O}_4$) C, H, N.

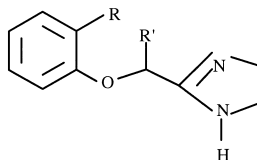
Synthesis of 24. A solution of **23** (0.1 g, 0.423 mmol) and 30% H_2O_2 (0.2 mL) in AcOH (1.25 mL) was left at room temperature for 0.5 h. The mixture was made basic with 2 N NaOH and extracted with CHCl_3 . Removal of the dried solvent gave an oil which was transformed into the hydrogen oxalate salt and crystallized from EtOH. Anal. ($\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_2\text{S}\cdot\text{H}_2\text{C}_2\text{O}_4$) C, H, N.

Compounds **8**, **9**¹³ and **19**¹³ as hydrochloride salts and **21** and **22** as hydrogen oxalate salts were obtained following the same procedure used for **23**, from the corresponding phenols (Scheme 1). The physical characteristics of all the compounds are reported in Table 4.

Pharmacology

In all cases, contractions were recorded isometrically by means of a force transducer connected to a two-channel Gemini polygraph (Basile, Comerio, Italy).

Table 4. Physical–chemical characteristics of compounds **8**, **9**, **19**, **21–24**



Compd	R	R'	Formula	Recrytn solvent	Mp ^a (°C)	Yield (%)	Analyses ^b
8	3-OCH ₃	H	C ₁₁ H ₁₄ N ₂ O ₂ ·HCl	EtOH	158–161	50	C, H, N
9	4-OCH ₃	H	C ₁₁ H ₁₄ N ₂ O ₂ ·HCl	<i>i</i> -PrOH	80–83	45	C, H, N
19	OC ₂ H ₅	H	C ₁₂ H ₁₆ N ₂ O ₂ ·HCl	<i>i</i> -PrOH	49–50	19	C, H, N
21	Oi-C ₃ H ₇	CH ₃	C ₁₄ H ₂₀ N ₂ O ₂ ·C ₂ O ₄ H ₂	<i>i</i> -PrOH	79–81	48	C, H, N
22	SCH ₃	H	C ₁₁ H ₁₄ N ₂ OS·C ₂ O ₄ H ₂	<i>i</i> -PrOH	120–122	43	C, H, N
23	SCH ₃	CH ₃	C ₁₂ H ₁₆ N ₂ OS·C ₂ O ₄ H ₂	<i>i</i> -PrOH	98–100	42	C, H, N
24	SOCH ₃	CH ₃	C ₁₂ H ₁₆ N ₂ O ₂ S·C ₂ O ₄ H ₂	EtOH	145–147	52	C, H, N

^aThe heating rate was 1 °C/min.

^bAnalyses for C, H, N were within $\pm 0.4\%$ of the theoretical value required.

Rat vas deferens. Male albino rats (175–200 g) were killed by a sharp blow on the head and both vasa deferentia were carefully removed, freed from adhering connective tissue and transversally bisected.

The epididymal portion was mounted in 20 mL organ baths containing a physiological salt solution (PSS) of the following composition (mM): NaCl 118, KCl 4.7, CaCl_2 2.5, MgCl_2 1.2, KH_2PO_4 1.2, NaHCO_3 25, glucose 11.1.

The initial loading tension was set at 1 g. The medium was maintained at 37°C and was aerated with 95% O_2 –5% CO_2 . The tissues were allowed to equilibrate for at least 1 h before the addition of any drug. Cirazoline concentration–response (contracture) curves were obtained cumulatively and taken as a control.

A 1 h washing period was followed and the tissues were challenged with the agonist under study. Responses were expressed as a percentage of the maximal response obtained in the control curve. It was verified in parallel experiments that the second cirazoline concentration–response curve was identical to the first because the change in ratio of equi-effective concentrations was less than 2, which usually entails a minimal correction. The results are expressed in terms of pD_2 , $-\log \text{ED}_{50}$, where ED_{50} is the concentration of agonist required to produce 50% of the maximum contraction.

When studying prazosin, dose-ratios at the ED_{50} values of the agonists were calculated at three antagonist concentrations (30 min incubation) and each concentration was tested six times. Dissociation constants (pA_2 values, Table 1) were estimated by Schild plot¹⁷ constrained to slope -1.0 . When applying this method, it was always verified that the experimental data generated a line whose derived slope did not significantly differ from unity ($P > 0.05$).

The prostatic portion was used to assess the agonistic activity on α_2 -adrenoreceptors. PSS composition was as given above for the epididymal portions, except that the MgCl_2 concentration was reduced to 0.54 mM and that it contained prazosin (0.1 mM) in order to block post-synaptic α_1 -adrenoreceptors. The tissues were stimulated electrically at 0.1 Hz with square pulses of 3 ms duration at a voltage of 10–15 V. When the twitch response became constant, cumulative concentration–response curves were run in each tissue, which was treated only once with the agonist under study. The results are expressed in terms of pD_2 , $-\log \text{ED}_{50}$, where ED_{50} is the concentration required to produce 50% inhibition of the twitch response.

Compounds devoid of agonist activity were tested as antagonist against clonidine. Dose-ratios at ED_{50} values of clonidine were calculated at one concentration which was tested six times. Dissociation constants were calculated according to the method of van Rossum.¹⁸

Pithed rat. Male normotensive rats (270–330 g) were housed 5 per cage and maintained on a 12 h light/dark

cycle. Food and water were available ad libitum. The animals were anesthetized with equithesin (9.6 g nembutal sodium, 42.6 g chloral hydrate, 21.2 g MgSO_4 , 400 mL propylene glycol, 50 mL ethyl alcohol and water to 1000 mL) 3 mL/kg bw ip. The right jugular vein was cannulated (PESO polyethylene tubing) for drug administration. Blood pressure was measured from the left common carotid artery through a PESO catheter connected to a pressure transducer (P23 ID, Statham, Hato Rey, Puerto Rico). The rats were then pithed by insertion of a steel rod (1.5 mm in diameter) through the skull and foramen magnum down into the spinal canal.²⁵ The tracheal cannula was inserted immediately after pithing and the animals were respired artificially by means of a Harvard Apparatus model 681 Rodent Respirator at a frequency of 35 cycles/min with a volume of 2 mL/100 g. Temperature was maintained at approximately 37°C. The pithed rats received heparin iv (150 iu/kg). The preparation was allowed to equilibrate for at least 30 min before drug administration. The increase in diastolic blood pressure was measured for single doses of the agonists given iv (0.5 mL/kg). Full recovery from the pressor effects with return to pre-injection values was ascertained between subsequent doses. After drug injection, the venous cannula was flushed with 50 mL of isotonic saline solution. The results are expressed in terms of ED_{50} , the concentration of the agonist required to increase the diastolic blood pressure by 50 mm Hg. The pressor response of the test compounds were evaluated after pretreatment with the α_1 -adrenoreceptor selective antagonist prazosin (0.1 mg/kg, iv) or α_2 -adrenoreceptor-selective antagonist yohimbine (1 mg/kg, iv), in order to establish the contribution made by each α -adrenoreceptor subtype to the pressor activity observed. In this case antagonists were given 15 min earlier than the agonist dose.

Statistical evaluation. The results are expressed as the means $\pm$ SEM. Student's-test was used to assess the statistical significance of the difference between two means.

Acknowledgements

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