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Role of the benzylic hydroxyl group of adrenergic catecholamines in eliciting α-adrenergic activity. Synthesis and α_1- and α_2-adrenergic activity of 3-phenyl-3-piperidinols and their desoxy analogs*

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Summary — In order to contribute to the definition of the role played by the benzylic hydroxyl group of adrenergic catecholamines in eliciting α-adrenergic activity, certain 3-phenyl-3-piperidinols (PPOs, **4**) and their corresponding desoxy 3-phenylpiperidine analogs (PPEs, **6**) were synthesized and tested for their α_1- and α_2-adrenergic activity by means of functional tests on isolated preparations. As regards the α_1-adrenergic activity, the values of the activity indices of the cyclic catecholic compounds (PPO **4a** and PPE **6a**) indicate that the benzylic hydroxyl does not play an essential role, provided that the other two active groups are in the pharmacophoric conformation. However, the fact that none of the other non-catecholic cyclic analogs are active on the α_1-receptor does not allow us to generalize this observation. As regards the α_2-adrenergic activity, the high values of the activity indices of PPEs **6**, compared with those of the corresponding 1-phenyl-2-aminoethanols (PAEs, **3**), PPOs (**4**) and 2-phenylethylamines (PEAs, **5**), confirm that when the aromatic moiety and the amino group are constrained into the pharmacophoric relationship, the presence of the alcoholic hydroxyl is not only unnecessary for the purposes of the expression of the activity at the level of the α_2-adrenoceptor, but often has a negative effect.

adrenergic drug / 3-phenyl-3-piperidinol / 3-phenylpiperidine / 1-phenyl-2-aminoethanol / 2-phenylethylamine / α_1-adrenergic agonist activity / α_2-adrenergic agonist activity

Introduction

The aromatic moiety, the alcoholic hydroxyl of the side chain and the amino group are the structural features of catecholamines and related drugs (fig 1), which, in accordance with the Easson–Stedman hypothesis [2], have most frequently been implicated in the interaction of these drugs with adrenergic receptors [3–11]. However, while it seems to be clear that the role played by the aromatic moiety and the amino group is that of interacting directly with particular receptor sites, the role of the alcoholic group is still open to discussion [7]. According to some authors [3, 5, 12, 13], the alcoholic hydroxylic group interacts directly with certain sites of the receptor protein. According to other authors [8, 14–17], the function of the hydroxyl is only that of keeping the other two active groups in a suitable spatial relationship. In the latter case, the hydroxyl would not be a requirement for binding and directly stimulating the adrenergic receptor, but its role would be that of having an indirect function in the interaction of catecholamines and correlated drugs with the adrenergic receptor. In fact, a semirigid analog of norepinephrine (**3a**) without the benzylic hydroxyl present in **3a**, compound **1**, has been found to maintain the α_2-adrenergic affinity of **3a** [17], and a conformationally restricted methoxamine (MA) analog **2** without the benzylic hydroxyl present in MA, has been found to maintain the α_1-adrenoceptor stimulating activity of MA [14, 15, 18].

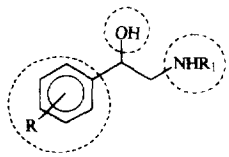
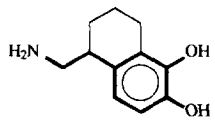


Fig 1. General formula of catecholamines and drugs structurally related to them, showing the moieties which are generally regarded as the three main reactive centers of these molecules.

*Eighteenth paper in the series 'Conformational effects on the activity of drugs'. For preceding paper, see reference [1].



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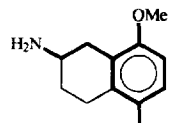
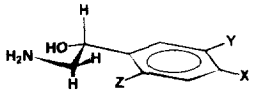


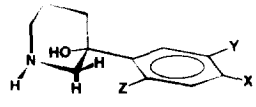
2

It appeared to be likely that the study of the α-adrenergic activity of a series of conformationally restricted analogs of adrenergic drugs, in which all three active groups are in a suitable spatial relationship for interaction with the receptor, together with a comparison of this activity with that of the corresponding analogs without the benzylic hydroxyl, but with the other active centers (aromatic ring and amino group) still constrained into the pharmacophoric spatial relationship, would throw light on the role played by the benzylic hydroxyl group of adrenergic catecholamines in the expression of the α-adrenergic activity.

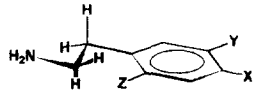
On the basis of the above considerations, the present paper examines the 3-phenyl-3-piperidinols **4** (PPOs), taken as semirigid analogs of 1-phenyl-2-aminoethanols **3** (PAEs), together with the 3-phenylpiperidines **6** (PPEs) which are the desoxy analogs of the PPOs **4** (fig 2). The PPEs **6** are also cyclic analogs of the 2-phenylethylamines **5** (PEAs) which, in turn, are desoxy analogs of PAEs **3**. We chose the same substituents of the aromatic ring of **3–6** as those present in adrenergic drugs with a stimulant activity: compounds **a** contain the hydroxyl groups found in the natural catecholamines in the 3,4-position of the aromatic ring; compounds **b** have the two methoxy groups found in MA [19] in the 2,5-position; compounds **c** have the hydroxyl group present in octopamine (**3c**) [20] in the 4 position; compounds **d** have the amino and hydroxyl groups found in 1-(3-amino-4-hydroxyphenyl)-2-isopropylaminoethanol [21]; and compounds **e** present, albeit in different positions, the two methyl groups found on the phenyl ring of 4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole (dexmedetomidine) [22].



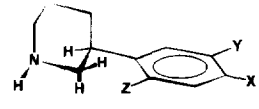
3a, X = Y = OH, Z = H
3b, X = H, Y = Z = OCH_3
3c, X = OH, Y = Z = H
3d, X = OH, Y = NH_2, Z = H
3e, X = Y = CH_3, Z = H



4a, X = Y = OH, Z = H
4b, X = H, Y = Z = OCH_3
4c, X = OH, Y = Z = H
4d, X = OH, Y = NH_2, Z = H
4e, X = Y = CH_3, Z = H



5a, X = Y = OH, Z = H
5b, X = H, Y = Z = OCH_3
5c, X = OH, Y = Z = H
5d, X = OH, Y = NH_2, Z = H
5e, X = Y = CH_3, Z = H



6a, X = Y = OH, Z = H
6b, X = H, Y = Z = OCH_3
6c, X = OH, Y = Z = H
6d, X = OH, Y = NH_2, Z = H
6e, X = Y = CH_3, Z = H

Fig 2. Perspective view of the molecular structure of PAEs (**3**), PPOs (**4**), PEAs (**5**) and PPEs (**6**) in their preferred conformation.

Chemistry

1-Phenyl-2-aminoethanols (PAEs, 3)

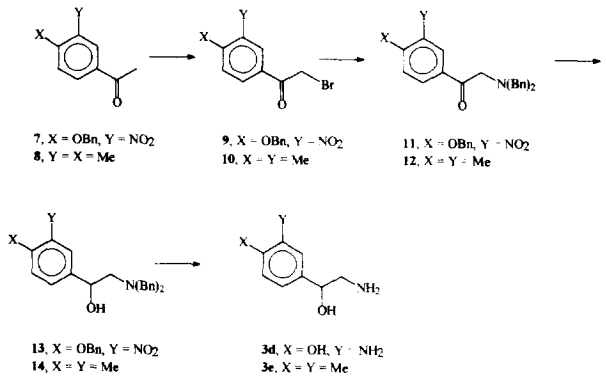
The PAE **3b** was obtained following a previously described synthetic route [23]. The PAEs **3d** and **3e** [24] were synthesized as shown in scheme 1, starting from the acetophenones **7** [25] and the commercially available **8**, respectively, which by reaction with bromine in $CHCl_3$ gave the corresponding ω-bromoacetophenones **9** [21] and **10** [26]. Treatment of **9** and **10** with dibenzylamine yielded the corresponding aminoketones **11** and **12** [27]. Reduction of **11** and **12** with $NaBH_4$ afforded the aminoalcohols **13** and **14** [27], which were transformed into the desired PAEs **3d** and **3e** by catalytic hydrogenolysis with Pd on charcoal

3-Phenyl-3-piperidinols (PPOs, 4)

The PPO **4a** was prepared as previously described [28]. The PPOs **4b–e** were synthesized as shown in scheme 2. Treatment of the 3-piperidone **18** [29] with the appropriate arylmagnesium bromide **15–17** yielded the corresponding piperidinols **19–21**. Catalytic hydrogenolysis of **19–21** with Pd on charcoal afforded **4b**, **4c** and **4e**, respectively. Nitration of **4c** with fuming HNO_3 at –40°C yielded the mononitro derivative **22**, which by subsequent catalytic reduction with H_2 in the presence of Pd on charcoal afforded the desired PPO **4d**.

2-Phenylethylamines (PEAs, 5)

The PEAs **5d** [30, 31] and **5e** [32] were obtained as outlined in scheme 3. Treatment of the *p*-methoxybenzonitrile **23** with fuming HNO_3 at –40°C afforded the mononitro derivative **25** [33], which by reaction with H_2 and Pd on charcoal in the presence of HCl, afforded the diamine **27**. Subsequent demethylation of **27** with 48% aqueous HBr yielded the PEA **5d** [30, 31]. Treatment of the dimethyl-substituted benzyl-

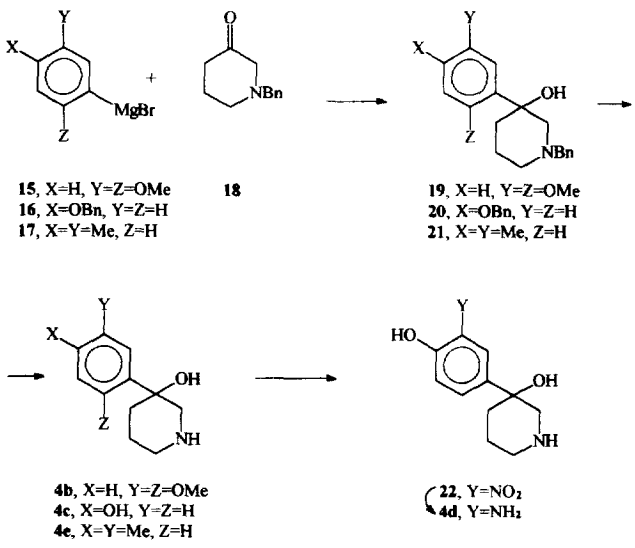


Scheme 1.

chloride **24** with tetraethylammonium cyanide yielded the corresponding benzonitrile **26** [34], which was transformed into the PEA **5e** [32] by catalytic reduction with H_2 and Pd on charcoal in the presence of HCl.

3-Phenylpiperidines (PPEs, 6)

The PPE **6a** was prepared as previously described [35]. The PPEs **6b–e** were synthesized as shown in scheme 4. The cross-coupling reaction of the appropriate arylmagnesium bromide **15**, **28** or **17** with 3-bromopyridine (**29**) catalyzed by dichlorobis-(triphenylphosphine)Ni(II) [36], yielded the corresponding 3-phenylpyridines **30** [37], **31** [34], and **32** [38], which by hydrogenation in the presence of PtO_2, afforded the PPEs **6b** [37, 39], **33** [40] and **6e**, respectively. Demethylation of **33** with 48% aqueous HBr gave the PPE **6c** [40]. Nitration of **6c** with fuming HNO_3 at –40°C yielded the mononitro derivative **34** which by subsequent catalytic reduction with H_2 in the presence of Pd on charcoal afforded the desired PPE **6d**.



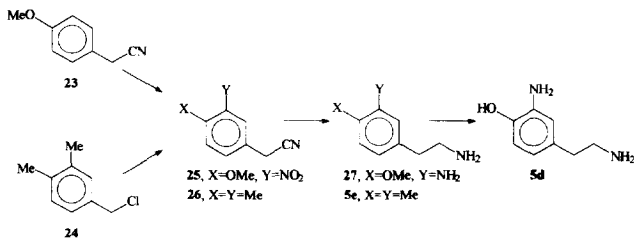
Scheme 2.

Conformation

As far as the conformation of PAEs (**3**) is concerned, the findings of both theoretical and experimental conformational studies [6, 23, 28, 41, 42] indicate that these aminoalcohols preferentially exist in the conformation along the C-C bond of the aminoethanol side-chain, in which the aryl and the amino groups are *anti*. This conformation, which corresponds to the one indicated as **A** in figure 3, is actually favored with respect to those of conformers **B** and **C**, because conformer **A** possesses the fewest non-bond interactions and is also stabilized by the formation of an intramolecular hydrogen bond between the alcoholic hydroxyl and the amino group, which are in a *gauche* relationship. In particular, for **3a** [28] and **3b** [23], as salts in solution, the rotameric distribution between the three conformers **A**, **B**, and **C** was found to be 72%, 11%, 17% and 70%, 16%, 14%, respectively.

The preferred conformation of PPOs **4** can be established through their IR spectra in the 3 μ region, simply on the basis of the presence or absence of the OH...N hydrogen bond; only in one of the possible chair conformers is there the possibility of intramolecular hydrogen bonding between the hydroxyl and the ring nitrogen [29, 43–45]. The presence in the IR spectra of the PPOs **4b**, **4c** and **4e** (see *Experimental protocols*) of a strong band due to this interaction [44, 46–48] indicates that their preferred conformation is the one with the hydroxyl group axial and the bulky aryl group in the more favorable equatorial position (see **A** figure 5 below). As regards **4a** and **4d**, the instability of their free bases unfortunately makes it impossible to record their IR spectra in dilute solution. However, their preferred conformation can be assumed on the basis of that determined for **4b**, **4c**, **4e** and other previously studied 3-piperidinols [29, 45].

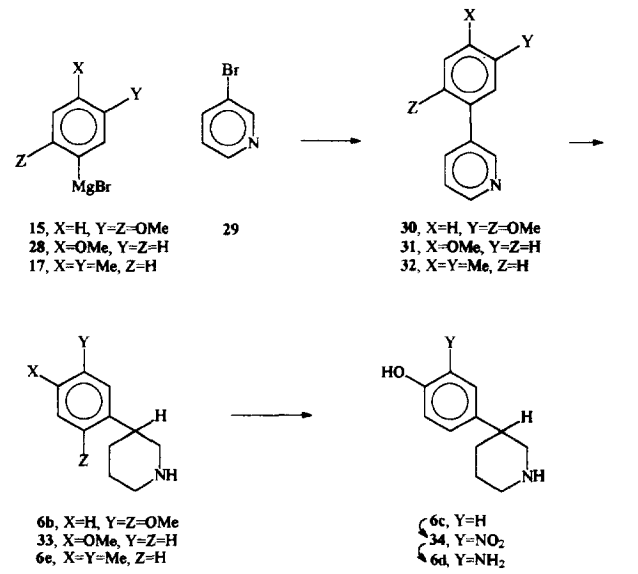
The conformational data [49, 50] concerning dopamine (**5a**) indicate that it presents a very high conformational freedom. However, both theoretical and NMR spectral data [51] indicate that the preferred conformation of **5a·HCl** (43%) is the *trans*-extended conformation (the **A** conformer of fig 4). The conformational situation of the other PEAs (**5b–e**) can be



Scheme 3.

assumed by analogy with the known conformation of **5a**, because the substitutions on the aromatic ring of **5b–e** should not substantially influence the conformation distribution around the side-chain C-C bond.

The conformational situation of PPEs **6** can be assigned on the basis of findings for the analog 3-(*m*-hydroxyphenyl)-*N*-propylpiperidine; theoretical [52] and experimental [53] studies have shown that this compound exists in the conformation in which the piperidine ring is in the chair conformation and the aromatic ring is in the equatorial position (see **B** fig 5).



Scheme 4.

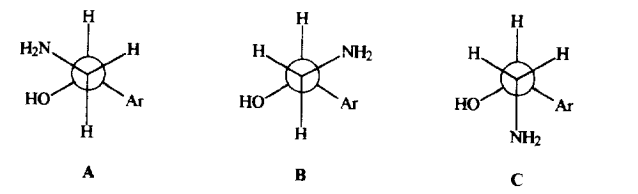


Fig 3. Newman's projections along the side chain C-C bond of the conformers of PAEs, **3**.

Pharmacology

Results

α-Adrenergic activity

Compounds **3–6** were tested on isolated rat vas deferens for their activity on α_1-receptors and on isolated guinea-pig ileum for their activity on α_2-receptors (table I).

Rat vas deferens α_1-receptors

Norepinephrine **3a** contracted smooth musculature of isolated vas deferens *via* activation of α_1-receptors, exhibiting a pD_2 value of 5.20, in agreement with the value previously reported by us [11]. The PPO **4a** and the PPE **6a** showed a stimulating activity comparable to that of **3a**; their pD_2 and ia (intrinsic activity) values did not differ significantly from those of the full agonist **3a**. The PEA **5a** maintained the stimulating activity, with an ia practically identical to that of **3a**, but with a pD_2 value which is about one order of magnitude lower than that of the reference drug [54].

The pD_2 value found for the PAE **3b** was in agreement with a previously reported value [55]. The PEA **5b** presented an agonistic activity with a decrease in both receptor sensitivity and ia with respect to **3b**. The cyclic compounds **4b** and **6b** were completely devoid of any agonistic activity.

Octopamine **3c** presented an agonistic activity characterized by a lower pD_2 value and a higher ia value than that of **3a**. Tiramine (**5c**) presented a pD_2 value comparable to that of **3c**, but its ia decreased to such a level that the profile of the compound is that of a partial agonist of very low efficacy. The cyclic compounds **4c** and **6c** did not show any agonistic activity.

The PAE **3d** showed a pD_2 value of the same level as that of **3c**, with a further increase in the ia. Compounds **4d**, **5d** and **6d** were completely devoid of any agonistic activity on α_1-receptors.

The PAE **3e** and the PEA **5e** showed pD_2 values of about 1.5 units below that of **3a**, and low values of intrinsic activity. The corresponding cyclic compounds **4e** and **6e** were found to be completely inactive.

All the effects observed were produced by a direct mechanism. In the case of the compounds which

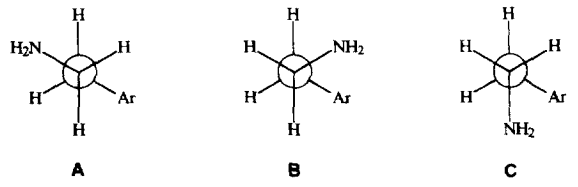


Fig 4. Newman's projections along the side chain C-C bond of the conformers of PEAs, **5**.

possess direct and indirect mechanisms (**3c** and **5c**), the assays were performed on tissues obtained from reserpinized animals. For the other compounds, some additional tests performed on tissues from reserpine-pretreated rats gave practically identical results to those obtained from tissues from untreated animals.

The PPOs **4b–e**, the PEA **5d** and the PPEs **6b–e**, which did not exhibit any agonist activity were tested for antagonism on this receptor population. Compounds **4b–d**, **5d** and **6c–d** were completely devoid of any antagonist properties, while compounds **4e**, **6b**, and **6e** showed only a very weak activity ($-\log IC_{50} \leq 3.50$).

Guinea pig-ileum α_2-receptors

All the compounds **3a–6a** exhibited a stimulating effect on the α_2-adrenoceptors located on cholinergic fibers of the guinea-pig ileum. The physiological mediator **3a** exhibited a pD_2 and ia values in agreement with previously reported values [11]. The PPO **4a** and the PEA **5a** showed pD_2 values about one or two orders of magnitude lower than that of **3a**, respectively. The PPE **6a** showed an activity index (7.03) higher than that of **3a** (6.60). The ia was equal to 1.0 for all four compounds.

The PAE **3b** exhibited a modest affinity and a modest ia on α_2 receptors. Both activity and ia indices increased on passing to PEA **5b**, PPO **4b** and then to PPE **6b**, which presented a pD_2 value of 6.27 and an ia value of 1.0.

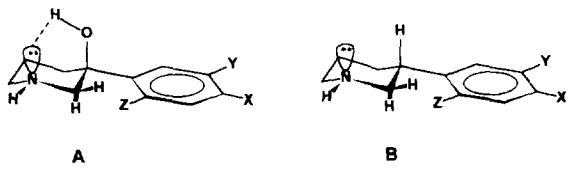


Fig 5. Preferred chair conformers of PPOs, **4** (**A**) and PPEs, **6** (**B**). Broken lines indicate the intramolecular hydrogen bond.

Octopamine **3c** showed an appreciable activity index, which is accompanied by a low ia. The PPO **4c** and the PPE **6c** exhibited pD_2 values lower than that of **3c**, but a higher ia. Tiramine **5c** was practically devoid of any stimulating activity.

The PAE **3d** showed an appreciable affinity index, together with a weak ia. The PPO **4d**, the PEA **5d** and the PPE **6d** exhibited a pD_2 value similar to that of **3d**, which is accompanied by a higher ia value.

The PAE **3e** showed a modest affinity, which is accompanied by an ia equal to that of **3a**. The PPO **4e** and the PEA **5e** presented an activity similar to that of **3e**, as far as the pD_2 and the ia indices are concerned. The PPE **6e** showed an ia similar to that of **3e–5e**, which is accompanied by a higher pD_2 value.

Pharmacological discussion

α_1-Adrenergic activity

An examination of the data presented in table I indicates that on passing from the PAEs **3** to the corresponding PEAs **5**, the α_1-adrenergic activity decreases in the case of compounds **a**, **b** and **c**, and disappears in the case of compounds **d**; as regards compounds **e**, the pD_2s of **3** and **5** are practically identical considering the standard errors, whereas **5e** has a higher intrinsic activity than **3e**.

The cyclization of PAEs **3** to PPOs **4** leads to the disappearance of the activity, except in the case of catecholic compounds, which display a slightly lower level of activity.

Moreover, the cyclization of PEAs (**5**) to their corresponding PPEs (**6**) induces the disappearance of the α_1-adrenergic activity, when it is present in the reference phenylethylamine (**5a–c**, **e**). The behavior of catecholic derivatives is also an exception in this case; the activity increases quite appreciably on passing from **5a** to **6a**.

A comparison of the α_1-adrenergic activity of PPOs (**4**) with that of their desoxy analogs, the PPEs (**6**), shows that in the only case in which the PPO is active (**4a**), the corresponding desoxy analog (**6a**) exhibits an improvement in the activity.

A comparison of the activity of PAEs (**3**) with that of their cyclic analogs without the benzylic hydroxyl (PPEs, **6**) shows that the α_1-adrenergic activity disappears in this kind of cyclization, the only exception being that of the catecholic derivatives, among which the piperidine derivative **6a** shows a slightly higher activity than the corresponding open-chain compound **3a**.

α_2-Adrenergic activity

The results of the pharmacological tests on α_2-adrenoceptors are shown in table I. They show that no homogeneous variations are found in the activity on passing

Table I. α-Adrenoceptor agonistic activity of compounds **3a–e** (PAEs), **4a–e** (PPOs), **5a–e** (PEAs) and **6a–e** (PPEs) on isolated preparations.

Compound	*α-Adrenoceptor activity*[a]			
	Isolated rat vas deferens (α_1)		*Isolated guinea-pig ileum (α_2)*	
	pD_2	*ia*[b]	pD_2	*ia*[b]
PAE **3a**	5.20 ± 0.09[c]	1.00[c]	6.60 ± 0.10[d]	1.00[d]
PPO **4a**	4.80 ± 0.18[e]	0.96[e]	5.49 ± 0.10[f]	1.00[f]
PEA **5a**	4.31 ± 0.15[g]	0.91[g]	4.62 ± 0.11[h]	1.00[h]
PPE **6a**	5.37 ± 0.12	0.92	7.03 ± 0.09	1.00
PAE **3b**	5.05 ± 0.15[i]	1.20[i]	4.10 ± 0.18[j]	0.68[j]
PPO **4b**			4.74 ± 0.12	1.00
PEA **5b**	4.26 ± 0.11[k]	0.48	4.58 ± 0.13	0.84
PPE **6b**			6.27 ± 0.10	1.00
PAE **3c**	4.20 ± 0.10	1.33	4.98 ± 0.09	0.57
PPO **4c**			4.20 ± 0.10	1.00
PEA **5c**	3.78 ± 0.12	0.17	< 3.50	
PPE **6c**			4.22 ± 0.11	1.00
PAE **3d**	4.38 ± 0.15	1.48	4.73 ± 0.15	0.63
PPO **4d**			4.38 ± 0.09	0.90
PEA **5d**			5.15 ± 0.10	1.00
PPE **6d**			4.67 ± 0.13	1.00
PAE **3e**	3.85 ± 0.14	0.36	4.33 ± 0.22	1.00
PPO **4e**			4.01 ± 0.15	1.00
PEA **5e**	3.75 ± 0.17	0.61	4.67 ± 0.21	1.00
PPE **6e**			5.12 ± 0.12	0.97

[a]The agonist activity of each compound on the receptors was evaluated by means of pD_2 value, *ie* the negative logarithm of the drug molar concentration, that produces 50% of the maximal effect; the values represent the mean of 4–6 experiments for each drug ± standard error; [b]intrinsic activity, *ie* the ratio between the maximal response elicited by the compound under test and that elicited by the full agonist, namely NE; [c]reference 11: pD_2 5.12 ± 0.10, ia 1.00; [d]reference 11: pD_2 6.56 ± 0.11, ia 1.00; [e]reference 11: pD_2 4.76 ± 0.14, ia 0.95; [f]reference 11: pD_2 5.56 ± 0.21, ia 1.40; [g]reference 54: pD_2 4.38 ± 0.09, ia 0.91; [h]unpublished results: pD_2 4.66 ± 0.16, ia 1.00; [i]reference 55: pD_2 5.09 ± 0.10, ia 1.45; [j]reference 55: pD_2 4.06 ± 0.09, ia 0.70; [k]reference 14: pD_2 4.96 ± 0.10 (for rabbit central ear artery).

from PAEs (**3**) to their corresponding desoxy analogs, the PEAs (**5**). While the dimethoxy- (**b**), aminohydroxy- (**d**) and dimethyl-substituted (**e**) derivatives display an increase in the activity, there is a reduction of the activity, or it even disappears, in the case of the catecholic (**a**) and *p*-hydroxy-substituted (**c**) derivatives, respectively.

The cyclization of PAEs (**3**) to their corresponding PPOs (**4**) also leads to conflicting results. A slight improvement in the activity is found in the case of the dimethoxy-substituted derivatives (**b**), with a reduction in the case of the catecholic compounds (**a**) and a substantial maintenance of the same activity in the remaining cases (**c–e**).

The cyclization of PEAs (**5**) to their corresponding PPEs (**6**) is accompanied by an improvement in the α_2-adrenergic activity, with the exception only of type **d** derivatives for which the activity undergoes a slight reduction.

A comparison of the activity of piperidine derivatives with (PPOs, **4**) or without (PPEs, **6**) the benzylic hydroxyl, shows that the elimination of the piperidinolic hydroxyl group has a positive effect, which is particularly clear in the case of the catecholic (**a**) and the dimethoxy-substituted (**b**) derivatives; their activity remains practically unchanged only in the case of the *p*-hydroxy-substituted compounds (**c**).

The α_2-adrenergic activity generally improves on passing from PAEs (**3**) to their corresponding piperidine analogs without the benzylic hydroxyl group, the PPEs (**6**).

The PEA **5d** and the PPEs **6b** and **6e** are devoid of any α_1-adrenergic activity, and display a good activity on α_2-receptors. This high selectivity towards α_2-receptors might make it possible to use these compounds as a tool in the classification of this receptor's subtypes.

α_2-Adrenergic receptors play an important role in the regulation of many kinds of physiological processes, both in peripheral organs and within the central nervous system [56]. α_2-Selective agonists may be of therapeutic interest in the well-known field of central hypotensive agents, and also as drugs employable in the suppression of opiate withdrawal, analgesia, muscle relaxation in spasticity, attention-deficit hyperactivity disorder in adolescents and children, support for general anesthesia, glaucoma, constitutional growth delay, and diarrhea. Consequently, the possibility of using specific ligands for the various subtypes could have a remarkable impact on future therapy. For this reason, the PPE **6e** appears to be of particular interest, seeing that it displays an exclusive selectivity for α_2-receptors, is also devoid of any β_1-stimulating properties and has an extremely limited activity (pD_2 = 3.8) on β_2-receptors (unpublished results in this laboratory).

Discussion

In some papers that advance the hypothesis that the benzylic hydroxyl of adrenergic drugs does not interact directly with the receptor site [8, 14–17], it is suggested that the role of this group is that of maintaining the other two active groups (aryl group and amino nitrogen) in a steric situation favorable for binding to the receptor. It is commonly accepted [11, 23, 28, 42] that for catecholamines and structurally related drugs, like PAEs, the pharmacophoric conformation corresponds to the preferential one shown in **3**, the **A** conformer in figure 3. In the phenylethylamine derivatives, the preferential conformer shown in **5**, the **A** in figure 4, corresponding to the preferred and pharmacophoric conformer of **3**, is less favored compared with **3**, as a result of a lack of the stabilization afforded by the hydrogen bond between the alcoholic hydroxyl and the amino group. In the 3-piperidinol derivatives (PPOs, **4**) the three active centers of catecholamines are constrained into the steric relationship (see **A** fig 5), which corresponds to that present in the pharmacophoric conformation of **3**. In the piperidine derivatives (PPEs, **6**) the benzylic hydroxyl is not present, but the other two active groups (aryl group and amino nitrogen) are in the same steric relationship (see **B** fig 5) found in the preferred conformations of **3**, **4** and **5**. A comparison of the α-adrenergic activity of PPEs (**6**), which do not contain the alcoholic hydroxyl but have the two remaining active groups in the pharmacophoric conformation, with that of the corresponding derivatives **3**, **4** and **5**, and in particular with that of the PAEs (**3**), may offer an insight into the role played by the benzylic hydroxyl of adrenergic catecholamines in the interaction with α-adrenoceptors.

As regards the activation of the α_1-adrenergic receptor, the similar values of the activity indices of the PPE **6a** and **3a** would appear to indicate that the benzylic hydroxyl of **3a** should not play an essential role, provided that the other two active groups are in the pharmacophoric conformation. On the other hand, the insertion of the hydroxyl into the piperidine structure of **6a** leads to a fall in the activity, even if this group is placed in the correct steric relationship with respect to the other two active groups; the 3-piperidinolic derivative **4a**, however, is not appreciably less active than **3a**. However, the fact that none of the other non-catecholic cyclic analogs are active does not allow us to generalize the observations made with the catecholic compounds. The lack of any α_1-adrenergic activity in the dimethoxy-substituted PPE (**6b**) was not to be expected in the light of the good activity at this receptor type found for compound **2** [14], seeing that the two pharmacophoric groups (aryl group and amino nitrogen) may assume in the same spatial

relationship in both compounds. The only difference is that in **2** (see fig 6), the aryl moiety is conformationally restrained due to its insertion into the tetrahydronaphthalene system. This would appear to indicate that the piperidine nucleus of PPEs **6** is detrimental to the interaction with the α_1-adrenergic receptor. Compound PPE **6a**, in which the aryl is a catechol, has a high α_1-adrenergic activity (even higher than that of **3a** itself). This might support the hypothesis [14, 23, 55, 57] that certain variations in the adrenergic activity, which are observed when adrenergic drugs with an ethanolamine structure are cyclized to conformationally restrained analogs, may also depend on the characteristic features of the aromatic moiety.

As regards the α_2-adrenergic activity, the cyclization of PAEs (**3**) to PPEs (**6**) led to an improvement in the activity in all cases. This fact undoubtedly lends support to the hypothesis that it may also be possible to obtain a stimulating activity at the level of α_2-adrenergic receptors with compounds not including the benzylic hydroxyl group, provided that the other two active groups are in a suitable pharmacophoric steric relationship. The presence of a benzylic hydroxyl in the piperidine structure also has a negative effect at the level of α_2-adrenergic receptors, as in the case of PPOs (**4**), even if the activity of these compounds is not appreciably different from that of the PAEs (**3**).

The good α_2-adrenergic activity observed for the PPE **6a** is in agreement with the value of the affinity for the same receptor reported for the tetrahydronaphthalene derivative **1** [17]; in one of the conformations allowed for the latter compound (see **1** fig 6), the two pharmacophoric groups are in a spatial relationship that corresponds to that found for the same groups in the preferred conformation of **6a**.

The irregular behavior of the activity of the PAEs (**3**) and the corresponding PEAs (**5**) does not allow us to advance any hypothesis about the importance of the differences in the rotameric distribution of **3** and **5** for the purposes of the α_2-adrenergic activity.

Conclusions

The lack of any homogeneous structure–activity relationship (SAR) in the findings for the α_1-adrenergic activity makes it impossible to propose any suggestions about the role played by the benzylic hydroxyl of catecholamines and structurally related drugs in the interaction with this receptor sub-type.

The SAR obtained for the α_2-adrenergic activity, on the other hand, consistently indicates that when the aromatic moiety and the amino group are constrained into the pharmacophoric spatial relationship by the piperidine structure the presence of the benzylic hydroxyl is not only unnecessary for the purposes of the expression of the activity at the level of the α_2-adrenoceptor, but, on the contrary, almost always induces a decrease in the activity. It would therefore appear that the α_2-adrenergic activity of catecholamines and structurally related compounds is not linked with any direct intervention of the benzylic hydroxyl in the drug–receptor interaction.

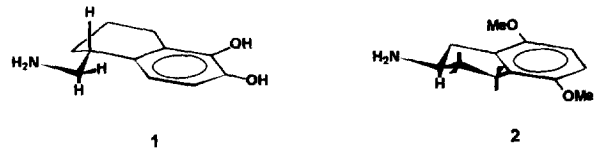
Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparisons between compounds were recorded with a Perkin-Elmer Infracord Model 137 instrument or with an FTIR Mattson 1000 Unicam spectrometer, as Nujol mulls in the case of solid substances, or as liquid film in the case of liquids. IR spectra for the determination of OH...N stretching bands were taken in CCl_4 with a Perkin-Elmer Model 257 double-beam grating spectrophotometer for compounds **19–21** and **4b**, using the indene band at 3110 cm^{-1} as a calibration standard, or with an FTIR Mattson 1000 Unicam spectrometer, for compounds **4c** and **4e**; a quartz cell of 1 cm optical length was employed and the concentration of the solution was 5 x 10^{-3} M or lower, to prevent intermolecular association. ^{1}H-NMR spectra were routinely recorded with a Varian EM 360 A instrument in *ca* 5% solution of $CDCl_3$ (for the neutral compounds or the free bases) or D_2O (for the salts), using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ as the internal standard, respectively. ^{1}H-NMR spectra were also measured for compounds **3e**, **4d–6d**, **6b**, **19** (as salts) and **25** on a Varian CFT-20 spectrometer operating at 80 MHz, and for hydrochloride of **3d** on a Brucker AC-200 instrument. Evaporations were made *in vacuo* (rotating evaporator). Column chromatography was carried out on 70-230 mesh silica gel. $MgSO_4$ was always used as the drying agent. Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within 0.4%.

4-Benzyloxy-3-nitroacetophenone 7

The compound **7** was obtained following the previously described synthetic route. **7**: mp 133–135°C (EtOH) (lit mp [25] 135.5–137°C (*i*-PrOH/2-butanone)); ^{1}H-NMR δ 2.53 (s, 3H, Me), 5.32 (s, 2H, CH_2Ph), 7.12–8.60 (m, 8H, Ph and Ar).



1 2

Fig 6. Perspective view of the molecular structure of tetrahydronaphthalene derivatives **1** and **2** in their allowed conformations.

*ω-Bromo-4-benzyloxy-3-nitro- **9** and ω-bromo-3,4-dimethyl-acetophenone **10***

Compounds **9** and **10** were synthesized by direct bromination of **7** and **8** in $CHCl_3$ as previously described for the preparation of compound **9** [21]. **9**: mp 135–138°C (EtOAc/hexane) (lit mp [21] 141–143°C (EtOAc)); ^{1}H-NMR δ 4.42 (s, 2H, CH_2Br), 5.38 (s, 2H, CH_2Ph), 7.13–8.73 (m, 8H, Ph and Ar). **10**: mp 60–62°C (hexane) (lit mp [26] 63°C); ^{1}H-NMR δ 2.43 (s, 6H, 3,4-Me), 4.55 (s, 2H, $COCH_2Br$), 7.23–8.16 (m, 3H, Ar).

*1-(3-Amino-4-hydroxyphenyl) **3d•2HCl** and 1-(3,4-dimethyl-phenyl)-2-aminoethanol hydrochloride **3e•HCl***

A solution of the appropriate ω-bromoacetophenone **9** or **10** (8.6 mmol) in anhydrous benzene (45 ml) was added dropwise at room temperature to a stirred solution of dibenzylamine (17.2 mmol) in anhydrous benzene (15 ml). After 72 h, the reaction mixture was filtered and the resulting solution was evaporated to yielded crude ω-(dibenzylamino)-4-benzyloxy-3-nitroacetophenone **11** (^{1}H-NMR δ 3.68 (brs, 4H, $N(CH_2Ph)_2$), 3.97 (s, 2H, $COCH_2$), 5.32 (s, 2H, CH_2Ph), 6.90–8.60 (m, 18 H, Ph and Ar)) or ω-(dibenzylamino)-3,4-dimethylacetophenone **12**, which were used in the following reaction without further purification. An analytical sample of **12** had mp 84–86°C (MeOH) (lit mp [27] 84–85°C); ^{1}H-NMR δ 2.36 (s, 6H, 3,4-Me), 3.88 (brs, 6H, $CH_2N(CH_2Ph)_2$), 7.18–7.90 (m, 13H, Ph and Ar).

A cooled (0°C) solution of **11** or **12** (3.2 mmol) in anhydrous EtOH (20 ml) was treated portionwise with solid $NaBH_4$ (0.36 g, 9.5 mmol). The resulting mixture was stirred at room temperature for 2 h, neutralized with 10% aqueous H_2SO_4, concentrated, alkalinized with solid K_2CO_3, and extracted with EtOAc. Evaporation of the washed (H_2O) and dried organic layer, yielded a residue consisting essentially of 1-(4-benzyloxy-3-nitrophenyl)-2-dibenzylaminoethanol **13** or 1-(3,4-dimethylphenyl)-2-dibenzylaminoethanol **14**. **13**: oil ^{1}H-NMR δ 2.65 (d, 2H, J = 7.0 Hz, $CHCH_2N$), 3.50, 3.95 (2d, 4H, J = 13.8 Hz, $N(CH_2Ph)_2$), 4.66 (t, 1H, J = 7.0 Hz $CHOH$), 5.23 (s, 2H, CH_2Ph), 6.95–7.83 (m, 13 H, Ph and Ar). **14**: mp 55–57°C (hexane) ((lit mp [27] 52–53°C (dry Et_2O)); ^{1}H-NMR δ 2.21 (s, 6H, 3,4-Me), 2.63 (d, 2H, J = 7.0 Hz, $CHCH_2N$), 3.45, 3.96 (two d, 4H, J = 13.8, $N(CH_2Ph)_2$), 4.66 (t, 1H, J = 7.0 Hz, $CHOH$), 6.3 (brs, 3H), 7.36 (br, 10H, Ph and Ar).

The crude solution of **13** or **14** (2.14 mmol) in EtOH (25 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of 10% Pd on charcoal (0.5 g). When the absorption stopped, the catalyst was filtered off. The resulting solution was treated with an excess of Et_2O•HCl and the solvent was evaporated to give a residue consisting of **3d•2HCl** or **3e•HCl** which were crystallized from the proper solvent. **3d•2HCl**: amorphous solid (*i*-PrOH/*i*-Pr_2O); ^{1}H-NMR δ 3.28–3.49 (2 dd, 2H, J = 13.0, 8.7 and 4.3 Hz, CH_2NH_2), 5.10 (dd, 1H, J = 8.4 and 4.3 Hz, $CHOH$), 7.21–7.65 (m, 3H, Ar). Anal for $C_8H_{14}Cl_2N_2O_2$ (C, H, N). **3e•HCl**: mp 141–143°C (EtOH/Et_2O) (lit mp [24] 213–215°C (EtOH)); ^{1}H-NMR δ 2.27 (s, 6H, -Me), 3.12–3.28 (m, 2H, CH_2NH_2), 4.93 (dd, 1H, J = 8.0 and 4.8 Hz, $CHOH$), 7.20 (br, 3H, Ar). Anal for $C_{10}H_{16}ClNO$ (C, H, N).

*General procedure for the preparation of 1-benzyl-3-(2,5-dimethoxyphenyl)-**19•HCl**, 1-benzyl-3-(4-benzyloxyphenyl)-**20•HCl** and 1-benzyl-3-(3,4-dimethylphenyl)-3-piperidinol hydrochloride **21•HCl***

A solution of the 1-benzyl-3-piperidone **18** [29] (7.9 g, 41.8 mmol) in anhydrous Et_2O (35 ml) was added dropwise to a stirred solution of the appropriate arylmagnesium bromide **15–17** prepared from Mg (1.20 g, 49.4 mmol) and arylbromide (47.5 mmol) in anhydrous THF (45 ml). The reaction mixture was stirred at room temperature overnight, hydrolyzed to pH 5 with 5% aqueous HCl, and extracted with Et_2O. The combined organic phases were washed (H_2O) and extracted with 5% aqueous HCl. The aqueous extracts were washed with Et_2O, basified with solid KOH, and then extracted with Et_2O. Evaporation of the washed (H_2O) and dried extracts yielded crude **19–21** as an oil, which was dissolved in anhydrous Et_2O and treated with an excess of Et_2O•HCl. The solid precipitate was filtered and crystallized from the appropriate solvent to give pure hydrochloride salt of **19–21**. **19•HCl** (23%): mp 212–214°C (*i*-PrOH). Anal for $C_{20}H_{26}ClNO_3$ (C, H, N). **20•HCl** (31%): mp 191–193°C (MeOH/Et_2O). Anal for $C_{25}H_{28}ClNO_2$ (C, H, N). **21•HCl** (45%): mp 240–241°C (MeOH/Et_2O). Anal for $C_{20}H_{26}ClNO$ (C, H, N).

The hydrochloride salts of **19–21** were converted into the free bases by treating an aqueous solution of the salts with solid KOH and extracting the free bases with $CHCl_3$. The $CHCl_3$ layer was washed (H_2O), filtered and evaporated to give pure **19–21** as oils. **19**: IR (CCl_4) ν 3480 cm^{-1} (OH...N); ^{1}H-NMR δ 1.38–2.99 (m, 8H), 3.55 (s, 2H, NCH_2Ph), 3.78 (s, 6H, 2,5-OMe), 6.56–7.45 (m, 8H, Ph and Ar). **20**: IR (CCl_4) ν 3500 cm^{-1} (OH...N); ^{1}H-NMR δ 1.40–3.10 (m, 8H), 3.56 (s, 2H, NCH_2Ph), 5.06 (s, 2H, CH_2Ph), 6.80–7.73 (m, 14H, Ph and Ar). **21**: IR (CCl_4) ν 3493 cm^{-1} (OH...N); ^{1}H-NMR δ 1.53–3.06 (m, 14H), 3.63 (s, 2H, NCH_2Ph), 7.11–7.60 (m, 8H, Ph and Ar).

*General procedure for the preparation of 3-(2,5-dimethoxy-phenyl)- **4b•HCl**, 3-(4-hydroxyphenyl)- **4c•HCl** and 3-(3,4-dimethylphenyl)-3-piperidinol hydrochloride **4e•HCl***

A solution of the appropriate hydrochloride salt of **19–21** (2.0 mmol) in EtOH (50 ml) was stirred under hydrogen at room temperature and atmospheric pressure in the presence of 10% Pd on charcoal (0.080 g). When the absorption stopped, the catalyst was filtered off and the solution was evaporated to yield a solid residue which was crystallized from the appropriate solvent to afford the pure hydrochloride of **4b**, **4c** and **4e**. **4b•HCl** (60%): mp 135–137°C (*i*-PrOH); ^{1}H-NMR δ 1.56–2.47 (m, 4H), 2.63–3.40 (m, 4H), 3.63 (s, 3H, OMe), 3.70 (s, 3H, OMe), 6.48–6.77 (m, 3H, Ar). Anal for $C_{13}H_{20}ClNO_3$ (C, H, N). **4c•HCl** (89%): mp 200–202°C (MeOH/Et_2O); ^{1}H-NMR δ 1.93–2.20 (m, 4H), 2.86–3.53 (m, 4H), 7.00–7.50 (2d, 4H, J = 8.0 Hz, Ar). Anal for $C_{11}H_{16}ClNO_2$ (C, H, N). **4e•HCl** (90%): mp 220–225°C dec (MeOH/Et_2O); ^{1}H-NMR δ 1.73–2.57 (m, 10H), 2.83–3.63 (m, 4H), 7.22–7.43 (m, 3H, Ar). Anal for $C_{13}H_{20}ClNO$ (C, H, N).

Compounds **4b**, **4c** and **4e**, as salts were converted into the free bases by treating an aqueous solution of the salt with solid KOH for **4b** and **4e** or with concentrate aqueous NH_3 for **4c**, and extracting the free base with $CHCl_3$. Evaporation of the filtered $CHCl_3$ extracts afforded the practically pure piperidinols **4b**, **4c** and **4e** as oils. **4b**: IR (CCl_4) ν 3445 cm^{-1} (OH...N); **4c**: IR (CCl_4) ν 3447 cm^{-1} (OH...N); **4e**: IR (CCl_4) ν 3493 cm^{-1} (OH...N).

*3-(3-Nitro-4-hydroxyphenyl)-3-piperidinol hydrochloride **22•HCl***

Solid **4c•HCl** (2.0 g, 8.7 mmol) was added in portions to a cooled (–40°C) and stirred solution of fuming HNO_3 (14.7 ml). After completion of the addition, the mixture was stirred at –40°C for 0.5 h, poured into ice-water and basified with concentrated aqueous NH_3. The solid precipitate was collected by filtration, dissolved in MeOH and treated with an excess of Et_2O•HCl to yield a solid which was crystallized from MeOH/Et_2O to give pure **22•HCl** (0.5 g, 21%): mp 163–165°C; ^{1}H-NMR δ 1.76–2.47 (m, 4H), 2.83–3.56 (m, 4H), 8.06–8.56 (m, 3H, Ar). Anal for $C_{11}H_{15}ClN_2O_4$ (C, H, N).

3-(3-Amino-4-hydroxyphenyl)-3-piperidinol ***4d·2HCl***
A solution of **22·HCl** (0.2 g, 0.73 mmol) was dissolved in EtOH (20 ml) and then treated with hydrogen in the presence of Pd on charcoal, as reported for the preparation of the hydrochloride salts of **4b**, **4c** and **4e**. Crystallization of the crude product from MeOH/Et_2O yielded **4d·2HCl** (0.10 g, 49%): mp 170–172°C dec; ^{1}H-NMR δ 1.62–2.41 (m, 4H), 2.74–3.63 (m, 4H), 6.91–7.46 (m, 3H, Ar). Anal for $C_{11}H_{18}Cl_2N_2O_2$ (C, H, N).

(3-Nitro-4-methoxyphenyl)acetonitrile ***25***
Compound **25** [33] was obtained by the reaction of **23** (5.15 g, 35.0 mmol) with fuming HNO_3 acid (22.5 ml), following the synthetic procedure previously described for the preparation of **22·HCl**. **25**: mp 84–86°C (hexane/$CHCl_3$); ^{1}H-NMR 3.76 (s, 2H, CH_2CN), 3.98 (s, 3H, OMe), 7.11 (d, 1H, J = 8.8 Hz, Ar), 7.28–7.79 (m, 2H, Ar).

(3,4-Dimethylphenyl)acetonitrile ***26***
A solution of **24** (1.0 g, 6.5 mmol) in acetonitrile (30 ml) was treated with tetraethylammonium cyanide (1.02 g, 6.5 mmol) and the resulting mixture was stirred at room temperature for 12 h. The solution was then treated with 5% aqueous $NaHCO_3$ (30 ml) and extracted with Et_2O. Evaporation of the washed (brine) and dried organic phase gave an oily residue consisting essentially of **26** [34], which was used for the subsequent reaction without further purification.

2-(3-Amino-4-methoxyphenyl)- ***27·2HCl*** *and 2-(3,4-dimethylphenyl)ethylamine hydrochloride* ***5e·HCl***
A solution of **25** or **26** (5.7 mmol) in 10 N aqueous HCl (1 ml) and EtOH (26 ml) was shaken under hydrogen at room temperature and atmospheric pressure in the presence of 10% Pd on charcoal (0.24 g). When the absorption stopped, the catalyst was filtered off, and the solution was evaporated to give a crude residue which was crystallized from MeOH/Et_2O to yield the pure hydrochloride salt of **27** or **5e**. **27·2HCl** (65%): mp 150–151°C dec; ^{1}H-NMR δ 3.57–4.10 (m, 7H), 7.00–7.60 (m, 3H, Ar). Anal for $C_9H_{16}Cl_2N_2O$ (C, H, N). **5e·HCl** (70%): mp 216–218°C (lit mp [32] 218–220°C (EtOH)); ^{1}H-NMR δ 2.36 (s, 6H, 3,4-Me), 2.83–3.50 (m, 4H, CH_2CH_2N), 7.03–7.45 (m, 3H, Ar).

(4-Hydroxy-3-aminophenyl) ethylamine hydrobromide ***5d·2HBr***
A mixture of **27·2HCl** (0.75 g, 3.12 mmol) and 48% aqueous HBr (36 ml) was stirred and refluxed under nitrogen for 1.5 h, then evaporated to dryness. The solid residue was dissolved in MeOH and treated dropwise with anhydrous Et_2O to give a hygroscopic amorphous solid consisting of practically pure **5d·2HBr** [30] (0.50 g, 51%): ^{1}H-NMR δ 2.69–4.08 (m, 4H, CH_2CH_2N), 6.82–7.81 (m, 3H, Ar). Anal for $C_8H_{14}Br_2N_2O$ (C, H, N).

General procedure for the synthesis of 3-(2,5-dimethoxyphenyl)- ***30·HCl****, 3-(4-methoxyphenyl)-* ***31·HCl*** *and 3-(3,4-dimethylphenyl) pyridine* ***32·HCl***
A stirred solution of the appropriate arylmagnesium bromide **15**, **28** or **17** prepared from the corresponding aryl bromide (60.0 mmol) and Mg (1.5 g, 61.7 mmol) in anhydrous THF (150 ml) was treated at 10°C dropwise with a solution of 3-bromopyridine **29** (9.48 g, 60.4 mmol) and dichlorobis-(triphenylphosphine) Ni(II) [36] (0.70 g, 1.1 mmol) in anhydrous THF (135 ml). When the addition was complete the reaction mixture was stirred at room temperature under N_2 for 24 h and then poured into ice-cold 5% aqueous HCl and washed with Et_2O. The aqueous layer was basified to pH 8.0 with solid K_2CO_3 and extracted with Et_2O. Evaporation of the washed (H_2O) and dried extracts yielded a crude residue which was purified by chromatography on a silica-gel column, eluting with 2:3, 3:5 or 3:7 AcOEt/hexane mixtures for **30**, **31** and **32** respectively. **30** (20%): ^{1}H-NMR δ 3.75 (s, 3H, OMe), 3.80 (s, 3H, OMe), 6.93 (br, 3H, Ar), 7.23–8.93 (m, 4H, Py). **31** (35%): ^{1}H-NMR δ 3.84 (s, 3H, OMe), 6.91–8.77 (m, 8H, Ar and Py). **32** (22%): ^{1}H-NMR δ 2.27 (s, 6H, Me), 7.00–8.90 (m, 7H, Ar and Py). Compounds **30–32** were converted into hydrochlorides by dissolving the bases in Et_2O and treatment with an excess of Et_2O·HCl. The solid precipitate was filtered and crystallized from MeOH/Et_2O to yield pure **30·HCl–32·HCl** [35, 37, 38]: **30·HCl**: mp 170–173°C (lit mp [37] 170–173°C (MeOH-Et_2O)). **31·HCl**: 181–183°C (lit mp [35] 55–57°C as free base). **32·HCl**: mp 198–200°C.

General procedure for the synthesis of 3-(2,5-dimethoxyphenyl)- ***6b·HCl****, 3-(4-methoxyphenyl)-* ***33·HCl*** *and 3-(3,4-dimethylphenyl)piperidine hydrochloride* ***6e·HCl***
A solution of the hydrochloride of the appropriate pyridine derivative **30–32** (5.95 mmol) in MeOH (150 ml) was treated with PtO_2 (0.75 g) and the resulting mixture was shaken at room temperature under hydrogen at atmospheric pressure. When the absorption stopped, the catalyst was filtered off, and the solvent was evaporated to yield a solid residue which was crystallized from the proper solvent to give the hydrochloride salts of **6b**, **33** and **6e**. **6b·HCl** (65%): mp 164–167°C (Me_2CO) (lit mp [37] 164–167°C (Me_2CO), lit mp [39], 169.5–170.5°C (*i*-PrOH/hexane) as hydrobromide); ^{1}H-NMR δ 1.75–2.13 (m, 4H), 2.93–3.52 (m, 5H), 3.81 (s, 3H, OMe), 3.84 (s, 3H, OMe), 6.88–7.08 (m, 3H, Ar). **33·HCl** (80%): mp 143–145°C (MeOH/Et_2O) (lit mp [40] 145°C (EtOH)); ^{1}H-NMR δ 1.67–2.20 (m, 4H), 2.83–3.67 (m, 5H), 3.83 (s, 3H, OMe), 7.00, 7.33 (2d, 4H, J = 8.5 Hz, Ar). **6e·HCl** (58%): mp 138°C (MeOH/Et_2O); ^{1}H-NMR δ 1.68–2.20 (m, 4H), 2.23 (s, 6H, 3,4-Me), 2.83–3.76 (m, 5H), 7.10–7.48 (m, 3H, Ar). Anal for $C_{13}H_{20}ClN$ (C, H, N).

3-(4-Hydroxyphenyl)piperidine hydrobromide ***6c·HBr***
A solution of **33·HCl** (2.0 g, 8.8 mmol) in 48% aqueous HBr (20 ml) was stirred under N_2 at 120°C for 2 h, and then evaporated to dryness. Crystallization of the solid residue with Me_2CO/Et_2O yielded pure **6c·HBr** (2.0 g, 88%): mp 189–191°C (lit mp [40] 195°C (EtOH)); ^{1}H-NMR δ 1.63–1.76 (m, 4H), 2.70–3.96 (m, 5H), 6.93, 7.26 (2d, 4H, J = 8.0 Hz, Ar).

3-(4-Hydroxy-3-nitrophenyl)piperidine hydrochloride ***34·HCl***
34·HCl was obtained by the reaction of **6c·HBr** (1.0 g, 3.9 mmol) with fuming HNO_3 (3.0 ml), following the synthetic procedure previously described for the preparation of **22·HCl**. **34·HCl** (0.5 g, 50%): mp 216–218°C (*i*-PrOH); ^{1}H-NMR δ 1.76–2.23 (m, 4H), 2.90–3.70 (m, 5H), 7.73–8.00 (m, 3H, Ar). Anal for $C_{11}H_{15}ClN_2O_3$ (C, H, N).

3-(3-Amino-4-hydroxyphenyl)piperidine hydrochloride ***6d·2HCl***
This compound was prepared by reduction of **34·HCl** (0.50 g, 1.94 mmol) with hydrogen in the presence of 10% Pd on charcoal, following the synthetic procedure reported above for the preparation of compounds **3d·2HCl** and **3e·HCl**. **6d·2HCl** (0.30 g, 58%): mp 194–196°C dec; ^{1}H-NMR δ 1.65–2.35 (m, 4H), 2.78–3.66 (m, 5H), 7.00–7.51 (m, 3H, Ar). Anal for $C_{11}H_{18}Cl_2N_2O$ (C, H, N).

Pharmacological methods

The assays were conducted in accordance with the legislation of the Italian Authorities (DL 27/01/92, No 116) concerning animal experimentation. The animals under Et_2O anesthesia

were killed by cervical dislocation and bled, and then the abdominal cavity was opened by a midline incision. The organs were immediately explanted and placed in cold Tyrode solution (composition (μM): NaCl (1368); KCl (2.95); $CaCl_2$ (1.80); $MgSO_4 \cdot 7H_2O$ (1.05); $NaHPO_4$ (0.41); $NaHCO_3$ (11.9); glucose (5.5)) and gassed with carbogen (95% O_2; 5% CO_2).

Isolated rat vas deferens

α_1-Adrenoceptor activity was assayed on isolated vas deferens taken from Sprague–Dawley male albino rats (200–250 g body weight). Both vasa deferentia were carefully removed without stretching from the epididymis to the prostatic urethra, after moving the intestine to one side. The intact duct was carefully separated from extraneous surrounding tissues and placed in a 10-ml organ bath containing Tyrode solution (pH = 7.4) at 37°C, bubbled with carbogen. The preparation was suspended longitudinally between the organ holder and a force displacement transducer (Basile Model 7006) loaded with 0.5 g connected to a microdynamometer (Basile Model 7050).

For the additional experiments carried out on reserpinized animals, the tissues were obtained from rats pretreated with reserpine (1 mg/kg bw, ip) 24 h before the sacrifice.

Guinea-pig ileum

Dunkin–Hartley male guinea pigs weighing 250–300 g, deprived of food intake for 24 h before the experiments, were sacrificed by a blow on the back of the neck. Portions of ileum 2–3 cm in length, about 10 cm distal to the ileocecal valve, were carefully dissected, freed from the surrounding mesenteric tissue, attached with thread to the organ holder and to the recording system by opposite sides of their open ends, and suspended in a 10-ml organ bath containing Tyrode solution at 37°C gassed with carbogen. The ileum preparations were placed between two platinum electrodes (4 x 45 mm) set at a distance of 7 mm in the bath. The tissues were preloaded with a tension of 0. 5 g and left to stabilize for 45–60 min before beginning electrical stimulation, which was carried out with a digit stimulator (Biomedica Mangoni Model BM-ST3) using the following parameters: single rectangular pulses, 0.1 Hz frequency, 0.3 ms pulse width, 12 V supramaximal voltage. The activity of the tested drugs on α_2-adrenoceptors was evaluated as their ability to inhibit acetylcholine release evoked by electrical stimulation of nerve fibers. The effects of the released mediator on intestinal smooth muscle were recorded as longitudinal contractions by an isotonic transducer (Basile Model 7006) connected with a unirecord microdynamometer (Basile Model 7050).

Agonist activity was expressed in terms of pD_2 values (–log ED_{50}, *ie* the negative logarithm of a drug molar concentration producing 50% of a maximal response), and intrinsic activity (the ratio between the maximal response of a test compound and that of the reference agonist, **3a**).

Antagonist activity was measured by the ability of the compounds tested, after a contact period of 30 min with the tissue, to antagonize the stimulating activity of **3a** on α-receptors. This action was expressed as –log IC_{50} (*ie* the negative logarithm of the drug molar concentration which reduced the stimulating effect of the agonist by 50%).

Reserpine was used as the free base, whereas the following drugs were used as salts: *l*-norepinephrine (**3a**), as bitartrate; **3b**, octopamine (**3c**), tiramine (**5c**) (as oxalates), **3d**, **3e**, **4a–e**, dopamine (**5a**), **5b**, **5e** and **6b–e** (as hydrochlorides) and **5d**, **6a** (as hydrobromides).

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