# Conformational Effects on the Activity of Drugs. 13.[1] A Revision of Previously Proposed Models for the Activation of α- and β-Adrenergic Receptors

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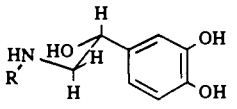
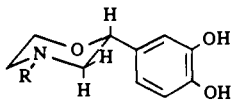
The $\alpha_1$-, $\alpha_2$-, $\beta_1$-, and $\beta_2$-adrenergic properties of the 2-(3,4-dihydroxyphenyl)morpholines **3** and **4** (2-DPMs), of the 3-(3,4-dihydroxyphenyl)-3-piperidinols **5** and **6** (3-DPPs), and of the *trans*-2-amino-5,6-dihydroxytetrahydronaphthalen-1-ols **7** and **8** and the *trans*-2-amino-6,7-dihydroxytetrahydronaphthalen-1-ols **9** and **10** (2-ADTNs) were evaluated in vitro both by radioligand binding assays and by functional tests on isolated preparations and compared with those of norepinephrine (NE, **1**) and isoprenaline (ISO, **2**). Through a comparison of the stereostructures of the compounds examined with their biopharmacological properties, it was possible to revise previously proposed molecular models for the direct activation of α- and β-adrenergic receptors. The revised models (A–C) provided information about the conformational requirements of adrenergic drugs, which substantially fit in with the results of several published studies involving conformationally-restricted adrenoceptor agonists. The different position of the catecholic hydroxyl groups in model B, which refers to the $\alpha_2$ receptors, and in model C, which refers to the β receptors, confirms the importance of the rotameric position of the aromatic ring of catecholamines in the interaction with the α- and β-adrenergic receptor.

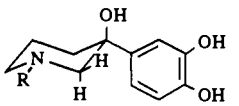
In a previous paper[2] in this series, a comparative study was made of the stereostructures and of the adrenergic stimulating activity of norepinephrine (NE, **1**), isoprenaline (ISO, **2**), and their morpholine (2-DPMs, **3** and **4**), piperidine (3-DPPs, **5** and **6**), and tetrahydronaphthalene (2-ADTNs, **7** and **8**) semirigid analogues.



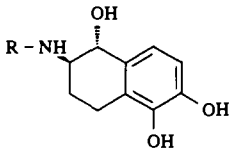
**1**, R = H
**2**, R = $CHMe_2$



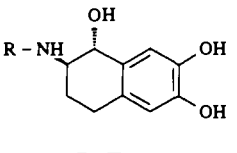
**3**, R = H
**4**, R = $CHMe_2$



**5**, R = H
**6**, R = $CHMe_2$



**7**, R = H
**8**, R = $CHMe_2$



**9**, R = H
**10**, R = $CHMe_2$

Even though compounds **3–8** present a more limited degree of conformational freedom compared with catecholamines **1** and **2**, they (**3–8**) may adopt a number of different conformations. It is not possible a priori to establish the conformation through which these cyclic analogs (**3–8**), as well as NE (**1**) and ISO (**2**), interact with the receptor site. However, it is reasonable to suppose that compounds possessing a similar adrenergic activity will, at the moment of the interaction with the receptor, present their presumed active groups (aryl moiety, amine nitrogen, and alcoholic or ethereal benzylic oxygen)[3] situated in a strictly similar spatial relationship, complementary to that of the active centers of the corresponding receptor. Therefore, those compounds which show a similar adrenergic receptor activity should interact with the receptor in conformations that respond to this requirement.

On the basis of this assumption, molecular models were combined of molecules biologically active on a given receptor, in conformations which allowed the spatial coincidence of the pharmacologically active groups. This combination led to the construction of two three-dimensional molecular models, **a** and **b** (see Figure 1), deriving **a** from drugs active on the α-adrenergic receptor and **b** from drugs active on the β-adrenergic receptor.[2] NE and its morpholine and piperidine analogues (**3** and **5**, respectively) were utilized for the construction of model **a**, while NE, ISO, and their morpholine (**3** and **4**), piperidine (**5** and **6**), and tetrahydronaphthalene (**7** and **8**) analogues were used to obtain model **b**.[2]

These molecular models provided information about steric requirements for direct activation of α and β re-

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(1) For paper 12, see: Balsamo, A.; Breschi, M. C.; Lapucci, A.; Macchia, B.; Macchia, F.; Martinotti, E.; Nencetti, S.; Nieri, P.; Orlandini, E. Synthesis and Evaluation of the Pharmacological Activity of Rigid Analogues of Sympathomimetic Catecholamines Derived from Bicyclo[2.2.1]heptane. *J. Med. Chem.* **1989**, *32*, 856–859.

(2) Macchia, B.; Balsamo, A.; Epifani, E.; Lapucci, A.; Nencetti, S.; Macchia, F.; Breschi, M. C.; Martinotti, E.; Ceserani, R. Conformational Effects on the Activity of Drugs. 11. Stereostructural Models for the Direct Activation of the α- and β-Adrenergic Receptor. *J. Med. Chem.* **1986**, *29*, 740–747.

(3) See for example: (a) Ariens, E. J. The Structure-Activity Relationships of Beta Adrenergic Drugs and Beta Adrenergic Blocking Drugs. *Ann. N.Y. Acad. Sci.* **1967**, *139*, 606–631. (b) Lands, A. M.; Brown, T. G. *Drugs Affecting the Peripheral Nervous System*; Burger, A., Ed.; Dekker: New York, 1967; Chapter 8, pp 399–472. (c) Brittain, R. T.; Jack, D.; Ritchie, A. C. Recent β-Adrenoceptor Stimulants. *Adv. Drug Res.* **1970**, *5*, 197–253. (d) Petrongolo, C.; Tomasi, J.; Macchia, B.; Macchia, F. Molecular Orbital Studies on the Mechanism of Drug-Receptor Interaction. Adrenergic Drugs. Conformation and Reactivity of Isoproterenol and 1-(*p*-Nitrophenyl)-2-isopropylaminoethanol. *J. Med. Chem.* **1974**, *17*, 501–507. (e) Triggle, D. J. Adrenergics: Catecholamines and Related Agents. *Burger's Medicinal Chemistry*; Wolff, M. E., Ed.; Wiley-Interscience: New York, 1981; Chapter 41, pp 225–283. (f) Albert, A. *Selective Toxicity: The Physico-Chemical Basis of Therapy*. Chapman and Hall: London, 1985; Chapter 12. (g) Hoffman, B. B.; Lefkowitz, R. J. Catecholamines and Sympathomimetic Drugs. *The Pharmacological Basis of Therapeutics*; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press: New York, 1990; Chapter 10, pp 187–220.

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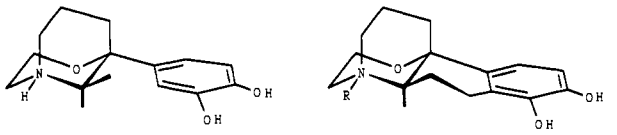


a b

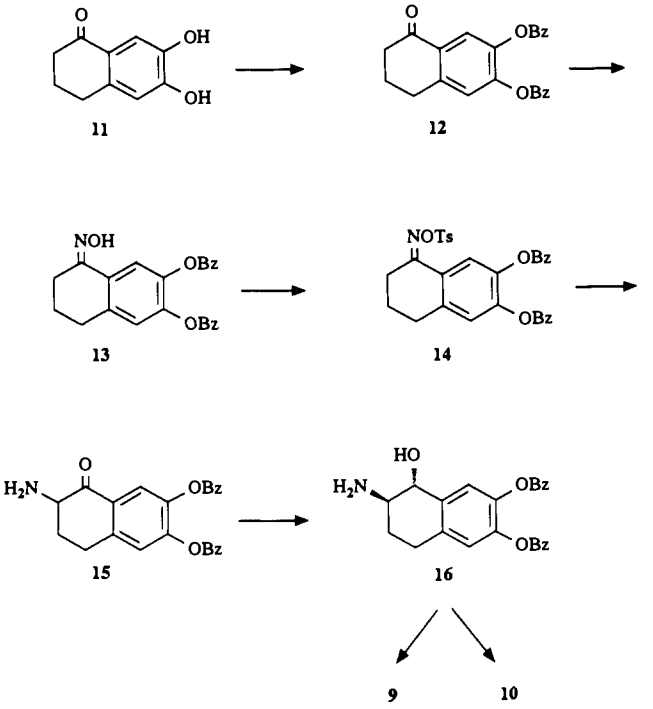
Figure 1.

ceptors, respectively. Moreover, the models indicated molecular portions that should not hinder a "fit" of the drug with the receptor.

In the course of a subsequent study, during which radioligand binding assays on compounds **1–6** were performed, we realized that the values of binding affinities were not always in agreement with the results of the functional tests carried out on the same compounds by pharmacologists from this university. In particular, the practically complete absence of binding affinity found for the 3-DPPs **5** and **6** toward both $\beta_1$ and $\beta_2$ receptors was in poor agreement with the $\beta_1$-adrenergic activity found for the N-unsubstituted 3-DPP (**5**) (comparable to that of NE) and with the $\beta_1$- and $\beta_2$-adrenergic activity found for the *N*-isopropyl-substituted 3-DPP (**6**) (practically equal to that of ISO). At this point, our pharmacologists made a check of the data previously obtained for compounds **1–6**, using the same types of functional tests. The new data showed that **5** and **6**, in agreement with the indications provided by the binding tests, possess a considerably lower $\beta_1$- and $\beta_2$-adrenergic activity than the level previously reported.[2] The rest of the new pharmacological data were found to be in agreement with those previously found, within the limits of variability acceptable for these types of tests.[4] The dramatic change in the level of activity now found for **5** and **6** on $\beta_1$ and $\beta_2$ receptors, with respect to the data previously published, is difficult to explain, unless the hypothesis is admitted that a banal, albeit unjustifiable, exchange of samples may unfortunately have taken place.

In order to allow a more homogeneous comparison of all the pharmacological data, the 2-ADTNs **7** and **8** were synthesized by us and tested: descriptions of their activity were taken from the data of other authors.[5] The evaluation of the $\alpha$-adrenergic activity of all the compounds examined (**1–8**) was also extended to their activity on $\alpha_2$ receptors. Furthermore, during the revision of the pharmacological data of **1–8**, the study was extended also to the analogues of **7** and **8** (**9**[6] and **10**) which differ from **7** and **8** in the position of the phenolic hydroxyls. Although compounds of type **9** and **10** were reported some time ago in literature,[6,7] the only comment made with regard to their adrenergic properties is that they possess no noticeable

Scheme I



11 12 13 14 15 16 9 10

$\beta$-adrenergic activity. The present work is a report on the vitro evaluation of the $\alpha_1$-, $\alpha_2$-, $\beta_1$-, and $\beta_2$-adrenergic activity of compounds **1–10**, based on both radioligand binding assays and functional tests on isolated preparations. On the basis of these results, the molecular models proposed in the above-mentioned work[2] are accordingly revised.

## Chemistry

The 2-ADTNs **7** and **8** were obtained following the synthetic route previously described.[8] The 2-ADTNs **9** and **10** were synthesized as indicated in Scheme I, starting from 6,7-dihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (**11**).[9] Treatment of **11** with benzylchloride yielded the bis(benzyloxy)tetralone **12** which by reaction with hydroxylamine was transformed into the corresponding oxime **13**. Reaction of **13** with *p*-toluenesulfonyl chloride afforded the *O*-*p*-toluenesulfonate (**14**) which by reaction with potassium ethoxide afforded the aminochetone **15**. Reduction of **15** with sodium borohydride yielded exclusively the *trans*-amino alcohol **16** which was catalytically hydrogenolized to *trans*-2-ADTN **9**. Treatment of **16** with acetone and hydrogen in the presence of palladium on charcoal gave the *N*-isopropyl-substituted 2-ADTN **10**; in the course of the catalytic reductive alkylation of **16**, the removal of the benzylic protecting groups also takes place.

The configuration and conformation of the 2-ADTNs **9** and **10** was determined on the basis of their $^1$H NMR spectra. The relatively high values of the coupling constants between the protons linked to the C(1) and C(2) carbons (8.1 and 9.1 Hz for **9** and **10**, respectively) are in agreement with a trans diaxial relationship between them,

(4) There is evidence that various factors, such as species, strain, maturation, aging, etc. of the animals utilized in the experiments can affect the pharmacological responses. See for example: (a) Williams, M.; Sills, M. A. Quantitative Analysis of Ligand-receptor Interactions. *Comprehensive Medicinal Chemistry*; Emmet, J. C., Ed.; Pergamon Press: Oxford, 1990; Vol. 3, pp 45–80 and references therein cited. (b) Docherty, J. R. Cardiovascular Responses in Ageing: a Review. *Pharmacol. Rev.* **1990**, *42*, 103–125.

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(8) Itoh, K.; Motohashi, M.; Kuriki, H.; Sugihara, H.; Inatomi, N.; Nishikawa, M.; Oka, Y. The Syntheses and $\beta$-Adrenoceptor Activities of N-Substituted 2-Amino-5,6-Dihydroxy-1,2,3,4-tetrahydro-1-naphthalenols. *Chem. Pharm. Bull.* **1977**, *25*, 2917–2928.

(9) Ninomiya, I.; Naito, T.; Ishii, H.; Ishida, T.; Ueda, M.; Harada, K. Syntheses of Dihydro-derivatives of the Benzo[c]-phenanthridine Alkaloids Avicine and Nitidine by Enamide Photocyclization. *J. Chem. Soc., Perkin Trans. 1* **1975**, *8*, 762–764.

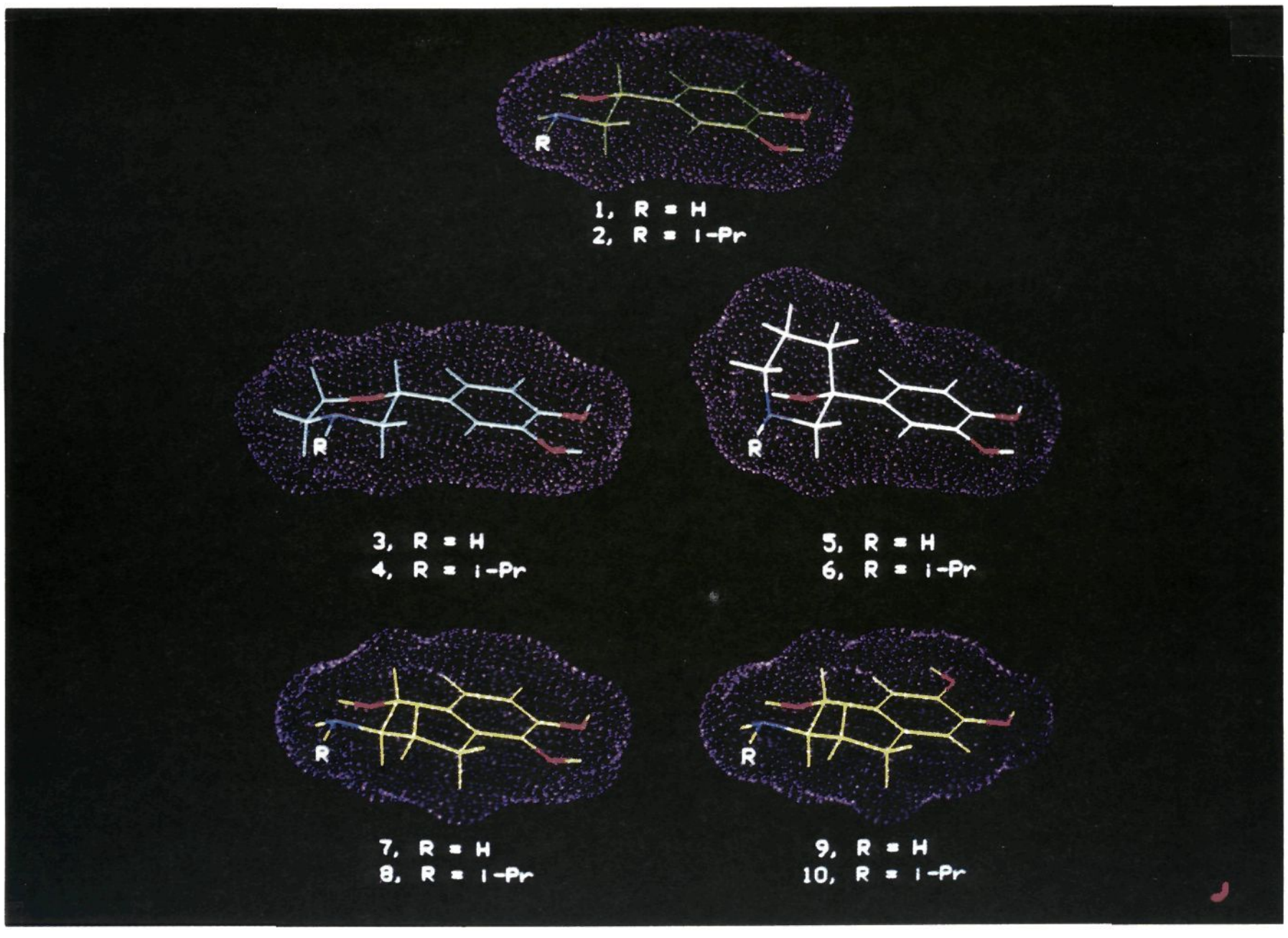



**Figure 2.** Drugs **1–10** in their preferred conformations.[2] NE (**1**) and ISO (**2**) are colored green, the 2-DPMs **3** and **4** cyan, the 3-DPPs **5** and **6** white, and the 2-ADTNs **7–10** yellow; nitrogen and oxygen atoms are blue and red, respectively. The dot clouds indicate the molecular volumes.

thus indicating that the benzylic hydroxyl and the amino group are in the trans diequatorial situation. Furthermore, values both of the $J_{1,2}$ and of the chemical shift of the benzylic proton signals (4.67 and 4.70 ppm for **9** and **10**, respectively) are in close agreement with the values of the corresponding constants in the 2-ADTNs **7** and **8**, which preferentially exist in the half-chair conformation shown in Figure 2.[10] This makes it possible to assign also to **9** and **10** an analogous half-chair preferred conformation (see Figure 2).

## Radioligand Binding Assays

**α-Adrenergic Affinity.** The affinity of compounds **1–10** (see Table I) for α-adrenergic receptors was determined by binding tests carried out on rat brain membrane preparations. [$^3$H]Prazosin and [$^3$H]rauwolscine were used as specific tritiated ligands for $\alpha_1$ and $\alpha_2$ receptors, respectively. The results obtained for NE and ISO on these preparations were found to be in agreement with previous reports.[11]

**Rat Brain $\alpha_1$ Receptors.** The N-unsubstituted 2-DPM (**3**) and 3-DPP (**5**), and the *N*-isopropyl-substituted 2-DPM (**4**) showed a similar inhibitory activity in the [$^3$H]prazosin-labeled binding assays, which was lower than that of NE. The *N*-isopropyl-substituted 3-DPP **6** showed a very low affinity. Both 2-ADTNs **7** and **8** appeared to be practically inactive; a good affinity was found, on the contrary, for the 2-ADTNs **9** and **10**.

**Rat Brain $\alpha_2$ Receptors.** Among the cyclic analogues of NE, the greatest affinities for the $\alpha_2$ brain receptors were shown by the 2-DPM **3** and the 2-ADTN **9**; the affinity decreased passing to the 3-DPP **5** and then to the 2-ADTN **7**. The *N*-isopropyl-substituted compounds showed $K_i$ values higher than those of the corresponding N-unsub-

(10) (a) Sugihara, H.; Urakawa, K.; Kuriki, H.; Nishikawa, M.; Sanno, Y. Syntheses and β-Adrenoceptor Activities of 2-Alkylamino-6-hydroxy-5-hydroxymethyl-1,2,3,4-tetrahydro-1-naphthalenols. *Chem. Pharm. Bull.* **1977**, *25*, 2988–3002. (b) Motohashi, M.; Mizuta, E.; Nishikawa, M. Nuclear Magnetic Resonance Studies of 2-Amino- and 2-Substituted Amino-5,6-dihydroxy-1,2,3,4-tetrahydro-1-naphthalenols. *Chem. Pharm. Bull.* **1981**, *29*, 1501–1509. (c) Motohashi, M.; Wada, Y.; Kamiya, K.; Nishikawa, M. Crystal Structure of (±)-*trans*-2-Cyclobutylamino-5,6-dihydroxy-1,2,3,4-tetrahydro-1-naphthalenol Hydrobromide. *Chem. Pharm. Bull.* **1980**, *28*, 3656–3661. (d) Motohashi, M.; Nishikawa, M. Conformational Analysis of Beta$_2$-Adrenoceptor-Stimulating Agents. *Mol. Pharmacol.* **1981**, *20*, 22–27.

(11) (a) U'Prichard, D. C.; Greenberg, D. A.; Snyder, S. H. Binding Characteristics of a Radiolabeled Agonist and Antagonist at Central Nervous System Alpha Noradrenergic Receptors. *Mol. Pharmacol.* **1977**, *13*, 454–473. (b) DeBernardis, J. F.; Kerkman, D. J.; Winn, M.; Bush, E. N.; Arendsen, D. L.; McClellan, W. J.; Kyncl, J. J.; Basha, F. Z. Conformationally Defined Adrenergic Agents. 1. Design and Synthesis of Novel $\alpha_2$ Selective Adrenergic Agents: Electrostatic Repulsion Based Conformational Prototypes. *J. Med. Chem.* **1985**, *28*, 1398–1404. (c) Macchia, B.; Balsamo, A.; Lapucci, A.; Macchia, F.; Manera, C.; Nencetti, S.; Breschi, M. C.; Lucacchini, A.; Martini, C.; Martinotti, E. An Anomalous Effect of N-Isopropyl Substitution in Determining β-adrenergic Activity. *Drug Des. Delivery* **1988**, *2*, 257–262.

**Table I.** Radioligand Adrenergic Binding Affinities of Compounds 1–10

| | $K_i$, nM[a] | | | |
|---|---|---|---|---|
| | α-adrenergic binding affinity | | β-adrenergic binding affinity | |
| compd | rat brain ($\alpha_1$) | rat brain ($\alpha_2$) | rat brain ($\beta_1$) | bovine lung ($\beta_2$) |
| NE (1) | 450 (390–520) | 4.8 (4.5–7.1) | 126 (108–144) | 6000 (5200–6600) |
| 2-DPM (3) | 7400 (7000–7800) | 26 (17–28) | 15000 (12000–18000) | 9200 (8500–9800) |
| 3-DPP (5) | 15000 (12500–17500) | 500 (410–580) | >100000 | >100000 |
| 2-ADTN (7) | >100000 | 2900 (2400–3200) | 8000 (5400–10500) | 120 (105–140) |
| 2-ADTN (9) | 300 (150–380) | 90 (81–103) | >100000 | 71000 (60000–85000) |
| ISO (2) | 35500 (32000–39500) | 21000 (16000–24000) | 80 (53–100) | 110 (100–130) |
| 2-DPM (4) | 6700 (6000–7500) | 5100 (4300–5600) | 48000 (42000–53500) | 36000 (32000–40000) |
| 3-DPP (6) | 36000 (30000–40000) | 48000 (44000–59000) | 60000 (48000–73000) | 92000 (85000–98000) |
| 2-ADTN (8) | >100000 | 5800 (5200–6600) | 760 (710–810) | 62 (40–80) |
| 2-ADTN (10) | 1200 (800–1500) | 1570 (1350–1690) | >100000 | >100000 |

[a] Geometric means with confidence limits shown in parentheses.

**Table II.** Adrenoceptor Agonistic Activities[a] of Compounds 1–10 on Isolated Preparations

| | α-adrenoceptor activity[a] | | | | β-adrenoceptor activity[a] | | | |
|---|---|---|---|---|---|---|---|---|
| | isolated rat vas deferens ($\alpha_1$) | | isolated guinea pig ileum ($\alpha_2$) | | isolated guinea pig atria ($\beta_1$) | | isolated guinea pig tracheal strip ($\beta_2$) | |
| compd | p$D_2$ | ia[b] | p$D_2$ | ia[b] | p$D_2$ | ia[b] | p$D_2$ | ia[b] |
| NE (1) | 5.12 (±0.10) | 1.00 | 6.56 (±0.11) | 1.00 | 6.32 (±0.07) | 1.00 | 6.03 (±0.06) | 1.00 |
| 2-DPM (3) | 4.88 (±0.09)[c] | 0.91[c] | 7.34 (±0.12) | 1.08 | 5.31 (±0.30)[d] | 0.91[d] | 4.76 (±0.20)[e] | 0.70[e] |
| 3-DPP (5) | 4.76 (±0.14)[f] | 0.95[f] | 5.56 (±0.21) | 1.40 | 3.81 (±0.06) | 1.00 | 4.44 (±0.28) | 0.77 |
| 2-ADTN (7) | 3.70 (±0.15)[g] | 0.87 | 5.32 (±0.22) | 1.53 | 4.96 (±0.21)[h] | 1.00[h] | 7.78 (±0.05)[i] | 0.75[i] |
| 2-ADTN (9) | 4.60 (±0.11) | 0.50 | 6.63 (±0.32) | 1.42 | 4.57 (±0.42) | 0.81 | 5.36 (±0.15) | 1.10 |
| ISO (2) | 3.50 (±0.14) | 0.83 | 4.95 (±0.14) | 0.74 | 8.45 (±0.12) | 1.00 | 8.33 (±0.18) | 1.00 |
| 2-DPM (4) | – | – | 5.00 (±0.21) | 1.02 | 4.62 (±0.08)[j] | 0.77[j] | 5.91 (±0.09)[k] | 0.87[k] |
| 3-DPP (6) | – | – | 4.57 (±0.09) | 0.89 | 3.50 (±0.12) | 0.16 | 4.64 (±0.15) | 0.98 |
| 2-ADTN (8) | –[g] | – | 5.60 (±0.19) | 0.72 | 6.72 (±0.08)[l] | 1.00[l] | 8.06 (±0.09)[m] | 0.97[m] |
| 2-ADTN (10) | <3.50 | – | 5.72 (±0.24) | 1.35 | 4.44 (±0.22) | 0.75 | 6.17 (±0.51) | 0.47 |

[a] The values represent the mean of 4–6 experiments for each drug ± standard error in parentheses. [b] Intrinsic activity, i.e., the ratio between the maximal response elicited by the compound under test and that elicited by the full agonist, namely NE and ISO for α and β adrenoceptors, respectively. [c] Reference 2: p$D_2$ 4.75 (±0.25); ia 0.91. [d] Reference 2: p$D_2$ 5.31 (±0.30); ia 0.91. [e] Reference 2: p$D_2$ 4.76 (±0.20); ia 0.70. [f] Reference 2: p$D_2$ 4.53 (±0.30); ia 0.95. [g] Reference 5: p$D_2$ <3.5 on isolated rabbit aortic strips. [h] Reference 5: p$D_2$ 6.74 (±0.21); ia 1. [i] Reference 5: p$D_2$ 7.49 (±0.05); ia 1. [j] Reference 2: p$D_2$ 5.74 (±0.34); ia 0.88. [k] Reference 2: p$D_2$ 6.34 (±0.19); ia 0.93. [l] Reference 5: p$D_2$ 7.56 (±0.08); ia 1. [m] Reference 5: p$D_2$ 8.41 (±0.09); ia 1.

stituted ones; the affinity of the 2-DPM 4 and of the 2-ADTNs 8 and 10 was found to be higher than that of ISO.

The $K_i$ values shown in Table I indicate that compounds 1–10 interact more selectively on $\alpha_2$ than on $\alpha_1$ rat brain adrenoceptors.

**β-Adrenergic Affinity.** The β-adrenergic affinity of compounds 1–10 (see Table I) was checked by binding tests on rat brain membranes for the $\beta_1$ receptors and on bovine lung membranes for $\beta_2$ receptors. [$^3$H]CGP was used as a specific tritiated ligand for rat brain $\beta_1$ receptors, whereas [$^3$H]DHA was used to label bovine lung $\beta_2$ receptors.

**Rat Brain $\beta_1$ Receptors.** The $K_i$ values obtained by us for NE and ISO were substantially in agreement with those previously obtained by us,[11c] on heart bovine atria, and by other authors,[12] on chick atria, using [$^3$H]DHA. As far as the cyclic analogues of NE are concerned, the 2-DPM 3 and the 2-ADTN 7 exhibited comparable $K_i$ values which were, however, higher than that of NE; the 3-DPP 5 and the 2-ADTN 9 appeared to be practically inactive. With regard to the cyclic analogues of ISO, the 2-ADTN 8 showed the lowest $K_i$ value, 10 times higher than that of ISO, while a marked increase in the $K_i$ values was found on passing to the 2-DPM 4 and the 3-DPP 6; 2-ADTN 10 appeared to be inactive.

**Bovine Lung $\beta_2$ Receptors.** The affinity values obtained for NE and ISO on this isolated preparation were in agreement with others previously obtained from tests on bovine tracheal smooth muscle[11c] and rat lung,[13] using [$^3$H]DHA and [$^{125}$I]iodohydroxybenzylpindolol, respectively. Among the N-unsubstituted cyclic compounds, the 2-ADTN 7 was the most active, with a $K_i$ value markedly lower than that of NE. An increase in $K_i$ was observed on passing to the 2-DPM 3 and then to the 2-ADTN 9; the 3-DPP 5 was inactive. The 2-ADTN derivative (8) was the most active of the cyclic analogues of ISO, with a $K_i$ value slightly lower than that of the parent open-chain compound (ISO). A marked increase in $K_i$ values was observed also in this case, on passing to the 2-DPM 4 and the 3-DPP 6; the 2-ADTN 10 was found to be inactive also on this kind of β-receptors.

The trend of the affinity for the β receptors of the compounds examined (1–10) was similar on both types of preparations with the only exception of the 2-ADTN 7 which in one case ($\beta_1$ receptor) showed a $K_i$ value markedly higher than that of NE, while in the other case ($\beta_2$ receptor) showed $K_i$ value clearly lower than that of NE.

## Functional Tests

**α-Adrenergic Activity.** Compounds 1–10 were tested on isolated rat vas deferens for their activity on $\alpha_1$ receptors and on isolated guinea pig ileum for their activity on $\alpha_2$ receptors (Table II).

**Rat Vas Deferens $\alpha_1$ Receptors.** The 2-DPM 3 and the 3-DPP 5 showed a stimulating activity comparable with that of NE; their p$D_2$ and ia (intrinsic activity) values

(12) Williams, K.; Strange, P. G.; Bennett, T. Alterations in β-Adrenoceptor Number and Catecholamine Content of Chick Atria after Reversible Sympathetic Denervation with 6-Hydroxydopamine. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *336*, 64–69.

(13) Minneman, K. P.; Hegstrand, L. R.; Molinoff, P. B. The Pharmacological Specificity of Beta$_1$- and Beta$_2$-Adrenergic Receptors in Heart and Lung in Vitro. *Mol. Pharmacol.* **1979**, *16*, 21–33.

were not significantly different from those of the full agonist NE. The 2-ADTN **7** showed a very weak activity; the 2-ADTN **9** showed an appreciable $pD_2$ value, accompanied, however, by a low value of ia. The *N*-isopropyl-substituted compounds **4**, **6**, **8**, and **10** did not show any activity at a concentration of $10^{-3}$ M. All the effects observed were produced by a direct mechanism, because some additional tests performed on tissues from reserpine-pretreated rats gave practically identical results to those obtained from tissues from untreated animals.

**Guinea Pig Ileum $\alpha_2$ Receptors.** The effects of compounds **1–10** on $\alpha_2$ receptors were evaluated on electrically stimulated guinea pig ileum. This tissue was chosen after some experimental attempts on electrically stimulated rat vas deferens. However, when the latter organ was employed, it was not possible to discriminate between the pre- and postsynaptic activity of some compounds, because at certain doses, they simultaneously activated both $\alpha_1$ and $\alpha_2$ receptors; consequently, the postsynaptic excitatory response masked the presynaptic inhibitory component in this tissue. In the ileum, the lack of postsynaptic contractile $\alpha$ receptors[14] made it possible to evaluate the $\alpha_2$-inhibitory action on the release of acetylcholine.

All four N-unsubstituted cyclic analogues (**3**, **5**, **7**, and **9**) exhibited a noteworthy stimulating effect on the $\alpha_2$ adrenoceptors located on cholinergic fibers of the guinea pig ileum. In particular, the 2-DPM **3** showed an activity higher than that of the standard agonist and the physiological mediator, NE. ISO and its cyclic analogues (**4**, **6**, **8**, and **10**) showed an appreciable activity on this receptor subtype, with an ia lower than that of the N-unsubstituted compounds. The direct effect of the tested compounds was assessed by blocking the responses with yohimbine $10^{-5}$ M.

The values of the $pD_2$ and of the ia shown in Table II indicate that, in general, compounds **1–10** act more selectively on $\alpha_2$ than on $\alpha_1$ adrenoceptors.

**$\beta$-Adrenergic Activity.** Compounds **1–10** were tested on isolated guinea pig atria for their activity on $\beta_1$ receptors and on isolated guinea pig tracheal strips for their activity on $\beta_2$ receptors (Table II). In order to exclude an indirect mechanism of receptor activation, some additional tests were performed on tissues obtained from reserpine-pretreated animals; the results did not differ from those obtained in tissues of untreated guinea pigs.

**Guinea Pig Atria $\beta_1$ Receptors.** As far as the N-unsubstituted cyclic analogues are concerned, the 2-DPM **3** and the 2-ADTN **7** exhibited similar $pD_2$ values which are 1 order of magnitude lower than that of NE. The $pD_2$ value decreased on passing to the 2-ADTN **9** and then to the 3-DPP **5**. With regard to the *N*-isopropyl-substituted semirigid compounds, the 2-ADTN **8** showed a high $pD_2$ value which was still comparable, even if lower, to that of ISO; the $pD_2$ decreased on passing to the 2-DPM **4** and the 2-ADTN **10**, and then to the 3-DPP **6**. The stimulating activity shown by all compounds was completely blocked by propranolol $10^{-5}$ M.

The trend of the ia was similar to that of the $pD_2$, with the exception of the 3-DPP **5**, which, despite its low $pD_2$ value, showed a high value for the ia.

The differences observed between the $pD_2$ values obtained by us for the two 2-ADTNs **7** and **8** and those previously obtained (see Table II) by other authors[5] on the same isolated tissue may be due to the fact that the old values were obtained by measuring the chronotropic effects of the drugs, while we evaluated the inotropic response to the same treatment.

**Guinea Pig Tracheal Strip $\beta_2$ Receptors.** Among the cyclic analogues of NE, the 2-ADTN **7** exhibited a $pD_2$ value higher than that of the parent open-chain compound NE; the other three cyclic analogues of NE, i.e. the 2-DPM **3**, the 3-DPP **5**, and the 2-ADTN **9**, showed a $pD_2$ value lower than that of NE itself. With regard to the semirigid analogues of ISO, the highest activity was shown by the 2-ADTN **8**, which exhibited a $pD_2$ value practically equal to that of ISO; the $pD_2$ values decreased on passing to the 2-DPM **4** and the 2-ADTN **10**, and then to the 3-DPP **6**. The agonistic action shown by all the compounds was completely abolished by butoxamine $5 \times 10^{-6}$ M.

With regard to the intrinsic activity, its trend is similar to that of the affinity index, with three exceptions: the low value of ia of the 2-ADTNs **7** and **10**, in spite of their high $pD_2$, and the high value of ia shown by the 3-DPP **6**, which exhibited a relatively low value of $pD_2$.

The data obtained by us for the two 2-ADTNs **7** and **8** on the tracheal tissue were in substantial agreement with those previously reported (see Table II).[5]

The trend of the $pD_2$ for the $\beta$ receptors of the compounds examined was similar in isolated guinea pig atria and tracheal strips, with the exception of the 2-ADTN **7** which exhibited a markedly different $pD_2$ value in the two pharmacological preparations.

## Discussion

**$\alpha$-Adrenoceptor Activity.** An examination of the data presented in Tables I and II indicates that the results of the functional tests show an activity trend that is substantially similar to the affinity trend revealed in the binding tests.[15] Furthermore, it may be noted that in the case of both the open-chain compounds and their cyclic analogues, the activity toward the $\alpha_2$ receptor is considerably higher than that on the $\alpha_1$ receptor.

The values of both the $K_i$ and the $pD_2$ shown in the tables indicate that the cyclization of NE to its corresponding morpholine (**3**) and piperidine (**5**) derivatives leads to compounds which show, on the whole, an appreciable activity on both types of $\alpha$ receptors, even if somewhat lower than that of the original parent compound. This conclusion is in agreement with our previous observations about $\alpha_1$ adrenergic receptors.[2,17-19]

The results obtained with the 2-ADTNs (see Tables I and II) show that the different type of limitation of the

(14) Bolton, T. B. Mechanism of Action of Transmitters and Other Substances on Smooth Muscles. *Physiol. Rev.* **1979**, *59*, 606–718.

(15) Quantitative differences between the responses of the functional tests and those of the binding tests might depend on differences between the animal species and the kinds of tissues used;[4a] in addition, for an agonist, both differences in spare receptors or receptor reserves and amplification factors may differentiate the curve of the functional response from that of the occupancy.[16]

(16) Kenakin, T. P. The Classification of Drugs and Drug Receptors in Isolated Tissues. *Pharmacol. Rev.* **1984**, *36*, 165–222.

(17) Del Tacca, M.; Bertelli, A.; Mazzanti, L.; Stacchini, B.; Balsamo, A.; Crotti, P.; Macchia, B.; Macchia, F. Conformational Effects on the Activity of Drugs. 5. Pharmacological Properties of 2-(*p*-Nitrophenyl)-4-isopropylmorpholine, a Cyclic Analog of INPEA. *J. Med. Chem.* **1975**, *18*, 836–838.

(18) Balsamo, A.; Crotti, P.; Lapucci, A.; Macchia, B.; Macchia, F.; Del Tacca, M.; Mazzanti, L.; Ceserani, R. Conformational Effects on the Activity of Drugs. 7. Synthesis and Pharmacological Properties of 2-(*p*-Nitrophenyl)-Substituted Morpholines. *J. Med. Chem.* **1979**, *22*, 738–741.

(19) Balsamo, A.; Lapucci, A.; Macchia, B.; Macchia, F.; Ceserani, R.; Longiave, D. An Approach to the Knowledge of the Steric Requirements for $\alpha$-Adrenoreceptor Direct Activation. $\alpha$-Adrenergic Properties of New 3-Piperidinols. *Eur. J. Med. Chem.* **1981**, *16*, 163–169.

conformational freedom of NE and ISO obtained by using tetrahydronaphthalene derivatives such as **7** and **8**, and **9** and **10**, respectively, is important for the fulfillment of the $\alpha$-adrenergic activity. The cyclization which leads to the derivatives **9** and **10** gives markedly better results than the one which leads to the derivatives **7** and **8**, especially with regard to activity on the $\alpha_2$ receptors: the activity of **9** and **10** on the latter type of receptor is comparable to that of NE and ISO, respectively.

**$\beta$-Adrenoceptor Activity.** Also for this type of receptor, the trend of the results of the functional tests is analogous to that of the binding tests, if the data referring to the *N*-isopropyl-substituted 2-DPM **4** are excluded. While the pharmacological assays show that this compound possesses a significant activity, even if considerably lower than that of ISO, the binding tests, on the contrary, reveal a very modest affinity for both types of $\beta$ receptors.

The results of these new pharmacological assays, together with those of the binding tests, indicate that, in contrast with previous findings of functional tests,[2] the 3-DPPs **5** and **6** present an extremely limited activity, compared with that of the corresponding parent open-chain compounds NE and ISO. It seems, therefore, that the cyclization of NE and ISO to their corresponding piperidine derivatives does not practically allow the maintenance of their $\beta$-adrenergic activity.

The situation of the 2-DPMs **3** and **4** is less clear. The N-unsubstituted compound **3** presents an appreciable activity, even if it is about 1 order of magnitude lower than that of NE; its $K_i$ value is very similar to that of NE, in the case of $\beta_2$ receptors, whereas in the case of $\beta_1$ receptors, it is clearly higher. The *N*-isopropyl-substituted 2-DPM (**4**) is found from functional tests to possess a significant, albeit much lower, activity than that of ISO; binding assays indicate that it has a very modest affinity for both types of $\beta$ receptors. These results would appear not to be in complete agreement: if only the N-unsubstituted 2-DPM (**3**) is considered, it is found that the cyclization of NE to its morpholine derivative does not dramatically affect its $\beta$-adrenergic activity; if, on the contrary, the *N*-isopropyl-substituted 2-DPM (**4**) is taken into consideration, the insertion of the ethanolamine portion of ISO into the morpholinic structure would appear to determine a marked fall in the receptor affinity. The decrease in the activity which is observed on passing from NE to its corresponding 2-DPM **3**, which lacks the proton of the hydroxyl group present in the side chain of NE, might be attributed to a negative effect of the substitution of this proton[20] on interaction with the $\beta$ receptor. The more marked decrease in the activity which is found on passing from ISO to its morpholine analogue **4**, might depend not only on the substitution of the hydroxylic hydrogen, but also on an excessive steric hindrance around the nitrogen atom which is completely substituted in **4**. It would therefore appear that the *N*-isopropyl substitution, which usually has a positive effect on the $\beta$-adrenergic activity,[3a-f] has, on the contrary, a negative effect on this kind of activity in the morpholinic derivative **4**, adding an extra steric hindrance to the one deriving from the additional atoms needed to make the molecule semirigid.[20b,21]

The results of the binding tests together with the pharmacological data, confirm the marked activity of the 2-ADTNs **7** and **8**,[5] which in the case of $\beta_2$-receptors proves to be comparable to that of NE and ISO, respectively. The last two 2-ADTNs **9** and **10** were found to be markedly less active than **7** and **8**. It is therefore clear that the cyclization of the catecholamines NE and ISO to their corresponding tetrahydronaphthalene derivatives **7** and **8** does not substantially modify their $\beta$-adrenoceptor activity, in contrast with the findings obtained when the conformational freedom of the catecholamines is restricted by means of the use of the tetrahydronaphthalene derivatives **9** and **10**.

**Molecular Models.** With regard to the comparison of the biofarmacological data of semirigid analogues with those of the corresponding open-chain compounds in order to obtain information about the conformation–activity relationship, it is necessary to consider that: (a) the steric and electronic effects, arising from the additional neighboring atoms necessary to make up the semirigid structure, lead to primary modifications of the physical and chemical properties of the flexible parent compound; (b) this in turn may cause a modification in the biological activity of the pharmacophoric groups in the new molecules, compared with the activity elicited by the same groups in the original flexible molecule; (c) consequently, when a conformationally restricted analogue presents an overall receptor activity of the same type as that of the corresponding open-chain compound, even if this activity is found to be quantitatively lower, it may reasonably be assumed that this analogue represents the pharmacophoric conformation of the corresponding conformationally mobile compound.

The 2-DPM **3** and the 3-DPP **5** show on the $\alpha_1$-receptor an activity comparable to that of NE. By superimposing, as described in the introduction, the molecular models of these three drugs in the conformations (see Figure 2) which allow the spatial coincidence between the three active groups, the molecular model A shown in Figure 3 is obtained. This molecular model corresponds to the one previously proposed by us (model **a** of Figure 1) for the $\alpha$ receptor.[2]

At the $\alpha_2$ receptor, the activity of NE (**1**) is found not only in the 2-DPM **3** and in the 3-DPP **5**, but also in the 2-ADTN **9**. The combination of the structure of **1**, **3**, **5**, and **9** in the conformations shown in Figure 2, which allow the spatial coincidence between the three active groups, makes it possible to obtain the three-dimensional model **B** shown in Figure 3.

The activity on the $\alpha_2$ receptor of the 2-ADTN **9** would appear to indicate that the steric hindrance created by the C(3)–C(4) ethylenic bridge of **9** and the limited freedom of movement of its aromatic moiety are not a hindrance to the expression of the activity on this kind of adrenergic receptor.

With regard to the activity on the $\beta$ receptors, an examination limited to the results of the binding tests would appear to indicate that only the 2-ADTNs **7** and **8** are able to maintain almost completely the $\beta$-adrenergic properties of NE and ISO, respectively. When the results of the functional tests are considered, however, it is found that besides the 2-ADTNs **7** and **8**, also the 2-DPMs **3** and **4** appear to possess an appreciable $\beta$-adrenergic activity.

On the basis of these results, it is possible, following a logical process of reasoning analogous to the one exploited for the construction of models referring to the $\alpha$ activation

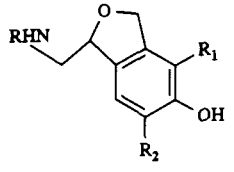
(20) See for example: (a) Biel, J. H.; Lum, B. K. B. The $\beta$-Adrenergic Blocking Agents; Pharmacology and Structure-Activity Relations. *Prog. Drug. Res.* **1966**, *10*, 46–89. (b) Balsamo, A.; Crotti, P.; Macchia, B.; Macchia, F.; Del Tacca, M.; Mazzanti, L. Conformational Effects on the Activity of Drugs. 4. Cyclic Analogs of 1-(*p*-Nitrophenyl)-2-isopropylaminoethanol. Synthesis and Evaluation of the Adrenergic $\beta$-Receptor Blocking Activity of 2-(*p*-Nitrophenyl)-4-isopropylmorpholine. *J. Med. Chem.* **1973**, *16*, 224–227.

(21) Burger, A. *A Guide to the Chemical Basis of the Drug Design*; Wiley: New York, 1983; pp 90–91.

(A and B of Figure 3), to propose the model C illustrated in Figure 3, which is the result of the combination of the structure of catecholamines 1 and 2 and of their morpholine (3 and 4) and tetrahydronaphthalene (7 and 8) cyclic analogues in the pharmacophoric conformations shown in Figure 2. This model differs from the one previously proposed by us[2] in that it does not include the molecular portion deriving from the use, in the construction of the model, of the piperidine derivatives 5 and 6.

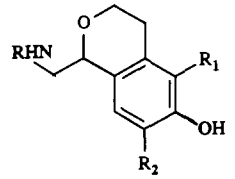
## Conclusions

Models A–C suggest a spatial situation in which the pharmacophoric groups of the adrenergic drugs examined (aryl moiety, amine nitrogen, and alcoholic or ethereal benzylic oxygen) should interact at the receptor site. This pharmacophoric spatial situation corresponds to the one found in the preferred conformations of catecholamines.[2,22]



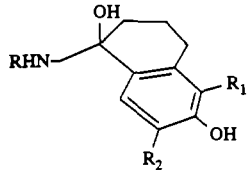
17, $R_1$ = OH $R_2$ = H

18, $R_1$ = H $R_2$ = OH



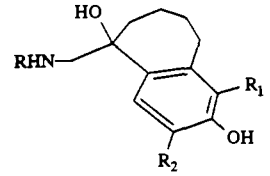
19, $R_1$ = OH $R_2$ = H

20, $R_1$ = H $R_2$ = OH



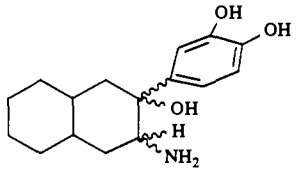
21, $R_1$ = OH $R_2$ = H

22, $R_1$ = H $R_2$ = OH



23, $R_1$ = OH $R_2$ = H

24, $R_1$ = H $R_2$ = OH



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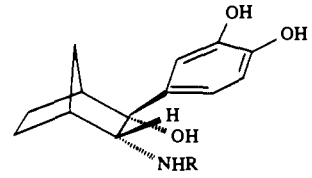

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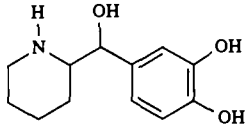

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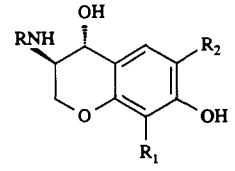
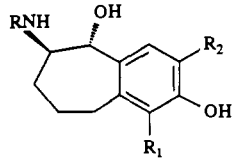


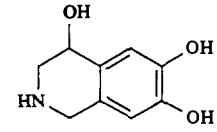
31, $R_1$ = OH $R_2$ = H

32, $R_1$ = H $R_2$ = OH



33, $R_1$ = OH $R_2$ = H

34, $R_1$ = H $R_2$ = OH



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There are some published studies involving cyclic catecholamine analogues in which the above-mentioned three pharmacophoric groups are incorporated into conformationally restricted structures. DeBernardis[23] described the 1-(aminomethyl)dihydroxyphthalans 17 and 18 in which the conformational freedom around the $C_\alpha$–$C_1$ bond of catecholamines is restricted through the incorporation of this bond in the framework of the phthalan. Srimal[24] studied the superior homologues of 17 and 18 (19 and 20) in which the same $C_\alpha$–$C_1$ bond is inserted into the framework of isochroman. The conformational freedom around the $C_\alpha$–$C_1$ bond is restricted also in the 1-(aminomethyl)dihydroxytetrahydro-1-naphthalenols 21[11c] and 22[25] and in their superior homologues with a benzocycloheptenic structure 23 and 24.[26] Smissman[27] synthesized the four possible 3-amino-2-(3,4-dihydroxyphenyl)-*trans*-decalols (25–28) in which the $C_1$–$C_2$ bond of NE is incorporated into the conformationally rigid *trans*-decaline system. In the *endo*-3-amino-*exo*-2-(3,4-dihydroxyphenyl)-2-hydroxybicyclo[2.2.1]heptanes (29), the $C_1$–$C_2$ side chain of catecholamines is fixed in the rigid norbornyl system.[1] In the *erythro*-(3,4-dihydroxyphenyl)-2-piperidylmethanol (30), the conformational freedom around the $C_2$–N bond of the NE is restricted through the insertion of this bond into a piperidine ring.[28] Sugihara[29] described the 3-aminodihydroxy-4-chromanols 31 and 32 in which the catecholamine moiety is incorporated into the chromane system; in these compounds, the conformational freedom is restricted around both the $C_\alpha$–$C_1$ and $C_1$–$C_2$ bonds. The conformational freedom of these bonds was restricted also through their incorporation into the seven-membered ring of the superior homologues of the 2-ADTNs 7 and 8, and 9 and 10 with a benzocycloeptenic structure (33 and 34).[7] In the 4,6,7-trihydroxytetrahydroisoquinoline (35), the entire catecholamine moiety is conformationally restrained through its insertion into the tetrahydroisoquinoline skeleton.[30]


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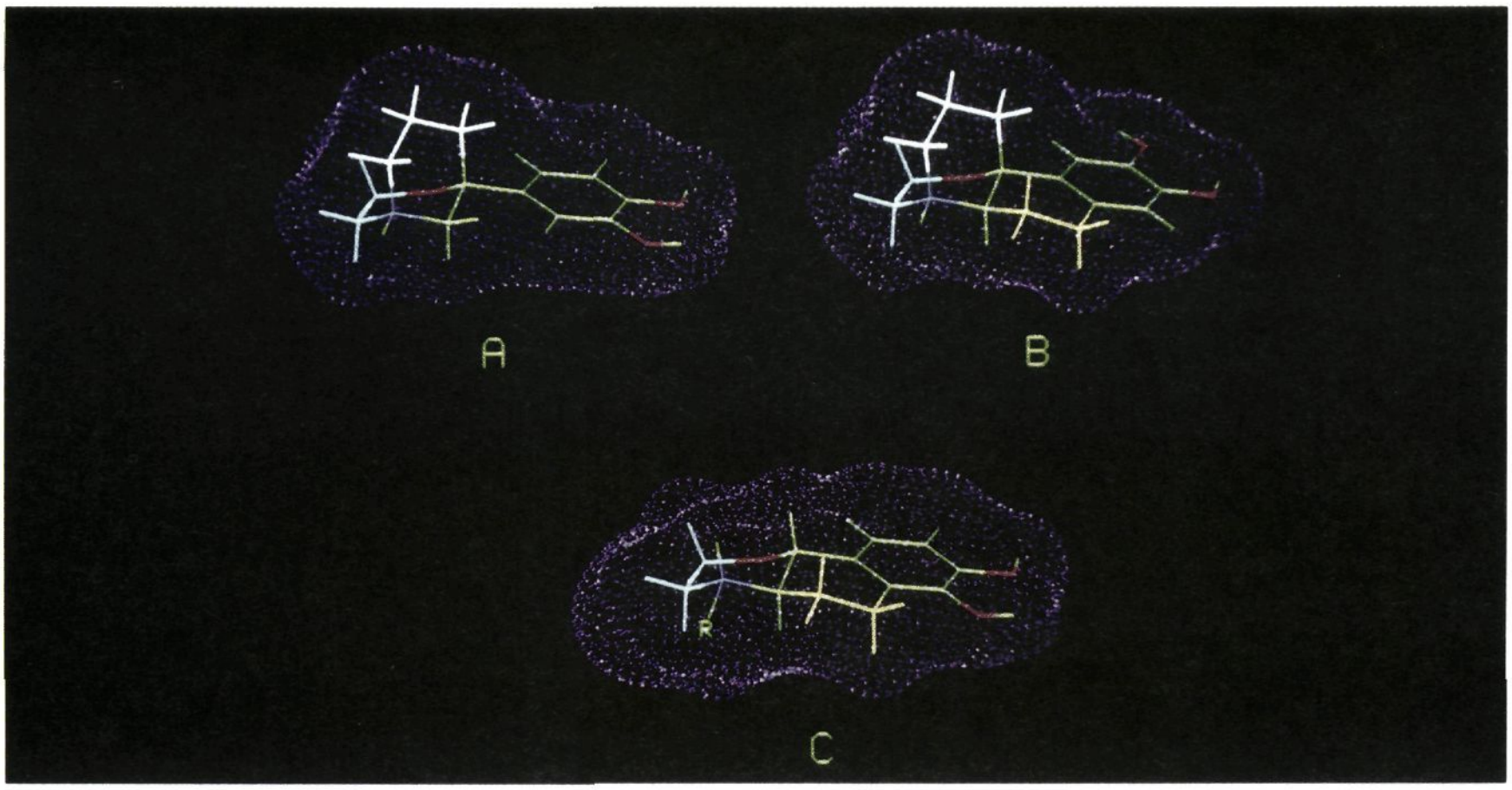


**Figure 3.** Molecular models arising from the superimposition of the pharmacophoric groups (aryl moiety, aminic nitrogen, alcoholic or ethereal oxygen) of drugs **1–10** in the conformations in which they should interact with the adrenergic receptors. The common arylethanolaminic portion is colored green as in NE and ISO; the other portions are colored depending on which drug (**3–10**) they arise from. The dot clouds indicate the molecular volumes; these volumes individuate steric hindrances that arise from atoms present in drugs **1–10** used in turn for the construction of the models. These regions of bulk should not therefore hinder a hypothetical "fit" of the models with the receptor.

Unfortunately, it is not always possible to compare the pharmacological data shown for compounds **17–35** with those obtained by us for compounds **1–10**. However, when the pharmacological data reported in literature can be used, an examination of the molecular models of the compounds which best interact with adrenergic receptors shows that each of them allows conformations that are in agreement with the information that can be gathered from models **A–C**.

Thus, for example, with regard to the cyclic analogues found to be active on $\alpha$ receptors, compound **17**, which is not substituted on the nitrogen, has been found to be active on both types of receptors,[23] in particular on the $\alpha_2$ one; this compound (**17**), in the half-chair conformation in which the amino group is in the anti position with respect to the aromatic ring, presents the pharmacophoric groups in a practically identical position to the one that the same groups may occupy in model **A**, where the aryl is free to rotate, and definitely occupy in model **B**, where the rotameric position of the aryl is also restricted.

Also compound **19** (R = H), for which a good level of activity on the $\alpha_1$-adrenergic receptor has been reported, while no data about its $\alpha_2$-adrenergic activity are available,[24] fits in with model **A**, as far as the $\alpha_1$-adrenergic receptor is concerned, in the conformation corresponding to the one indicated above for **17**.

For compound **21** (R = H), on the contrary, which possesses a good level of activity on $\alpha_2$-adrenergic receptors,[11c] it is not possible to determine an allowed conformation which permits a complete superimposition of its pharmacophoric groups with those of model **B**: when the benzylic oxygen and the amino nitrogen are superimposed, the aryl of **21** is found to be sensibly deviated with respect to the rotameric position that the same group presents in model **B**.

Among the various analogues of the catecholamines studied, only **30** (R = H) and **31** (R = *i*-Pr) have been found to possess an appreciable activity on $\beta$ receptors, in particular on $\beta_2$ ones.[28,29] For both these compounds, it is possible to determine allowed conformations in which a complete superimposition can be obtained between their active groups and the corresponding ones that are spatially blocked in model **C**. These findings are not wholly unexpected, in the case of both **30**, which presents a considerable conformational freedom, and **31**, which represents the analogue of the 2-ADTN **8** used in the construction of model **C**, from which **31** may be considered to be obtained by means of the isosteric substitution of its benzylic $CH_2$ with an oxygen atom.

The different position of the catecholic hydroxyls in model **B**, concerning the $\alpha_2$ receptor, and in model **C**, concerning the $\beta$ receptor, is in agreement with hypotheses advanced by other authors[5,10d,11b,23,31] about the importance of the rotameric position of the aromatic ring of catecholamines in the interaction with the $\alpha$- and $\beta$-adrenergic receptors.

## Experimental Section

**Chemistry.** Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken on paraffin oil mulls on a Perkin-Elmer Model 1310 instrument. $^1$H NMR spectra of all compounds were routinely detected 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. The $^1$H NMR spectra of **9** and **10**, as salts, were also detected in ca.

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2% $D_2O$ solution, at 23 °C, with a Bruker AC-200 instrument. Evaporations were made in vacuo (rotating evaporator). $MgSO_4$ was always used as the drying agent. Elemental analyses were performed by our analytical laboratory and agreed with the theoretical values to within ±0.4%.

***trans*-2-Amino- and *trans*-2-(Isopropylamino)-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalen-1-ol (7 and 8, Respectively).** Compounds 7 and 8 were obtained as oxalate salts, following the synthetic route previously described.[8] **7**·$H_2C_2O_4$: Mp 167–169 °C (MeOH–$Et_2O$) (lit.[8] mp 210–213 °C dec, as hydrobromide). Anal. ($C_{12}H_{15}NO_7$) C, H, N. **8**·$H_2C_2O_4$: mp 216 °C dec (MeOH–$Et_2O$) (lit.[8] mp 180–210 °C dec, as fumarate). Anal. ($C_{15}H_{21}NO_7$) C, H, N.

**6,7-Bis(benzyloxy)-3,4-dihydronaphthalen-1(2*H*)-one (12).** A stirred mixture of 6,7-dihydroxy-3,4-dihydronaphthalen-1-(2*H*)-one[9] (**11**) (1.7 g, 9.5 mmol), anhydrous $K_2CO_3$ (1.5 g, 10.5 mmol), KI (1.7 g, 10.5 mmol), $Na_2S_2O_3 \cdot 5H_2O$ (85 mg), and benzyl chloride (2.53 g, 20 mmol) in anhydrous EtOH (50 mL) was refluxed for 1.5 h and then treated dropwise with a solution of KOH (0.60 g, 10.7 mmol) in anhydrous EtOH (9.2 mL). After 2.5 h of further stirring at reflux temperature, the mixture was evaporated and the residue was treated with $H_2O$ (50 mL) and extracted with $CHCl_3$. Evaporation of the washed ($H_2O$) and filtered extracts yielded a solid residue which was crystallized from MeOH to afford pure **12** (2.2 g, 64%): mp 109–111 °C; $^1H$ NMR δ 1.88–2.30 (br, 2 H), 2.40–2.96 (br, 4 H), 5.21 (s, 4, $CH_2Ph$), 6.75 (s, 1 H), 7.41 (brs, 10 H), and 7.66 (s, 1 H). Anal. ($C_{24}H_{22}O_3$) C, H.

**6,7-Bis(benzyloxy)-3,4-dihydronaphthalen-1(2*H*)-one Oxime (13).** A stirred solution of **12** (3.0 g, 8.4 mmol) and $NH_2OH \cdot HCl$ (3.0 g, 43 mmol) in anhydrous pyridine (46 mL) was kept at 120 °C for 1 h, cooled to room temperature, and then poured into $H_2O$. Filtration of the resulting suspension yielded a solid product which was crystallized from MeOH to afford pure **13** (61%): mp 135–136 °C; $^1H$ NMR δ 1.63–2.06 (br, 2 H), 2.46–2.96 (br, 4 H), 5.18 (s, 4 H, $CH_2Ph$), 6.73 (s, 1 H), 7.46 (brs, 10 H), and 7.60 (s, 1 H). Anal. ($C_{24}H_{23}NO_3$), C, H, N.

**6,7-Bis(benzyloxy)-3,4-dihydronaphthalen-1(2*H*)-one *O*-*p*-Toluenesulfonyloxime (14).** A solution of *p*-toluensulfonyl chloride (2.0 g, 10.4 mmol) in anhydrous pyridine (18 mL) was added to a stirred and cooled (0 °C) solution of **13** (1.9 g, 5.1 mmol) in anhydrous pyridine (8 mL). The mixture was stirred at the same temperature for 30 min and at room temperature for 1 h and was then poured into $H_2O$. The resulting suspension was filtered and the solid product was crystallized from MeOH to afford pure **14** (1.9 g, 71%): mp 116–117 °C; $^1H$ NMR δ 1.56–1.98 (br, 2 H), 2.40 (s, 3 H), 2.43–2.87 (br, 4 H), 5.11 (s, 4 H), 6.63 (s, 1 H), 7.21 (s, 1 H), 7.23 (d, 2 H, $J$ = 7 Hz), 7.38 (brs, 10 H), and 7.86 (d, 2 H, $J$ = 7 Hz). Anal. ($C_{31}H_{29}NO_5S$) C, H, N.

**2-Amino-6,7-bis(benzyloxy)-3,4-dihydronaphthalen-1-(2*H*)-one (15).** A solution of EtOK, prepared from K (0.245 g, 6.27 mmol) and anhydrous EtOH (6.0 mL), was added to a stirred and cooled (0 °C) solution of **14** (2.95 g, 5.6 mmol) in anhydrous benzene (34 mL). After stirring at 0 °C for 5 h, the mixture was allowed to stand at 5 °C for 5 days. After removal by filtration of the insoluble material, the solution was treated with $Et_2O \cdot HCl$ and the solid precipitate was filtered and crystallized from MeOH/$Et_2O$ to yield pure **15**·HCl (1.0 g, 44%): mp 175–179 °C dec; IR $\nu$ 1660 $cm^{-1}$ (C=O). Anal. ($C_{24}H_{24}ClNO_3$) C, H, N.

***trans*-2-Amino-6,7-bis(benzyloxy)-1,2,3,4-tetrahydronaphthalen-1-ol (16).** Solid $NaBH_4$ was added in portions to a solution of **15**·HCl (0.65 g, 1.6 mmol) in anhydrous MeOH (40 mL), and the resulting mixture was stirred for 20 min at room temperature and then diluted with $H_2O$ (200 mL) and extracted with $CHCl_3$. Evaporation of the washed ($H_2O$) and dried extracts yielded a solid residue which by crystallization with $CHCl_3$/hexane afforded pure **16** (0.50 g, 84%): mp 97–100 °C dec; $^1H$ NMR δ 1.43–2.25 (br, 5 H), 2.57–3.05 (br, 3 H), 4.26 (d, 1 H, $J$ = 7.5 Hz), 5.08 (s, 2 H), 5.10 (s, 2 H), 6.61 (s, 1 H), 7.15 (s, 1 H), and 7.33 (br, 10 H). Anal. ($C_{24}H_{25}NO_3$) C, H, N.

The oxalate salt of **16** had mp 191–192 °C (MeOH-$Et_2O$). Anal. ($C_{26}H_{27}NO_7$) C, H, N.

***trans*-2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalen-1-ol Oxalate (9·$H_2C_2O_4$).** A solution of **16**·$H_2C_2O_4$ (0.25 g, 0.54 mmol) in MeOH (50 mL) was shaken under hydrogen at room temperature and atmospheric pressure in the presence of 10% Pd on charcoal (0.12 g). When the absorption stopped, the catalyst was filtered off and the solution was evaporated to yield a solid residue which was crystallized from MeOH/$Et_2O$ to afford the oxalate salt of **9** (0.080 g, 52%): mp 202 °C dec (lit.[6] mp 155 °C, as picrate); $^1H$ NMR δ 1.85–2.5 (m, 1 H), 2.15–2.30 (m, 1 H), 2.70–2.90 (m, 2 H), 3.28–3.45 (m, 1 H), 4.67 (d, 1 H, $J$ = 8.1 Hz, C*H*OH), 6.68 (s, 1 H), and 6.97 (s, 1 H). Anal. ($C_{12}H_{15}NO_7$) C, H, N.

***trans*-2-(Isopropylamino)-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalen-1-ol Oxalate (10·$H_2C_2O_4$).** A solution of **16** (0.25 g, 0.67 mmol) was dissolved in anhydrous MeOH (10 mL) and treated for 12 h at room temperature with $Me_2CO$ (3.0 mL). The solution was then shaken under hydrogen at room temperature and atmospheric pressure in the presence of 10% Pd on charcoal (0.10 g). When the absorption stopped the catalyst was removed by filtration, and the resulting solution was added to a solution of $H_2C_2O_4 \cdot 2H_2O$ (0.084 g, 0.67 mmol). The solid precipitate was collected by filtration and then crystallized from MeOH–$Et_2O$ to yield pure **10**·$H_2C_2O_4$ (0.080 g, 37%): mp 157–159 °C; $^1H$ NMR δ 1.36 and 1.41 (2d, 6 H, $J$ = 6.5 Hz), 1.63–2.00 (m, 1 H), 2.26–2.42 (m, 1 H), 2.70–3.00 (m, 2 H), 3.30–3.50 (m, 1 H), 3.72 (m, 1 H, $J$ = 6.5 Hz), 4.70 (d, 1 H, $J$ = 9.1 Hz, C*H*OH), 6.69 (s, 1 H), and 6.98 (s, 1 H). Anal. ($C_{15}H_{21}NO_7$) C, H, N.

**Radioligand Binding Methods. Rat Brain $\alpha_1$ and $\alpha_2$ Receptors.** $\alpha_1$ and $\alpha_2$-receptor bindings were determined in rat cerebral cortex membranes as elsewhere reported.[32]

**Rat Brain $\beta_1$ Receptors.** $\beta_1$ receptors were assayed in rat cortical membranes using [$^3H$]CGP 26505 [1-[[2-(3-carbamoyl-4-hydroxyphenoxy)ethyl]amino]-3-[4-[1-methyl-4-(trifluoromethyl)-2-imidazolyl]phenoxy]-2-propanol] as the specific ligand (Du Pont de Nemours, New England Nuclear Division, specific activity 28.4 Ci/mmol).

Rat cortices were rapidly isolated and homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer at pH 8. The homogenates were centrifuged at 48000*g* for 15 min at 5 °C. This step was repeated four times resuspending the pellets in 10 volumes of fresh buffer. The final crude membranes were suspended in Tris-HCl buffer containing 0.1% ascorbic acid. Protein concentration, as assayed by the method of Lowry et al.,[33] amounted to 4 mg/mL for displacement studies.

Routine [$^3H$]CGP 26505 binding assays were run by incubating 0.1 mL of crude rat brain membrane suspension at 25 °C for 60 min with [$^3H$]CGP 26505 (1 nM) in a total volume of 0.5 mL of Tris-HCl buffer. The inhibition of specific binding was determined in the presence of various concentrations of unlabeled competing drugs. Incubations were terminated by rapid vacuum filtration through Whatman GF/B glass-fiber filters. Filters were washed with three 5-mL portions of ice-cold Tris-HCl buffer and then counted. Specific binding was determined as the excess over blanks containing 30 μM *l*-isoprenaline.

**Bovine Lung $\beta_2$ Receptors.** $\beta_2$-Receptor bindings were studied in bovine lung using [$^3H$]DHA (dihydroalprenolol) as the ligand (Du Pont de Nemours, New England Nuclear Division, specific activity 48.1 Ci/mmol).

Membranes were obtained by lung homogenization in 1:20 volumes of 0.32 M sucrose, followed by centrifugation at 800*g* for 10 min at 5 °C. The supernatant was recentrifuged at 30000*g* for 10 min at 5 °C. The resulting pellet was suspended in 50 mM phosphate buffer at pH 7.4 containing 0.02% ascorbic acid and then centrifuged. This step was repeated twice. Crude lung membranes were suspended in phosphate buffer (~4 mg/mL proteins) and used for [$^3H$]DHA binding as elsewhere described.[34]

The affinity of drugs for specific binding sites was expressed as the molar concentration inhibiting specific binding by 50%

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($IC_{50}$). These values were calculated from the displacement curves by log probit analysis. The dissociation constant ($K_i$) was derived from the equation of Cheng and Prusoff.[35] The ligand affinities ($K_d$) of [$^3$H]CGP 26505 and [$^3$H]DHA were 0.7 and 1.0 nM, respectively.

**Pharmacological Methods. Isolated Rat Vas Deferens.** $\alpha_1$-Adrenoceptor activity was assayed on isolated vas deferens taken from normal Sprague-Dawley male albino rats (200–250 g body weight) sacrificed by cervical dislocation. 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. The organs were prepared and employed as previously described.[36]

**Guinea Pig Ileum.** Durkin-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 ($O_2$ 95%/$CO_2$ 5%). The ileum preparations were placed between two platinum electrodes (4 × 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 mod. 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 $\alpha_2$ 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 mod. 7006) connected with a unirecord microdynamometer (Basile mod. 7050).

**Guinea Pig Atria.** Isolated atria from Durkin-Hartley guinea pigs were used in order to test the activity of drugs on $\beta_1$ adrenoceptors. For the experiments carried out on reserpinized animals, the tissues were obtained from guinea pigs pretreated with reserpine (1 mg/kg bw, ip) 24 h before the sacrifice. The organs were suspended in Tyrode solution at 37 °C, gassed with $O_2$, and linked to an isometric force displacement transducer (Basile mod. 7005). The responses of the atria to the drugs were recorded after a stabilizing period of 45 min, by an unirecord microdynamometer (Basile mod. 7050).

**Guinea Pig Tracheal Strip.** Tracheae taken from the same type of guinea pigs, both normal and reserpine-pretreated, employed for atria assays, and prepared in accordance with the method described by Emmerson and MacKay,[37] were used to evaluate the activity of the tested drugs on $\beta_2$ adrenoceptors. One tracheal strip was obtained from each organ. Before starting the experiment, the tissues were allowed to stabilize for 1 h in an organ bath containing Krebs solution gassed with carbogen, under a resting tension of 0.5 g exerted by an isotonic transducer (Basile mod. 7006), connected with an unirecord microdynamometer (Basile mod. 7050).

The activities of the compounds on $\beta_2$-adrenoceptors were evaluated as the ability to inhibit the constant level of tracheal smooth muscle tone induced by carbachol 5.5 × $10^{-6}$ M.

Dose–response curves to the agonists were obtained by using the method of cumulative doses described by Van Rossum.[38]

The stimulating properties of the drugs on the receptors were evaluated by means of the apparent affinity index ($pD_2$), which is the negative log of the $ED_{50}$ value (M), and the intrinsic activity, expressed as the ratio between the maximal response obtained with the tested compounds and that of a full agonist. Norepinephrine and isoprenaline were taken as full agonists for the $\alpha$ and $\beta$ adrenoceptors, respectively. Reserpine was used as the free base, whereas the following drugs were used as salts: **1** (*l*-norepinephrine) as bitartrate, **2** (*l*-isoprenaline), butoxamine, carbachol, yohimbine, phentolamine, propranolol, the morpholine and piperidine derivatives (**3–6**) as hydrochlorides, and the tetrahydronaphthalene analogues (**7–10**) as oxalates.

**Molecular Graphics.** The molecular models shown in Figures 2 and 3 were drawn by using the Insight II program. The molecular volumes were the solvent-accessible ones, calculated by Connolly's method;[39] in Figure 2 the volumes of **1–10** were calculated for R = H; in Figure 3 the volume of model C was calculated for R = *i*-Pr.

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