A Conformational Study of β-Phenethanolamine Receptor Sites. 8. Pharmacological Study of 3-Isopropylamino-2-phenyl-*trans*-2-decalols†

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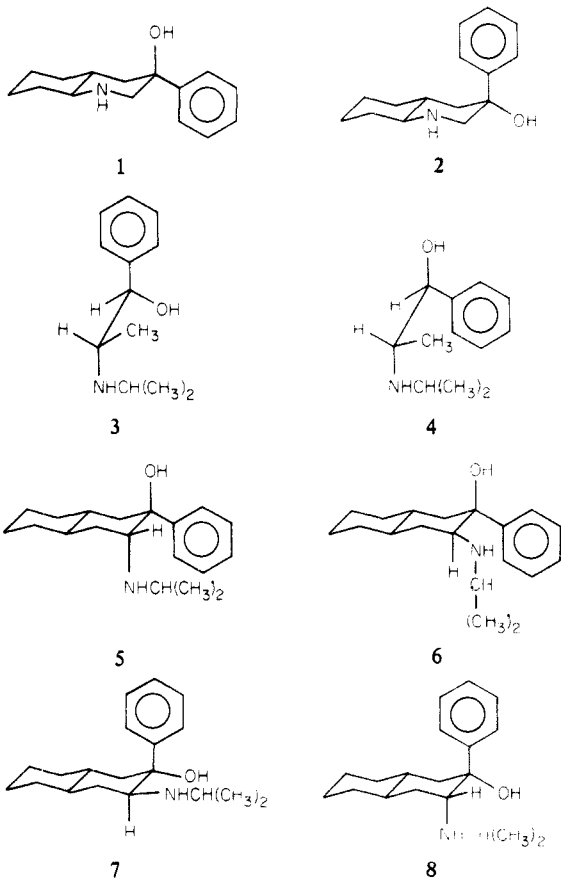
Two *N*-isopropylnorephedrines and their four possible decalol analogs were compared pharmacologically. Four of the six compounds at a concentration of 1×10^{-4} *M* caused a potentiation of D(−)-norepinephrine (NE) contraction of the rat vas deferens and increased the maximal response of the preparation to NE. Pretreatment in vivo with reserpine (5 mg/kg ip) 24 hr before the experiment in vitro did not change these effects. At a concentration higher than 1×10^{-3} *M* all of the decalin analogs antagonized the effects of NE. All the analogs lowered the dog blood pressure briefly. The lowering of blood pressure was augmented by α-adrenergic blockade and was not changed by β-adrenergic blockade, atropine, or a ganglionic blocking agent. Tachyphylaxis was not observed. The spontaneous contraction of isolated rabbit atria was depressed by the substances at concentrations of 1×10^{-4}–1×10^{-3} *M*. Catecholamine uptake1 in rat vas deferens was lowered by the substances and all of them produced a release of catecholamines from vas deferens. By virtue of the conformational rigidity of the decalols, possible inferences concerning the stereochemical aspects of the interaction of the ephedrine analogs with adrenergic neurone–receptor sites are discussed.

Smissman and Chappell[1] synthesized and reported the testing of *trans*-perhydroquinolines 1 and 2 on the rat vas deferens (Chart I). No α-adrenergic agonist response was found for these substances but they potentiated the contraction evoked by D(−)-norepinephrine (NE). Compound 1 was more effective than compound 2, thus indicating a true steric dependency. These compounds can be viewed as *N*-isopropyl derivatives of ephedrine. It is known that ephedrine enhances the effects of bioamines by the release of catecholamines and also by direct stimulation of the α-adrenergic receptor. Higher concentrations of ephedrine block the receptor. Ephedrine also affects the β-adrenergic receptor and blocks the uptake of catecholamines. Large doses lower the force of cardiac contraction. The potentiation of the NE effects by ephedrine is believed to be related to its ability to block the uptake of catecholamines.[2]

In order to study the possible mechanism and steric requirements of the observed potentiation, *N*-isopropylnorephedrine (3), *N*-isopropylnor-ψ-ephedrine (4), and the four *trans*-decalin analogs, 5–8, were prepared[3] (Chart I) and pharmacologically tested.

Chart I. Analogs of *N*-Isopropylnorephedrine and *N*-Isopropylnor-ψ-ephedrine



Results

On the rat vas deferens substances 3 and 4 at a concentration of 1×10^{-4} *M* potentiated the effect of NE only slightly but increased the maximal response by 58 and 45%, respectively. Substances 5 and 6 potentiated the effect of NE and increased the maximal response by 28 and 32%, respectively. Substances 7 and 8 were without effect on the NE dose–response curves at a concentration of 1×10^{-4} *M*. At a concentration of 1×10^{-3} *M* all of the decalin analogs antagonized the NE contraction, with compounds 7 and 8 being the most effective. The substances 3 and 4 showed antagonism in concentration of 10^{-2} *M* (Table I). Pretreatment with reserpine did not change the effects of substances 3–8 on rat vasa deferentia.

All of the substances lowered the blood pressure of the dog briefly at a dose of 1–2 mg/kg, with 3 and 7 being the most effective. Lowering was increased and prolonged by an α-blocker, phenoxybenzamine (3 mg/kg). The effect consisted of two phases: first, a 30-sec extreme drop in blood pressure (50–75 mm), both diastolic and systolic with little effect on pulse pressure; and second, a long-lasting effect of 30 min in which the systolic fell (30–40 mmHg) and the diastolic and pulse pressure fell (15–20 mmHg). Tachyphylaxis was not observed. In the same animal, ephedrine caused an increase in systolic, diastolic, and pulse pressures, by 40 mmHg, respectively, at a dose of 2

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Table I. Effects of *N*-Isopropylnorephedrines on Rat Vas Deferens NE Contractions[a]

Substance	Concn, *M*	PD_2NE[b]	% of NE max contraction[b]	Concn, *M*	PD_2NE[b]	% of NE max contraction[b]
Control		6.37 ± 0.06	100		6.37 ± 0.06	100
3	10^{-4}	6.56 ± 0.22	158 ± 9*	10^{-2}	6.12 ± 0.15	90 ± 9
4	10^{-4}	6.54 ± 0.19	145 ± 12*	10^{-2}	6.01 ± 0.17	70 ± 9*
5	10^{-4}	6.91 ± 0.08*	128 ± 7*	10^{-3}	5.84 ± 0.27*	85 ± 12
6	10^{-4}	7.32 ± 0.11*	132 ± 11*	10^{-3}	6.10 ± 0.21	88 ± 11
7	10^{-4}	6.35 ± 0.17	96 ± 9	10^{-3}	4.3 ± 0.6*	10 ± 4*
8	10^{-4}	6.31 ± 0.23	94 ± 8	10^{-3}	4.7 ± 0.5*	16 ± 5*
Reserpine pretreatment + **6**	10^{-4}	7.29 ± 0.14*	130 ± 12*	10^{-3}	6.05 ± 0.23	84 ± 13

[a] PD_2 values are means of ten dose-response curves evaluation with SE. Percent of maximal contraction was calculated individually for each preparation. NE cumulative maximal response = 100%. The values were not significantly changed in preparations from reserpinized rats as shown with substance **6**. [b] *, significantly different from control at $p < 0.05$.

Table II. Effects of *N*-Isopropylnorephedrines on the Uptake and Release of NE in Rat Vas Deferens[a]

	U_1, NE content, μg/g[b]	Release, NE content, μg/g[b]
Control	13.2 ± 0.2	9.8 ± 0.3
3 (10^{-4} *M*)	9.2 ± 0.4*	7.0 ± 0.5*
4 (10^{-4} *M*)	9.3 ± 0.5*	7.4 ± 0.4*
5 (10^{-4} *M*)	9.3 ± 0.3*	7.7 ± 0.4*
6 (10^{-4} *M*)	9.1 ± 0.5*	7.9 ± 0.5*
7 (10^{-4} *M*)	9.6 ± 0.4*	7.8 ± 0.3*
8 (10^{-4} *M*)	9.5 ± 0.6*	8.1 ± 0.6*

[a] Values = means from five experiments ± SE. U_1 = uptake$_1$. [b] *, significantly different from control at $p < 0.05$.

mg/kg. The hypotensive effect was not inhibited by β-blockade with propranolol (2 mg/kg). In the same dog, ephedrine's (2 mg/kg) α-adrenergic responses disappeared after treatment of the animal with phenoxybenazamine, and its β effect appeared. This β effect could be erased with propranolol. α- and β-receptor blockades were always checked with 30 μg/kg of norephinephrine and 30 μg/kg of isoproteronol, respectively, before and after blockade.

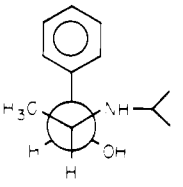
The hypotensive effect of *N*-isopropylnorephedrine also remained uneffected after blockade of cholinergic receptors with hexamethonium (4 mg/kg) and atropine (1 mg/kg). These blockades were checked for adequacy with effective doses of acetylcholine. All substances blocked NE uptake at a concentration of 10^{-4} *M*, the most active being **5** and **6**. All of the compounds caused a release of catecholamines with the most active being **3** and **4** (Table II).

The spontaneous contractions of isolated rabbit atria were stopped by substances **4**, **6**, **7**, and **8** at a concentration of 10^{-3} *M* and by **3** and **5** at 10^{-4} *M*. At a concentration of 10^{-4} *M*, **6**, **7**, and **8** increased the contractions.

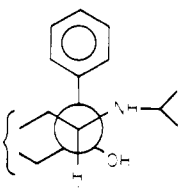
Discussion

The *trans*-decalin allows the conformation of groups at C-2 and C-3 to be fixed rigidly in space and enables us to offer postulations as to the importance of the steric aspects of the arrangement of the hydroxyl, phenyl, and amino functions with the understanding that these aspects are not responsible for all of the pharmacological results and differences in activities obtained with these compounds. Patil[4] and Swamy[5] showed that the configuration about the β-carbon in β-phenethanolamines was important in the potentiation of the NE response. They reported that (+)-ψ-ephedrine was not effective in potentiating the NE response but the (−) isomer was effective. The work reported herein is based on racemates but several postulations can be offered. Future work will involve the resolved compounds.

The most effective of the substances tested in lowering the blood pressure in the dog were **3** and **7**. Compound **3** is the *N*-isopropyl derivative of norephedrine. Compound **7** is an analog of **3a** (a rotamer structure of **3**). It has a fixed conformational requirement and can be depicted in Newman projections as follows.

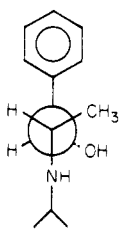


3a

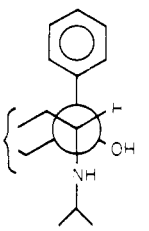


7

The nuclear magnetic resonance spectrum of **3** as the HCl salt shows a doublet for the benzylic proton at δ 5.2 (J = 4.5 Hz) which is indicative of the conformation shown (**3a**) or of a conformation in which the amino function forms a dihedral angle of ~180° with the phenyl but remains ~60° with respect to the hydroxyl function (**3b**). This is in agreement with the nuclear magnetic resonance study of ephedrine isomers by Portoghese.[6] Since the latter conformation of **3** would have the same conformational arrangement of functional groups as in **8**, and since **8** is of low activity, it is postulated that the conformation for **3** responsible for blood pressure lowering is as depicted in **3a**.

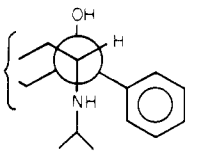


3b



8

Compounds **3** and **4**, the norephedrine and nor-ψ-ephedrine derivatives, showed only a slight potentiation of NE contraction in vas deferens while **5** and **6** showed a marked potentiation. This can be rationalized by assuming that the decalin molecule can bond hydrophobically to a greater extent with the NE uptake site and thus inhibits NE uptake. Compounds **7** and **8** both possess axial phenyl functions while **5** and **6** both have equatorial phenyl groups. This would tend to give an extended lipophilic profile to **5** and **6**. It is also possible that the axial hydroxyls in **5** and **6** contribute to the observed effect on uptake. The deoxy compounds have been prepared and will be examined to ascertain which of these groups is actually responsible for the inhibition observed.



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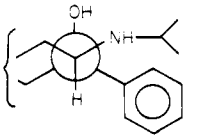
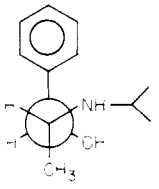
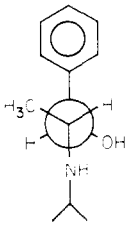


6

The increase in NE maximal response with **3**, the ephedrine analog, and **4**, the ψ-ephedrine analog, was almost identical, whereas the decalin **6**, related to ψ-ephedrine, increased the maximal response more than did the decalin **5**, related to ephedrine. Both **5** and **6** increased the PD_2NE over the effects noted with **3** and **4**. Both **3** and **4** can assume conformations in which the nitrogen is skewed (~60°) to the phenyl function and to the hydroxyl function. In these conformations (**3a**, **4a**) hydrogen bonding would be present between the hydroxyl and amino groups. While these conformations probably do exist, the populations in this form may be lower than that which is depicted by **3b** and **4b**. In these conformations the heavily



4a

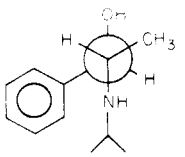


4b

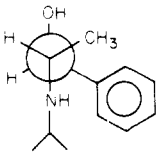
hydrated nitrogen function would not be in the sterically crowded area of the phenyl group but would be staggered (~180°) from the phenyl but still be at an angle which would allow hydrogen bonding to the hydroxyl function. If **3a** and **4a** are required for activity (dihedral angle of ~60° between phenyl and *N*-isopropyl groups) it can be postulated that their potentiation effect would be less than the rigid systems **5** and **6**. Compounds **5** and **6** possess equatorial phenyl groups in a fixed conformation with a 60° angle with the *N*-isopropyl group, thus explaining the higher activity for these two compounds.

The activity of **6** in potentiating NE contraction is greater than **5**. This can be rationalized as being due to the ability of **6** to approach the uptake site, with its two binding groups (phenyl, *N*-isopropyl) equatorial and the hydroxyl group axial.

With respect to the effect on the rabbit atria, it can be seen that compounds **3**, **4**, and **5** block heart contractions at their higher concentrations whereas they did not increase the heart contractions at their lower level. Compounds **6**, **7**, and **8** all increased the heart contractions at their lower levels. The structure–activity relationship here can be depicted as being due to the ability of **3** and **4** to act in the conformations **3c** and **4c** in which the hydroxyl group and the *N*-isopropyl function have a dihedral angle of ~180° and compound **5** has these two functions held rigidly in such a conformation. Compounds **6**, **7**, and **8** have fixed conformations in which the hydroxyl and *N*-isopropyl groups have a dihedral angle of ~60°. Furthermore, **3** and **5** possess the ephedrine configuration and are more active than **4** which is an analog of ψ-ephedrine.



3c



4c

The observations obtained with these racemic mixtures give an indication that different conformations and structural requirements are required by different receptor sites. It will be necessary to resolve these compounds into their optical antipodes for further studies in order to obtain more definitive results.

Experimental Section

NE dose–response curves[7] were obtained in isolated rat vasa deferentia[8] using concentrations from 3×10^{-7} to 3×10^{-5} *M*. Thirty Sprague–Dawley male rats (weight 300–350 g) were used. The isotonic contractions were recorded kymographically and standard intervals of 5 min after wash out were used between contractions. Tyrode's solution,[8] oxygenated by a carbon dioxide–oxygen mixture (5:95), was maintained at 37° in a 10-ml bath. Compounds **3**–**8** were added in concentration of 1×10^{-4} and 1×10^{-3} *M*.

To test the effects of catecholamine release on the NE dose–response curve, Serpasil-CIBA was injected ip in the dose 5 mg/kg 24 hr prior to the experiment.

NE uptake[1] was followed in isolated pieces of free floating vas deferens (weight 50–60 mg) using a concentration of 1.5×10^{-7} *M* of NE.[9] Cleaned and weighted pieces of rat vas deferens, two from each rat, were incubated in Tyrode's solution oxygenated with a mixture of carbon dioxide–oxygen (5:95), in Tyrode's solution with NE, or in Tyrode's solution with NE and the tested substance in a concentration of 1×10^{-4} *M*, for 60 min at 37°. After incubation, the tissue was washed three times for 5 sec in fresh Tyrode's solution neutralized with 1 ml of acetate buffer (0.1 *M*, pH 6) and 1 ml of this solution was used for fluorometric estimation of total catecholamines by Technicon autoanalyzer.[10] For release studies the muscles were incubated for 30 min with the individual agents at a concentration of 1×10^{-4} *M* in Tyrode's solution.

Blood pressure was examined in 12 adult mongrel dogs of both sexes weighing 12–18 kg, anesthetized with pentobarbital sodium (ip 35 mg/kg). A cannula was inserted into the right femoral artery and connected to a Statham transducer which in turn was connected to a Gilson recorder that had previously been calibrated with a mercury manometer. The compounds were administered iv via a cannula in the right femoral vein and were washed in each time with 0.5 ml of physiological saline. A tracheotomy was performed in all animals to maintain an adequate airway. Respiration was recorded by a bellows-type cuff connected to a Statham transducer which was in turn connected to the same Gilson recorder as used above. The electrocardiograph of dogs was continuously monitered with needle electrodes applied under the animal's skin after inducing anesthesia. The heart rate was computed from EKG.

The substances were also tested on 12 spontaneously contracting rabbit atria in Ringer–Locke solution in a 20-ml bath at 30°, aerated with 100% O_2. The isometric contractions were recorded on a Gilson polygraph using a strain-gauge transducer.

All statistical data are expressed as mean ± SE and the *t* test was used for statistical evaluation of the differences.

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References and Notes

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