PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 28–31 (1978)

# Conformational Requirements for Vascular $\alpha$-Adrenergic Activity (40132)

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Using the semi-rigid analogs of catecholamines with their side-chain fixed in cisoid or transoid conformations, Grey *et al.* (1) found that the transoid conformers were more active as $\alpha$-adrenergic agonists than the cisoid. Similarly, it has been shown that transoid conformation was required for action on the dopamine receptors in the central nervous system (2, 3) and in the renal vascular bed (4). These studies, however, did not provide information concerning the preferred rotameric conformation at the active site, which the catecholamines may take due to the freedom of rotation about the phenyl-C1 bond. The two extreme conformations resulting from 180° rotation around the phenyl-C1 bond are shown in Fig. 1.

In a recent study we have determined the rotameric conformations required for action on the dopamine vascular and $\beta_2$-adrenergic receptors (5). It was observed that a conformation of dopamine similar to that found in 2-amino-6,7-dihydroxytetrahydronaphthalene (trans $\beta$-rotameric form) was required for dopamine-like activity while a conformation found in 2-amino-5,6-dihydroxytetrahydronaphthalene (trans $\alpha$-rotameric form) was preferred for action on the $\beta_2$-adrenergic receptors, confirming the earlier report by Nishikawa *et al.* (6) with respect to the $\beta$-adrenergic receptor. Using the same analogs, we have now studied the conformational requirements for action on the $\alpha$-adrenergic receptor in a vascular smooth muscle. A brief report of this study has been made previously (7).

*Methods.* Rabbit aortic strips were prepared according to the method of Furchgott and Bhadrakom (8). Four strips, 20–25 mm long and 2–3 mm wide were prepared from the descending thoracic aorta of each rabbit and suspended in 10 ml baths containing Krebs Henseleit solution maintained at 37° ± 0.5° and aerated with a gas mixture of 95% oxygen and 5% carbon dioxide. Isometric contractions were recorded on a Beckman polygraph type RM using Statham force transducers. A resting tension of 2 g was applied for 1½ hr before starting any tests. The bathing fluid was changed every 15–20 min during the equilibration period.

Cumulative dose-response curves for each agonist were generated by adding 10 and 20 $\mu$l of the tenfold increasing concentrations of drugs. Each addition of drug was made after the maximum effect of the previous addition had been attained. Propranolol, $10^{-5}$ *M*, was added 10 min before starting the dose-response curve and was present in the bath throughout the period. The drug was washed out after the near maximum response had been reached. Following this procedure, three cumulative dose-response curves of different agonists were obtained on each strip. Sufficient time, approximately 1 hr, was allowed between dose-response curves to any two agonists. The maximum contraction caused by norepinephrine (NE), $10^{-5}$ *M*, was recorded for each strip. Results were calculated as a percentage of the NE-induced contraction and the data were analyzed by using the student's *t* test.

Reserpine, 1 mg/kg, was injected intraperitoneally to five rabbits 40 and 16 hr before killing to obtain strips depleted of endogenous catecholamines. Dose-response curves were obtained for each agonist in these strips following the same procedure as described above.

$pA_{10}$ of phentolamine was determined against each agonist following the procedure previously described (9). The effect of tenfold concentration increments of the agonist in the

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0037-9727/78/1581-0028$01.00/0

presence of one selected concentration of the antagonist was compared to the effect of unit dose (~$ED_{50}$) of each agonist prior to the antagonist in each strip. Four concentrations of phentolamine increasing by 3-, 10-, and 30-fold, ranging between $3 \times 10^{-8}$–$10^{-6}$ g/ml were used in each experiment. The antagonist was added 5 min before testing the effect of tenfold concentration of the agonist.

Dopamine hydrochloride, Calbiochem; epinine hydrochloride, K and K Laboratories; norepinephrine bitartrate, Sigma Chemical Company; 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrochloride (A-6,7-DTN), Abbott Laboratories; phentolamine hydrochloride (Regitine), Ciba Pharmaceutical Company; and the following compounds synthesized by J. G. Cannon and T. Lee were used: 2-methylamino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (N-methyl-A-6,7-DTN), 2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (A-5,6-DTN), and 2-methylamino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (N-methyl-A-5,6-DTN). For structural formulas see Fig. 1. All solutions of catecholamines and tetralin derivatives contained 0.1% sodium metabisulfite to prevent oxidation.

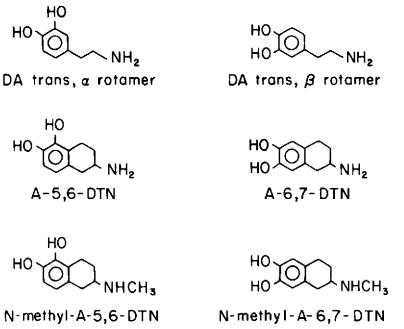


FIG. 1. Two rotameric conformations of trans DA and their semi-rigid structural analogs: 2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene (A-5,6-DTN) and its N-methyl analog (N-methyl-A-5,6-DTN); 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (A-6,7-DTN) and its N-methyl analog (N-methyl A-6,7-DTN). Epinine, N-methyl DA, which can exist in either rotameric conformation is not shown.

*Results.* Strips used in these experiments developed maximum tension ranging between 2.5–3 g, in response to $10^{-5}$ *M* NE. Cumulative concentration-response curves for each agonist obtained from such experiments normalized in terms of $10^{-5}$ *M* NE are shown in Fig. 2. The $ED_{50}$ values calculated from these dose-response curves are shown in Table I. Among the N-methylated analogs, epinine (N-methyl dopamine) with the open side-chain was significantly more active than N-methyl A-6,7-DTN which, in turn, was significantly more active than *N*-methyl A-5,6-DTN. Similar relative potencies were noted among the nor analogs: dopamine > A-6,7-DTN > A-5,6-DTN. The $ED_{50}$ value for A-5,6-DTN could not be determined as the contraction caused by the highest tested concentration of this compound did not reach 50% of the maximum contraction caused by NE.

Comparative activity of the six analogs was also measured on strips obtained from rabbits pretreated with reserpine. Hearts of these rabbits were used for estimation of catechol-

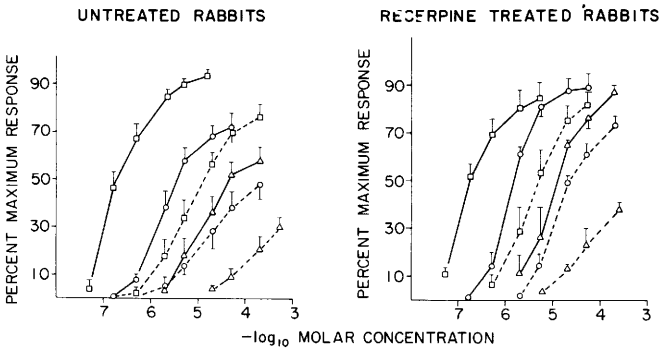


FIG. 2. Dose-response curves of the analogs in terms of the maximum contraction caused by $10^{-5}$ *M* NE (mean ± SE), on strips obtained from untreated and reserpine-treated rabbits. □——□ Epinine, □----□ Dopamine, ○——○ N-methyl-A-6,7-DTN, ○-----○ (A-6,7-DTN), △——△ N-methyl-A-5,6-DTN, △-----△ A-5,6-DTN. Number of experiments (4–7) for each analog are shown in Table I.

TABLE I. $ED_{50}$ VALUES OF THE ANALOGS ON NORMAL AND CATECHOLAMINE-DEPLETED RABBIT AORTIC STRIPS AND $pA_{10}$ VALUES OF PHENTOLAMINE AGAINST THEM (EXPOSURE TIME 5 MIN) MEAN ± SE.[a]

| Compound | $ED_{50}$ Values | | $pA_{10}$ of Phentolamine |
|---|---|---|---|
| | Untreated Rabbits | Reserpine-treated rabbits | |
| Epinine | 6.71 ± 0.22 (4) | 6.72 ± 0.14 (5) | 6.6 ± 0.04 (5) |
| N-methyl-A-6,7-DTN | 5.43 ± 0.14 (6) | 5.82 ± 0.05 (4) | 6.4 ± 0.14 (4) |
| Dopamine | 4.99 ± 0.17 (7) | 5.30 ± 0.21 (5) | 6.7 ± 0.12 (6) |
| A-6,7-DTN | 3.89 ± 0.36 (5) | 4.65 ± 0.10 (5) | 6.8 ± 0.62 (3) |
| N-methyl-A-5,6-DTN | 4.25 ± 0.20 (6) | 4.89 ± 0.10 (5) | 6.4 ± 0.08 (4) |

[a] Figures in parentheses are numbers of experiments.

amines and were found to contain less than 5% (0.026 ± 0.09 μg/g $n = 5$) of the endogenous catecholamine concentration of hearts from the control group (0.67 ± 0.09 μg/g $n = 3$). Cumulative concentration-response curves of these strips, based on the same type of experiments and treated similarly as described for normal strips, are shown in Fig. 2. The $ED_{50}$ values calculated from these curves are also shown in Table I. $ED_{50}$ values for N-methyl A-6,7-DTN and N-methyl A-5,6-DTN are significantly lower in catecholamine depleted strips than in the normal strips ($P < 0.05$) but for the other compounds the $ED_{50}$ values in two conditions are not different statistically. The compounds show the same order of relative potency with catecholamine-depleted strips as with the normal strips.

$pA_{10}$ values of phentolamine (exposure time 5 min) against each of the agonists are shown in Table I and range between 6.4 and 6.8, indicating that phentolamine causes similar antagonism of all the analogs in the series. The $pA_{10}$ value against A-5,6-DTN could not be determined because of its low potency.

*Discussion.* On the basis of the above results, the following generalizations can be made. First, the flexible dopamine and epinine molecules were more active than the semi-rigid analogs. Secondly, the addition of an N-methyl group to dopamine or the semi-rigid analogs increased α-adrenergic activity by approximately 50-fold. This data is in accordance with a previous investigation by Lands (10) with a series of catecholamines. Third, the A-6,7-DTN derivatives (trans β-rotamers) were significantly more potent than A-5,6-DTN (trans α-rotamers) as long as the compounds with the same substitutions on the nitrogen were compared.

The various analogs showed the same order of relative potency on strips depleted of endogenous catecholamines (reserpine-treated rabbits) as on the normal strips. Also, the individual analogs showed similar or somewhat greater potency on strips obtained from reserpine-treated rabbits compared to their potencies on normal strips. This fact, coupled with closely similar $pA_{10}$ values of phentolamine (6.4–6.8) against the open chain as well as the semi-rigid analogs, provides evidence that these compounds act directly on the α-adrenergic receptors in the vascular smooth muscle to produce contraction. Therefore, the relative activities of the various analogs reflect their potencies as α-adrenoceptor agonists. As such, out of the two extreme conformations, A-6,7-DTN (trans β-rotamer), being 30- to 50-fold more active than A-5,6-DTN (trans α-rotamer), appears to be the preferred conformation at the α-receptors. The fact that A-6,7-DTN and its N-methyl analog are less potent than dopamine and epinine, respectively, may appear to contradict the hypothesis that the β-rotamer represents the preferred conformation for α-adrenergic activity. However, α-methylation of catecholamines is known to result in weaker α-adrenergic agonists (9–11) and placing the α-carbon in the cyclohexene ring, as in these A-6,7-DTN derivatives, has an effect similar to methyl substitution on the α-carbon in the open chain compounds. Secondly, the ADTN derivatives are racemic mixtures and it is possible that one isomer may exert greater α-adrenergic activity.

We have previously determined the relative activities of these analogs on $\beta_2$-adrenergic receptors in the dog hind limb (5). Strikingly, the preferred conformations at the α- and $\beta_2$-adrenergic receptors are converse of each other, i.e., the 6–7 orientation of the catechol

group is preferred at the α-while 5–6 conformation is preferred at the $\beta_2$-receptors. In either case, N-methylation enhances the activity of the nor analog, but the open chain compounds are more active at the α-receptor than on the $\beta_2$-receptor. We have used only one test system for each of the adrenergic receptors. If, as a result of further studies on other adrenergic receptor preparations, a pattern of conformational preferences is established, it could provide deeper insight into the molecular and spatial disposition of the two adrenergic receptors.

Finally, it may be pointed out that the β-rotameric conformation is the preferred conformation for both α-adrenergic and dopamine receptor activation, indicating some similarity in the molecular structures of the two receptors. This is further supported by the overlap of antagonistic activity against both receptors in certain classes of compounds. Phenothiazines and butyrophenones, which are dopamine antagonists, also produce significant α-adrenergic blockade (12).

*Summary.* Semi-rigid analogs of trans α- and β-rotameric conformations of dopamine and epinine were studied for their α-adrenergic activity on isolated rabbit aortic strips. 2-Amino-6,7-dihydroxytetrahydronaphthalene and its N-methyl derivative (analogous to the trans β-rotamer of dopamine) were 30- to 50-fold more potent than 2-amino-5,6-dihydroxytetrahydronaphthalene and its N-methyl derivatives, respectively, indicating that the trans β-rotamer, rather than the trans α-rotamer, is the preferred conformation for action on the α-adrenergic receptors.

We wish to thank Mr. Morris Senegor for technical assistance, Mr. Ralph Earlandson for catecholamine assays, and Dr. C. W. Ours for the generous supply of 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene. This research was supported by NIH Grant Nos. GM-22220, GM-22365, GM-07019, and NS-12324.

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Received September 6, 1977. P.S.E.B.M. 1978, Vol. 158.