Pronounced Pharmacological Differences Arising from Minor Structural Changes in Conformationally Defined Amphetamine Analogues. Comparative Evaluation of *endo*- and *exo*-2-Amino- and *endo*- and *exo*-2-(Methylamino)benzobicyclo[2.2.1]heptene and -benzobicyclo[2.2.2]octene Analogues. Conformationally Defined Adrenergic Agents. 4

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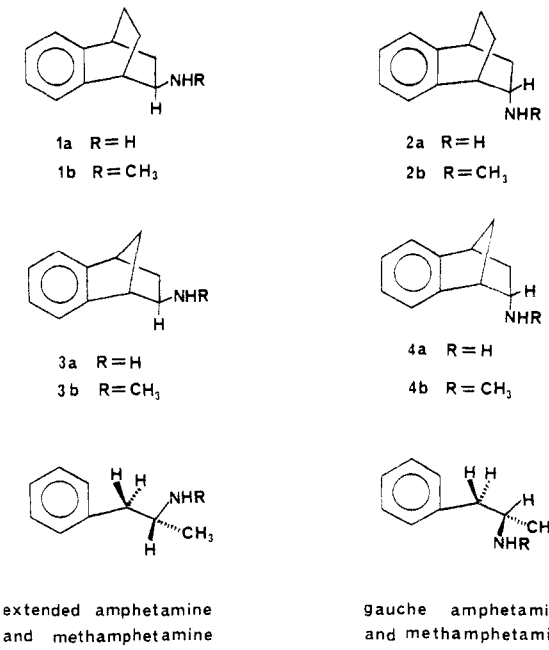
The conformationally defined analogues of amphetamine, *exo*- (**3a**) and *endo*-2-aminobenzobicyclo[2.2.1]heptene (**4a**), and methamphetamine, *exo*- (**3b**) and *endo*-2-(methylamino)benzobicyclo[2.2.1]heptene (**4b**), were synthesized and their stereochemical assignments confirmed by NMR analysis. Benzonorbornen-2-one (**7**) was converted to its oxime (**8**), followed by reduction to **4a**. Amine **4b** was produced from **7** by reductive amination with methylamine and $NaBH_3CN$. Amine **3a** was obtained by reduction of azide **5**, formed from benzonorbornadiene and mercuric azide. Formylation of **3a**, followed by hydride reduction, gave amine **3b**. These compounds were evaluated for their ability to inhibit the uptake of [^{3}H]norepinephrine ([^{3}H]NE) into chopped cerebral cortex and atrial tissue, to increase locomotor activity in mice, and to increase the beating rate of isolated rat atria. Pharmacological manipulations were performed to vary the releasable pool of NE from primarily vesicular to primarily extravesicular in nature. The data were compared to those previously reported for the closely related amphetamine analogues *exo*- (**1a**) and *endo*-2-aminobenzobicyclo[2.2.2]octene (**2a**) and methamphetamine analogues *exo*- (**1b**) and *endo*-2-(methylamino)benzobicyclo[2.2.2]octene (**2b**). As was found for the conformationally restricted gauche analogues **2a** and **2b** in the [2.2.2] ring system, the endo [2.2.1] analogues (**4a,b**) were consistently less active than the trans antiperiplanar (exo) analogues. However, in the exo analogues (**3a,b**) removal of one methylene unit from the ethylene bridge of the exo [2.2.2] ring system resulted in pronounced pharmacological differences. While **3b** was equipotent with methamphetamine in releasing [^{3}H]NE from extravesicular storage sites as measured by biochemical methods (tracer studies), release of extravesicular NE as measured by physiological methods (isolated atria, locomotor activity) was apparently masked by a nonspecific depressant effect induced by the compound. These complicating factors were not observed for the [2.2.2] analogue **1b**. Also, while both **1a** and **1b** showed a sensitivity to compartmentation of the releasable pool of [^{3}H]NE, **3a** showed a loss of sensitivity (possibly through monoamine oxidase inhibition). Since these pharmacological differences occurred upon relatively minor structural modifications, care should be exercised in utilizing such compounds as tools for assessing conformational preferences when the pharmacological evaluations employed rest upon physiological viability of effector membranes.

We have recently demonstrated conformational preferences for the amphetamine-induced inhibition of [^{3}H]-norepinephrine ([^{3}H]NE) uptake[1] and for the release[1,2] of previously accumulated [^{3}H]NE from adrenergic nerve terminals of rat brain and rat atria by utilizing as conformationally defined amphetamine analogues the exo (**1a**) and endo (**2a**) isomers of 2-aminobenzobicyclo[2.2.2]octene and as methamphetamine analogues the exo (**1b**) and endo (**2b**) isomers of 2-(methylamino)benzobicyclo[2.2.2]octene (Chart I). The rationale for this approach was based upon the excellent spatial approximations of amphetamine (or methamphetamine) rotamers afforded by this ring system. The endo and exo isomers of the 2-methylamine derivative provide excellent strain-free models of the gauche and fully extended (see Chart I) conformations, respectively, of methamphetamine, with minimal perturbation of bond orders, angles, and distances as determined by molecular orbital calculations[3] and X-ray crystallography.[4] The pharmacological results indicated that, while the fully

Chart I



extended conformation of amphetamine or methamphetamine was optimal for interaction with the uptake carrier and for displacement of NE from extravesicular storage sites, neither the gauche nor the fully extended confor-

(1) Bartholow, R. M.; Eiden, L. E.; Ruth, J. A.; Grunewald, G. L.; Siebert, J.; Rutledge, C. O. *J. Pharmacol. Exp. Ther.* **1977**, *202*, 532.
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(3) Grunewald, G. L.; Creese, M. W.; Walters, D. E. *ACS Symp. Ser.* **1979**, *no. 112*, 439.
(4) Grunewald, G. L.; Walters, D. E.; Flynn, D. L.; Atwood, S. D.; Creese, M. W.; Frenz, B. A.; Troup, J. M. *Acta Crystallogr., Sect. B* **1978**, *34*, 3462.

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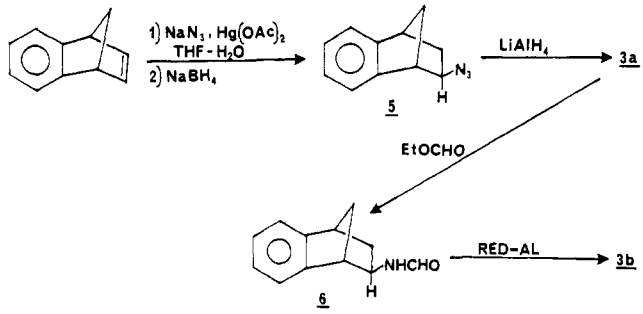
Scheme I



mation was effective in displacing NE from vesicular storage sites, where either conformational mobility or a conformation of amphetamine not approximated by either rigid analogue was required.

In an effort to further examine the validity of this general approach to the determination of conformational requirements for the interaction of sympathomimetic amines with the noradrenergic nerve ending, we have examined the pharmacological behavior of the closely related amphetamine analogues *exo*- (**3a**) and *endo*-2-aminobenzobicyclo[2.2.1]heptene (**4a**), as well as the corresponding methamphetamine analogues **3b** and **4b** (Chart I). The compounds were examined under varying conditions of NE compartmentation for ability to inhibit [^{3}H]NE uptake and to promote [^{3}H]NE release from noradrenergic nerve endings in rat brain (**3a,b** and **4a,b**) and rat heart (**3b**, **4b**), for their effects on locomotor activity in mice (**3a,b** and **4a,b**), and for their chronotropic effects on isolated beating rat atria (**3a,b** and **4a,b**). The effect of all four analogues on release of [^{3}H]dopamine ([^{3}H]DA) in rat brain was also measured.

While the [2.2.1] analogues (**3** and **4**) afford spatial arrangements quite similar to those of the [2.2.2] analogues (**1** and **2**), the former ring system is considerably more strained. We expected no significant differences in pharmacological behavior between the [2.2.1] (**3** and **4**) and [2.2.2] (**1** and **2**) analogues. On the contrary, the results to be described demonstrate that removal of a methylene unit from the ethylene bridge of the [2.2.2] analogues results in pronounced differences in pharmacological activity.

Brain and atrial tissue from untreated rats were employed to assess pharmacological effects under conditions of predominantly vesicular NE storage. An exaggerated extravesicular pool of NE was obtained by pretreatment of the tissue with pargyline in vitro,[5] while predominantly extravesicular compartmentation was obtained by loading tissue with [^{3}H]NE after reserpine (in vivo) and pargyline (in vitro)[6] treatment.

Results and Discussion

Chemistry. Although various pharmacological studies[7-9] as well as a ^{13}C NMR study[10] on all or some of these amines have been reported elsewhere by other authors, detailed experimental synthetic procedures and proof of structure have not been previously published. The exo amines **3a**[11] and **3b** were synthesized as outlined in Scheme I. Sodium borohydride reduction of the adduct of benzonorbornadiene[13] and mercuric azide afforded azide **5**, which was not purified but was reduced directly with lithium aluminum hydride to the primary amine **3a** in 49% overall yield. Treatment of **3a** with excess ethyl formate afforded formamide **6**, which was reduced with sodium bis(2-methoxyethoxy)aluminum hydride to give amine **3b** in 74% yield.

Scheme II

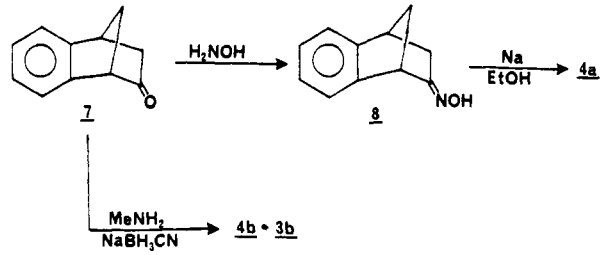
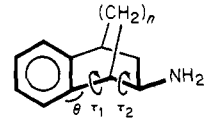


Table I. Comparison of Bicyclic Ring Systems as Frameworks for Phenethylamines[a-c]



compd	D, A	τ_1, deg	τ_2, deg	θ, deg
1 (n = 2)	5.07	60	179	127
3 (n = 1)	5.03	69	176	131
amphetamine sulfate	5.13	75	172	120

[a] Based on X-ray coordinate data; summarized from ref 3. [b] D = distance from the nitrogen to the center of the aromatic ring. [c] τ_1 and τ_2 are dihedral angles and θ is a bond angle as illustrated in the above structure.

The endo amines **4a**[9] and **4b**[9] were prepared from benzonorbornan-2-one (**7**)[13] as shown in Scheme II. Treatment of **7** with hydroxylamine gave oxime **8**,[9] which on reduction with sodium in ethanol in a similar manner to that reported[9] afforded the endo primary amine **4a**.

The corresponding methylamine **4b** was prepared by reductive amination[15] of ketone **7**. Treatment of **7** with methylamine and sodium cyanoborohydride in 5 N HCl–methanol afforded a mixture of the *endo*- and *exo*-methylamines **4b** and **3b** in approximately a 5:1 ratio (as determined by ^{1}H NMR).

Assignment of exo and endo orientation for the amine group in compounds **3** and **4** was based on their ^{1}H NMR spectra. The ^{1}H NMR spectra of substituted benzonorbornenes have been examined at length.[16,17a,b] Wilt et al. have shown that for the 2-chloro[17a] and 2-bromo[16] deriv-

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(8) Wood, L. E. *Res. Commun. Chem. Pathol. Pharmacol.* **1978**, *21*, 169.
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(10) Burn, P. K.; Crooks, P. A.; Meth-Cohn, O. *Org. Magn. Res.* **1978**, *11*, 370.
(11) This route to **3a** has previously been described; however, the amine was not isolated but was characterized as the *p*-toluenesulfonamide.[12]
(12) Dominianni, S. J.; Demerco, P. V. *J. Org. Chem.* **1971**, *36*, 2534.
(13) Prepared in 55% yield by the procedure of Kleinfelter[14] for the preparation of norbornanone from norbornene.
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Table II

drug	IC_{50}, μM, for uptake of [^{3}H]NE	EC_{50}, μM for release of [^{3}H]NE[a,b]	EC_{50}, μM for release of [^{3}H]DA[a,c]
1a	0.8[d]	147	192[d]
1b	0.8[d]	178	185
2a	39.0[d]	1000	681[d]
2b	48[d]	>1000	>1000
3a	1.1	16	185
3b	1.8	450	271
4a	100	630	794
4b	369	>1000	>1000
d-amphetamine	0.2[d]	0.8[d]	11[d]
l-amphetamine	0.5[d]	1.7[d]	61[d]

[a] Values for release are only estimates, since maximal effect was often not obtained even at millimolar concentration of drug. [b] Assume a maximum of 43% release. [c] Assume a maximum of 95% release. [d] Reference 1.

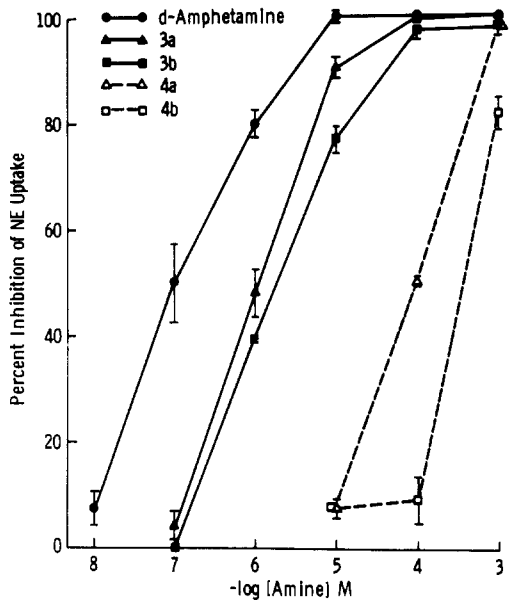

Figure 1. Inhibition of uptake of [^{3}H]norepinephrine into cerebral cortex tissue by amphetamine and rigid analogues of amphetamine. Chopped cerebral cortex was incubated for 10 min with various concentrations (10^{-8} to 10^{-3} M) of the compounds. [^{3}H]Norepinephrine (10^{-7} M) was then added, and the incubation was continued an additional 10 min. Tritium was measured in tissue and medium. Inhibition of neuronal uptake is expressed as a percentage of control values (mean ± SEM) for three to four experiments. The mean tissue/medium ratio for uptake in control samples was 7.1 ± 0.4.

atives the hydrogen attached to the halogenated carbon of the endo isomers resonates more than 0.5 ppm downfield from the corresponding hydrogen of the exo isomers. Also, in the endo derivatives the endo-C_3 hydrogen was distinguishable as a high-field doublet of triplets. Indeed, the signal for the endo-C_2 hydrogen of **3a** appeared at δ 2.95, while that for the exo-C_2 hydrogen of **4a** resonated at δ 3.62. Also, the pattern for the endo-C_3 hydrogen of **4a** was observed as a doublet of triplets at δ 0.60 ($CDCl_3$).

Pharmacology Our initial decision to utilize the [2.2.2] ring system over the [2.2.1] system was based on the closer spatial approximations to the amphetamine rotamers which it provided.[3] Also there is less strain inherent in the [2.2.2] ring system (Table I). As shown in Table II, the two [2.2.1] isomers (Figure 1) revealed no significant differences from the [2.2.2] analogues in the inhibition of uptake of [^{3}H]NE into chopped cerebral cortical tissue from rat brain. The IC_{50} for inhibition of [^{3}H]NE uptake

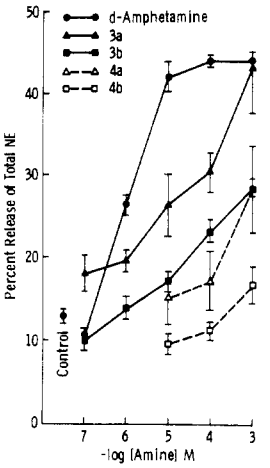

Figure 2. Release of [^{3}H]norepinephrine from isolated cerebral cortex by amphetamine and rigid analogues of amphetamine. Tissue which had previously accumulated [^{3}H]norepinephrine was incubated for 30 min with various concentrations (10^{-7} to 10^{-3} M) of the compounds. [^{3}H]Norepinephrine was separated from deaminated metabolites by cation-exchange chromatography. Release of [^{3}H]norepinephrine in the medium of the total [^{3}H]-norepinephrine (medium + tissue). Each value represents the mean ± SEM of three to four experiments. Control represents release in samples which were incubated in the absence of drug.

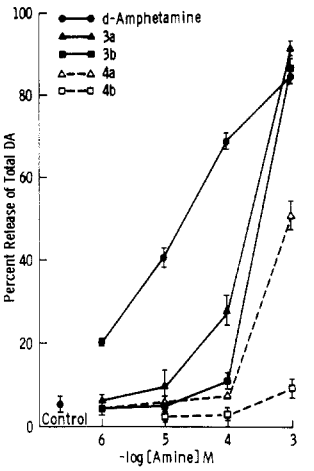

Figure 3. Release of [^{3}H]dopamine from isolated corpus striatum by amphetamine and rigid analogues of amphetamine. See legend to Figure 2 for design of experiment. Each value represents the mean ± SEM of three to four values. Control represents release in samples which were incubated in the absence of drug.

by **3a** was 1.1 μM, while the endo analogue **4a** was 100 μM. The data again support a fully extended conformation as optimal for interaction with and competitive blockade of the NE uptake carrier.

Similarly, examination of the analogues for the release of previously accumulated [^{3}H]NE from chopped cortex demonstrated that all of the conformationally defined analogues were much less potent than amphetamine (Figure 2 and Table II). Of the four exo analogues, the primary amine **3a** was at least ten times more potent than the other three. Thus, removal of the methyl substituent on the amine in the [2.2.1] series results in increased potency. This change in potency was not observed in the [2.2.2] series. In both the [2.2.1] and [2.2.2] series, the endo amines were considerably less potent than their corresponding exo isomers (Table II).

Examination of the release of previously accumulated [^{3}H]DA from chopped corpus striatum demonstrated that the [2.2.1] amines behaved similarly to their corresponding [2.2.2] analogues (Figure 3, Table II). In all cases, they

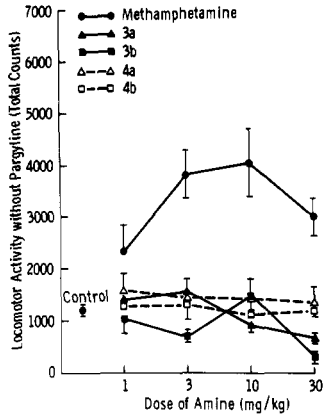

Figure 4. The effect of methamphetamine and rigid analogues of methamphetamine on mouse locomotor activity. Mice were injected ip with either drug or saline (control) and immediately placed into an activity cage. Locomotor activity is expressed as total counts in an Animex activity meter for the 1-h session. Each value represents the mean ± SEM of four determinations.

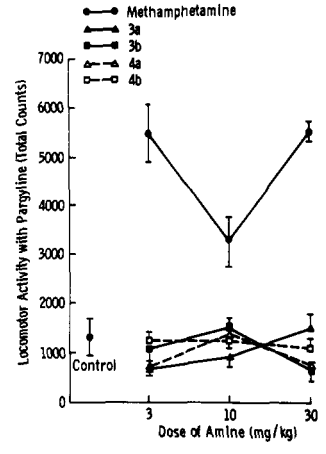

Figure 5. The effect of methamphetamine and rigid analogues of methamphetamine on locomotor activity of mice pretreated with pargyline. Mice were injected ip with pargyline, 100 mg/kg, 6 h before each session. Locomotor activity was determined as described under Experimental Section and the legend to Figure 4. Each value represents the mean ± SEM of four determinations.

were less potent than amphetamine. In each pair of amines (e.g., **3a,b**) there is little difference in potency between the primary and secondary derivatives (Table II).

However, examination of the ability of the compounds to increase locomotor activity in mice revealed an immediate difference in activity between the two ring systems. Whereas it had been demonstrated that the exo [2.2.2] analogue **1b** produced no increase in locomotor activity in untreated mice, pretreatment with pargyline (100 mg/kg, ip) 6 h prior to the experiment resulted in pronounced increases in locomotor activity,[1] similar in magnitude to that produced by methamphetamine. We felt this observation was consistent with the exaggeration of an extravesicular releasable NE pool known to result from monoamine oxidase inhibition.[5] Such was not the case with the exo [2.2.1] analogues **3a** and **3b**. These compounds and their endo isomers **4a** and **4b** were ineffective with (Figure 5) or without (Figure 4) pargyline pretreatment.

Using isolated beating atria from rat as a sensitive measure of NE release, we had previously demonstrated that NE release induced by the exo [2.2.2] analogue **1b** was quite sensitive to the intraneuronal compartmentation of the releasable NE pool. Thus, while the exo analogue **1b** produced no positive chronotropic responses under con-

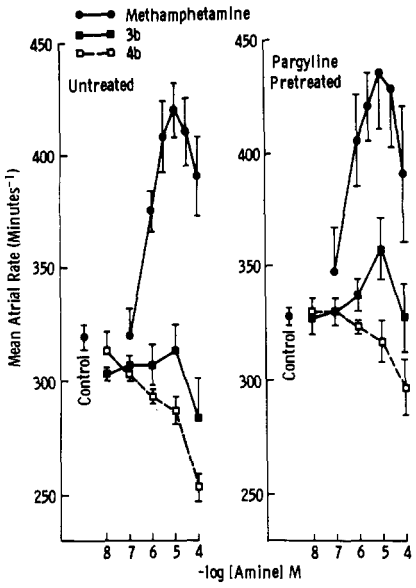

Figure 6. The effect of methamphetamine and the rigid analogues of methamphetamine on the beating frequency of isolated rat atria with and without pretreatment of the atria with pargyline (10^{-4} M). Drugs were added every 2 min until a cumulative dose–response curve was obtained. Each value represents the mean ± SEM of three experiments.

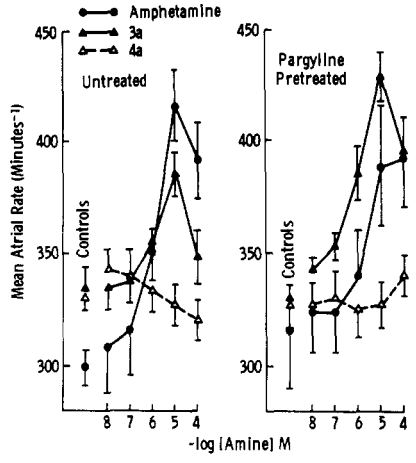

Figure 7. The effect of amphetamine and the rigid analogues of amphetamine on the beating frequency of isolated rat atria with and without pretreatment of the atria with pargyline (10^{-4} M). Drugs were added every 2 min until a cumulative dose–response curve was obtained. Each value represents the mean ± SEM of three experiments.

ditions of primarily vesicular storage, after treatment of the tissue with pargyline (10^{-4} M for 10 min, in vitro) to exaggerate extravesicular storage, **1b** approached methamphetamine in producing positive chronotropy.[2] Furthermore, upon repletion of a predominantly extravesicular releasable pool of NE by loading beating atria with NE after pretreatment with pargyline (in vitro) and reserpine (in vivo), methamphetamine and **1b** were equipotent in inducing release, with concommitant positive chronotropic response. In addition, release of previously accumulated [^{3}H]NE from similarly treated tissue showed **1b** and methamphetamine to have superimposable dose–response curves.[1]

Examination of the corresponding [2.2.1] methamphetamine analogues **3b** and **4b** revealed no increases in atrial rate before or after pargyline pretreatment (Figure 6). These results indicated no NE release from an extravesicular compartment by **3b** *as measured by atrial*

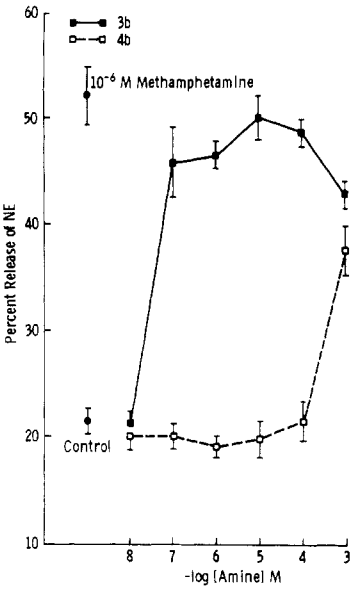

Figure 8. The effect of methamphetamine and the rigid analogues on the release of previously accumulated *l*-[^{3}H]NE from reserpine- and pargyline-treated rat atrial tissue. Tissue which had previously accumulated the ^{3}H-labeled amine was incubated for 15 min with various concentrations of the drugs. Release of [^{3}H]norepinephrine is expressed as a percentage of the total ^{3}H-labeled amine (medium + tissue). Each value is the mean ± SEM of three to six experiments. Control represents release in samples which were incubated in the absence of drug. Dose–response curves for methamphetamine are reproducible in these assays, so the value at only 10^{-6} M is given here. See ref 2 for the complete dose–response curve for methamphetamine.

rate. However, similar examination of the [2.2.1] amphetamine analogues **3a** and **4a** did reveal a positive chronotropic response induced by the exo analog (**3a**) both before and after pargyline pretreatment (Figure 7). In this case, the results suggested a lack of dependence upon compartmentation of releasable NE for the production of a positive chronotropic effect. This result was surprising in view of the minor structural difference (N-methylation) between the two exo analogues. Furthermore, it had been reported by Wood[8] that both the exo and endo [2.2.1] amphetamine analogues **3a** and **4a** did release previously accumulated [^{3}H]NE from rat atria. It should be noted, however, that the release reported for **4a** was considerably less than for **3a** and required a 10-fold higher concentration of drug. For the exo isomer **3a**, Wood[8] reported that the [^{3}H]NE release induced by this [2.2.1] analogue was not dependent upon compartmentation of the releasable NE pool, since release was observed both under conditions of predominantly vesicular and extravesicular storage.

In an effort to reconcile these observations, we performed release studies with the methamphetamine analogues **3b** and **4b** using pargyline- and reserpine-pretreated rat atrial tissue. The results, illustrated in Figure 8, indicated that the exo (**3b**) but not the endo (**4b**) [2.2.1] analogue did promote release of previously accumulated [^{3}H]NE from extravesicular storage sites quite similar to the release observed[2] with the exo [2.2.2] analogue **1b** and with methamphetamine.

Thus, upon removal of one methylene unit from the ethylene bridge of the exo [2.2.2] methamphetamine analogue **1b** to give **3b**, evidence of NE release as measured in vivo (locomotor studies) or in whole organs (isolated atria) was lost, while biochemical evidence of NE release demonstrated activity essentially identical with that of the [2.2.2] analogues. The same modification of the exo [2.2.2] amphetamine analogue (compare **1a** and **3a**) resulted in a loss of sensitivity to compartmentation of the releasable pool of [^{3}H]NE in release studies. The endo [2.2.1] isomers (**4a,b**) were inactive in all studies, as were the corresponding [2.2.2] isomers (**2a,b**).

It is not clear what factors are involved in the loss of activity of the [2.2.1] analogue **3b** in the isolated atrial rate and **3a** and **3b** in the locomotor activity evaluations mentioned. At this point we can only ascribe the phenomenon to a nonspecific depressant effect by **3b** which competes with the direct effects of NE release. This type of phenomenon has been observed before with substituted amphetamine analogues.[18] Thus, while we observed nonspecific membrane toxicity with **1b** at millimolar concentrations, if this phenomenon were operative at lower concentrations with **3b** it would counteract the positive chronotropy induced by NE release. However, it is not immediately apparent what factors would contribute to the observed membrane toxicity or what specific mechanisms might be involved. Since we did not see evidence for release by **4b** (at the same concentration used by Wood for **4a**[8]), we have no explanation for the release induced by the endo [2.2.1] analogue **4a** as reported by him.

The loss in sensitivity to compartmentation of the releasable NE pool observed with the exo [2.2.1] amphetamine derivative (**3a**) could be explained by two possible mechanisms. The first would involve displacement of vesicular NE by the [2.2.1] amphetamine analogue. We feel this is unlikely in view of the inability of either exo [2.2.2] analogue (**1a** or **1b**) to promote positive chronotropy in rat atria without pargyline pretreatment. Biochemical release studies reported in this paper for rat brain tissue add support to this argument. The second possibility involves inhibition of monoamine oxidase by the exo analogue (**3a**) itself, thus generating a releasable extravesicular pool of NE.

Thus, while the conformationally defined amphetamine analogues **1** and **3** are useful tools for studying conformational requirements for displacement of NE from noradrenergic nerve endings when biochemical (tracer) techniques are employed, care must be exercised when using physiological indices of NE release (locomotor activity, isolated beating atria), since direct membrane stabilization by the analogues, as observed for **3b** but not **1b**, or monoamine oxidase inhibition, as observed for **3a**, may mask or enhance the effects of NE release. Relatively minor structural changes in the analogues may result in pronounced differences in pharmacological activity.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained on a Beckman IR-33 spectrophotometer. ^{1}H NMR spectra were determined at 60 MHz with Varian T-60 and EM-360 spectrometers with Me_4Si as an internal standard. Electron-impact (70 eV) mass spectra were obtained on a Varian CH-5 mass spectrometer. Microanalyses were performed on a Hewlett-Packard Model 185B CHN analyzer at the University of Kansas. Where analyses are indicated only by symbols of the elements, the analytical results obtained for these elements were within ±0.4% of the theoretical values. Column chromatography was performed using silica gel (Brinkmann 70–230 mesh). R_f values reported were determined using Brinkmann precoated silica gel (Brinkmann F-254, 0.25 mm) plates and 5:2 $CHCl_3$–MeOH (saturated with NH_3) as the eluting solvent.

***exo*-2-Aminobenzobicyclo[2.2.1]heptene (3a).** To a solution of sodium azide (9.75 g, 150 mmol) and mercuric acetate[19] (15.95 g, 50 mmol) in 50% aqueous THF (100 mL) was added benzo-

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norbornadiene[20] (7.1 g, 50 mmol). After stirring at 50 °C for 16 h, the two-phase mixture was diluted with 15% aqueous KOH (50 mL) and treated with a solution of $NaBH_4$ (1 g, 26 mmol) in 15% aqueous KOH (50 mL). The THF layer was separated and the H_2O layer extracted with Et_2O (3 × 50 mL). The combined organic fractions were washed with H_2O and brine and dried ($MgSO_4$). The Et_2O solution (containing azide **5**) was carried on without further purification. The Et_2O solution of **5** was slowly added to a solution of $LiAlH_4$ (3.6 g) in anhydrous Et_2O (100 mL) maintained at 0 °C. After the addition of azide was complete, the ice bath was removed and the mixture stirred at room temperature for 1 h, at which time the reaction was quenched by the sequential addition of H_2O (4 mL), 15% NaOH (4 mL), and H_2O (16 mL). The mixture was filtered and the precipitate was washed with Et_2O. The filtrate was extracted with Et_2O (2 × 50 mL). The combined organic layers were washed with H_2O and brine and were dried (K_2CO_3). Evaporation of the solvent yielded a dark viscous oil, which was distilled to give 3.9 g (49% from benzonorbornadiene) of **3a** as a clear oil: bp 62–63 °C (0.1 mmHg); R_f 0.51; NMR ($CDCl_3$) δ 1.18–2.07 (2 H, m, C_3 methylene), 1.82 (2 H, s, NH_2, exchangeable in D_2O), 1.87 (2 H, br s, C_9 methylene), 2.83–3.13 (2 H, m, C_4 methine and NCH), 3.25 (1 H, br s, C_1 methine), 6.97 (4 H, s, aromatic). The hydrochloride salt was prepared and recrystallized from EtOH–Et_2O to give a white solid: mp 278–281 °C dec; MS M^+ 159 (P – HCl); IR (KBr) 3430 cm^{-1} (NH). Anal. ($C_{11}H_{14}NCl$) C, H, N.

***exo*-2-(Methylamino)benzobicyclo[2.2.1]heptene (3b).** Amine **3a** (1.0 g, 6.3 mmol) and ethyl formate (3.73 g, 50.4 mmol) were placed under N_2 in a 200-mL Wheaton pressure bottle. The mixture was heated at 95 °C for 6 h. After the mixture cooled, evaporation of the excess ethyl formate gave crystalline **6** in quantitative yield, which was used without further purification. To a suspension of formamide **6** in Et_2O (50 mL) was added dropwise a benzene solution of sodium bis(2-methoxyethoxy)-aluminum hydride (37.8 mmol) with cooling. The mixture was refluxed for 5 h and poured over ice. The aqueous layer was washed with Et_2O (2 × 50 mL). The combined organic portions were washed with H_2O and brine and were dried (K_2CO_3). Evaporation of the solvent gave an orange oil, which was distilled to give 800 mg (74%) of a colorless oil: bp 60–62 °C (0.1 mmHg). The oil was further purified by silica gel (100 g) column chromatography ($CHCl_3$–MeOH, 7:2, saturated with NH_3) to give 580 mg (53%) of amine **3b** as a colorless oil: R_f 0.52; NMR ($CDCl_3$) δ 1.10–2.03 (2 H, m, C_3 methylene), 1.27 (1 H, s, NH, exchangeable in D_2O), 1.82 (2 H, br s, C_9 methylene), 2.27–2.40 (1 H, m, NCH), 2.42 (3 H, s, NCH_3), 3.17 (2 H, br s, bridgeheads), 6.97 (4 H, br s, aromatic). The hydrochloride salt was prepared and recrystallized from EtOH–Et_2O to give a white solid: mp 188–188.5 °C; MS M^+ 173 (P – HCl); IR (KBr) 3440 cm^{-1} (NH). Anal. ($C_{12}H_{16}NCl$) C, H, N.

***endo*-2-Aminobenzobicyclo[2.2.1]heptene (4a).** Oxime **8**[9] was reduced with Na/EtOH in a similar manner to that reported.[9] Following distillation, **4a** was obtained as a clear oil: bp 52–53 °C (0.25 mmHg); R_f 0.60; NMR ($CDCl_3$) δ 0.60 (1 H, dt, J = 12 and 3 Hz, C_3 endo methylene), 1.23 (2 H, br s, NH_2, exchangeable in D_2O), 1.55–1.98 (2 H, m, C_9 methylene), 2.32 (1 H, ddd, J = 12, 9, and 4 Hz, C_3 exo methylene), 3.17 (2 H, br s, bridgeheads), 3.62 (1 H, dt, J = 9 and 4 Hz, NCH), 7.03 (4 H, br s, aromatic). The hydrochloride salt was prepared and recrystallized from EtOH–Et_2O to give a white solid: mp 224–225 °C (lit.[9] mp 230–231 °C); MS M^+ 159 (P – HCl); IR (KBr) 3450 cm^{-1} (NH). Anal. ($C_{11}H_{14}NCl$) C, H, N.

***endo*-2-(Methylamino)benzobicyclo[2.2.1]heptene (4b).** To a solution of anhydrous $MeNH_2$ (4.65 g, 150 mmol) in absolute MeOH (950 mL) was added MeOH saturated with gaseous HCl (10 mL), followed by ketone **7** (3.95 g, 25 mmol) and $NaBH_3CN$ (942 mg, 15 mmol). The solution was stirred for 45 h under N_2 in the presence of 3 Å molecular sieves. Concentrated HCl was then added to adjust the pH to below 2, and the MeOH was evaporated in vacuo. The residue was taken up into water and extracted with Et_2O. The aqueous layer was made basic (solid KOH) and extracted with Et_2O (4 × 50 mL). The combined Et_2O fractions were washed with H_2O and brine and were dried (K_2CO_3). Evaporation of the solvent gave a yellow oil which on distillation afforded 2.4 g (55%) of a clear liquid (bp 66 °C at 0.1 mmHg), which by NMR was ca. a 5:1 mixture of **4b** and **3b**. The mixture was separated by silica gel (200 g) column chromatography ($CHCl_3$–MeOH, 7:2, saturated with NH_3) to give 1.5 g (35%) of amine **4b** as a clear oil: R_f 0.58; NMR ($CDCl_3$) δ 0.22 (1 H, s, NH_2, exchangeable in D_2O), 0.67 (1 H, dt, J = 12 and 3 Hz, C_3 endo methylene), 1.47–2.0 (2 H, m, C_9 methylene), 2.0–2.5 (1 H, m, C_3 exo methylene), 2.32 (3 H, s, NCH_3), 3.1–3.6 (3 H, m, 2 bridgeheads and NCH), 7.03 (4 H, br s, aromatic). The hydrochloride salt was prepared and recrystallized from EtOH–Et_2O to give a white solid: mp 229–230.5 °C (lit.[9] mp 226–229 °C dec); MS M^+ 173 (P – HCl); IR (KBr) 3420 cm^{-1} (NH). Anal. ($C_{12}H_{16}NCl$) C, H, N. The slower moving (R_f 0.52) amine (0.3 g, 6%) was shown to be identical with **3b** prepared above.

Pharmacology. Methamphetamine hydrochloride was obtained from the A. H. Robbins Co. (Richmond, Va). *l*-[7-^{3}H]NE (5.8 Ci/mmol) and [^{3}H]DA (5.2 Ci/mmol) were obtained from Amersham/Searle (Arlington Heights, IL). Reserpine (Serpasil) and desipramine hydrochloride were obtained from Ciba-Geigy Corp. (Summit, NJ). Pargyline hydrochloride was obtained from Abbott Laboratories (North Chicago, IL), and β-thujoplicin was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Brain Studies. Adult male Sprague–Dawley rats (200–300 g) were killed by decapitation, the brains were quickly removed, and the cerebral cortex (white matter removed) or the corpus striatum was dissected and chopped with a mechanical tissue chopper at 0.3-mm intervals.

Uptake of [^{3}H]Norepinephrine into Chopped Brain Cortex. Uptake of the ^{3}H-labeled amine was studied by the method of Ziance and Rutledge.[21] The tissue was preincubated for 10 min at 37 °C in physiological salt solution equilibrated with 95% O_2–5% CO_2.[22] [^{3}H]NE was added to achieve a final concentration of 10^{-7} M and to give a total incubation volume of 2.0 mL. After incubation at 37 °C for an additional 20 min, the uptake was immediately terminated by centrifugation (10000*g* for 5 min, 4 °C). The supernatant was removed and the tissue was resuspended in 1.0 mL of physiological salt solution. The suspension was centrifuged and supernatant combined with the medium. The tissue was homogenized in 95% ethanol and then centrifuged at 10000*g* for 10 min. The radioactivity in the media and the ethanol extract was determined by liquid scintillation spectrometry. The protein content of the tissue homogenate was determined by the method of Layne.[23]

Release of [^{3}H]Norepinephrine from Chopped Cortex and [^{3}H]Dopamine from Chopped Striatum. The method of Ziance and Rutledge[21] was used for studying the release of ^{3}H-labeled amines from isolated brain. The selected tissue was incubated with 10^{-6} M *dl*-[^{3}H]norepinephrine or [^{3}H]dopamine and then washed to remove the unbound and nonspecifically bound ^{3}H-labeled amine. The drug was incubated with the tissue for 30 min, followed by centrifugation to separate the tissue from the medium. The ^{3}H-labeled amines in the medium and tissue extracts were separated from deaminated metabolites by adsorption and elution from Dowex 50, Na^+. The radioactivity in each fraction was determined by liquid scintillation spectrometry, and the ^{3}H-labeled amines were adjusted for recoveries in the individual fractions. The recoveries of both norepinephrine and dopamine were between 80 to 85%. The results were expressed as the percentage of ^{3}H-labeled amine in the medium of the total ^{3}H-labeled amine (medium plus tissue). EC_{50} represents the concentration that produces a half-maximal effect.

Measurement of Locomotor Activity. Locomotor activity was measured with an Animex locomotor activity apparatus (Stoelting Co., Chicago, Ill.), which measures changes in oscillator circuits as animals move across electromagnetic fields.[24] Adult male Swiss Webster mice (20–40 g) were injected with the test

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drug (ip) and immediately placed in a motor activity cage which rests on the Animex apparatus. The activity meter was pretuned and the sensitivity set at 40 μA before each test session. The activity cage was a blackened box which contained a light and a ventilation fan which produced constant low-level noise. Thus, extraneous audible and visual distractions were minimized. The activity of each animal was measured separately, and the animals were used once. Results are expressed as cumulative activity counts for the 1-h session.

Atrial Studies. Adult male Sprague–Dawley rats (180–250 g) were used in all experiments. When employed, reserpine pretreatment consisted of a single ip injection (2 mg/kg) 18 h before the animals were killed. For atrial release studies, a Tris buffer (pH 7.4) of the following composition (millimolar concentrations) was employed: NaCl, 140; KCl, 5; $CaCl_2$, 1.5; $MgSO_4$, 1.2; Tris, 10; and *d*-glucose, 10.[25] The medium was equilibrated with 100% O_2, and all incubations and manipulations were performed at 37 °C.

For atrial rate studies, a Krebs–Henseleit buffer[26] was employed (pH 7.4). The medium was equilibrated with 95% O_2–5% CO_2. All media contained disodium edetate (3×10^{-5} M) and ascorbic acid (10^{-4} M) to prevent chemical oxidation of NE and β-thujoplicin (10^{-4} M) as an inhibitor of catechol *O*-methyltransferase.[27]

In isolated atrial rate experiments, the heart was quickly removed and placed in a dish of fresh Krebs–Henseleit medium.[26] A strand of 2-0 surgical silk was affixed to each atrial apex, and then the ventricles were removed. The atria were then mounted in a 20-mL jacketed (37 °C) tissue bath and attached to a Grass strain gauge (250–500 mg resting tension). Responses were recorded using a Grass Model 7 physiograph. The incubation medium was continuously equilibrated with 95% O_2–5% CO_2. After a constant basal rate was established, the atria were treated with the appropriate drug in a volume of 20 μL. After a constant response was obtained, usually after 2 min, the next higher concentration of drug was added. This process was repeated until a cumulative concentration–effect curve was obtained.

In experiments where monoamine oxidase inhibition was desired, the atria were incubated in the presence of pargyline (10^{-4} M) for 10 min, followed by washing, before test drugs were added. Results are expressed as mean atrial rate at peak response (2 min) after addition of the test drug.

When drug-induced efflux of [^{3}H]NE from reserpine- and pargyline-treated atrial tissue was measured, the method described by Paton[25] was used. Briefly, reserpine-pretreated (2 mg/kg ip 18 h prior to experiment) animals were killed by decapitation, and the hearts were quickly removed. The atria were dissected; fat and connective tissue were removed. Each pair of atria was cut into six pieces of tissue. The tissue pieces were incubated in medium containing pargyline (10^{-4} M) for 30 min, washed, and then incubated with 10^{-7} M [^{3}H]NE for 1 h. After each piece of tissue was suspended on a fine hook, the tissue samples were transferred every 5 min to a series of tubes containing fresh medium. Test drugs were present in the tubes from 60 to 100 min of efflux. At the end of the efflux period, each piece of tissue was homogenized in 3 mL of absolute ethanol. The amount of radioactivity in aliquots of the tissue and media samples was determined by liquid scintillation spectrometry. Samples were corrected for quenching by conversion of counts per minute to disintegrations per minute. The efflux of NE was expressed as an efflux coefficient *f*:

$$f\ (\text{min}^{-1}) = \frac{\Delta A}{\Delta t \cdot A_t}$$

where ΔA is the disintegrations per minute lost from the tissue during the time interval Δt and A_t is the ^{3}H-labeled amine content of the tissue midway through the interval Δt. The ^{3}H-labeled amine content of the tissue at each point in the experiment was obtained by adding in reverse order the amount of ^{3}H-labeled amine lost during each efflux period to the amount remaining in the tissue at the end of the experiment.

When concentration–effect curves were obtained, the atrial tissue accumulated [^{3}H]NE as described above. After incubation with the tracer, each piece of tissue was placed into a small, porous basket constructed as a loosely wound coil (4-mm diameter × 7-nm length) at the end of a 4-in. length of 26-gauge nichrome wire. The tissue holders were suspended in a beaker containing 50 mL of fresh medium. The samples were incubated for 10 min, during which time the medium was vigorously stirred (mechanical stirrer) and equilibrated with oxygen. The tissue samples were transferred every 10 min to a beaker of fresh medium until 1 h of washing had been achieved. Each sample was then placed in a tube containing 2 mL of fresh medium and the drug to be tested. The tubes were stoppered and agitated vigorously for 15 min. The tissue samples were removed and homogenized in 3 mL of absolute ethanol. The ^{3}H-labeled amine content of the tissue and medium fractions was determined by liquid scintillation spectrometry.[28] The results were expressed as percent release, where:

$$\%\ \text{release} = \frac{\text{dpm of media}}{\text{dpm of media + dpm of tissue}} \times 100$$

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Synthesis and β-Adrenergic Blocking Activity of New Aliphatic Oxime Ethers

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New β-adrenergic blocking agents, most of which do not contain an aromatic nucleus, were synthesized. They were derived either from alkylamino-aliphatic oxime ethers or alkylamino-aliphatic ethers. Most active among these are *O*-[3-(*tert*-butylamino)-2-hydroxypropyl]acetoxime (**8**; trachea pA_2 = 7.65) and 1-isobutoxy-3-(*tert*-butylamino)-2-propanol (**15**; trachea pA_2 = 7.49), both of which displayed bronchoselectivity (β_2/β_1 ratio ~15). The role and importance of the aromatic nucleus in this class of compounds are discussed.

Recently we reported the synthesis of a new series of acetophenone oxime derivatives having marked β-adrenoceptor blocking activity.[2a] Our results showed that the intercalation of a >C=N– bond in the side chain of the

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