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Sympathomimetic effects of *exo*- and *endo*-isomers of 2-aminobenzonorbornene in vitro and in vivo

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A stereospecific synthesis of *endo*-2-aminobenzornorbornene is described. Its sympathomimetic activities and those of its *N*-methyl derivative were compared with the equivalent *exo*-isomers using the isolated rat anococcygeus muscle and the anaesthetized rat blood pressure preparations. On the anococcygeus muscle preparation the *endo*- and *exo*-isomers of the primary amines had similar indirectly acting sympathomimetic activities. In contrast the *exo*-*N*-methyl derivative was a far more potent sympathometic in vitro than the *endo*-*N*-methyl isomer. In the anaesthetized rat the *exo*- and *endo*-isomers of 2-aminobenzonorbornene and their *N*-methyl derivatives all had similar pressor activities, though the successive injections of the two *exo*-derivatives suggested an additional α-adrenoceptor blocking activity. The actions of these rigid sympathomimetics are compared with those of the flexible amphetamine structure.

Amphetamine has a wide range of central and peripheral actions mediated via several molecular mechanisms of action. The flexibility of the amphetamine molecule enables it to adopt different conformations and contributes to its range of activity. Two extreme conformations of amphetamine are included in Fig. 1, the *anti*-conformation when the amino group is most distant from the benzene ring, and the *gauche*-conformation when it is closest. Various conformationally restrained analogues of amphetamine have been synthesized and compared for biological activities (Martin et al 1969; Barfknecht et al 1973; Smissman & Pazdernik 1973a,b; Solomons & Sam 1973; Nelson & Sherwood 1974). Several pairs of more rigid analogues approximating to the extreme conformations of amphetamine have been compared mainly in their affinity for noradrenergic uptake processes including 2-aminoindane and 1-aminoindane, tranylcypromine and its *cis*-isomer (Horn & Snyder 1972) and the *exo*- and *endo*-isomers of 2-aminobenzobicyclo[2.2.2.] octene (Grunewald et al 1976). Molecular model comparisons show that all of these conformationally restrained amphetamine analogues have varying degrees of rotational or conformational freedom making them of limited use in elucidating the topography of the receptor site. We now describe the synthesis of the *exo*- and *endo*-isomers of 2-aminobenzonorbornene (Fig. 1) which are rigid stereoanalogues of the *anti*- and *gauche*-conformers of amphetamine. We have initially compared their sympathomimetic effects and those of their *N*-methyl and *NN*-dimethyl derivatives in vitro and in vivo. Some of the results were presented to the British Pharmaceutical Conference (Burn et al 1976).

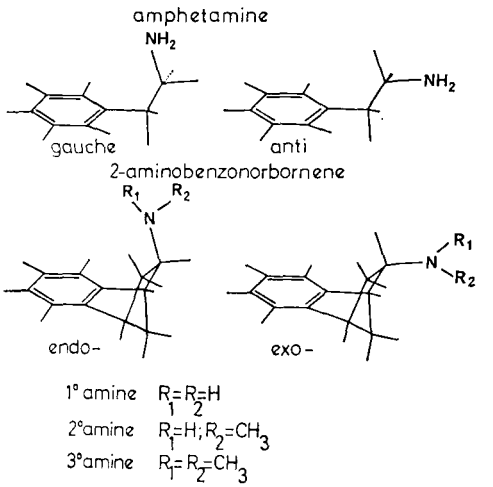

FIG. 1. The *anti* and *gauche* conformations of amphetamine, and the structures of the *endo*- and *exo*-isomers of 2-aminobenzonorbornene.

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MATERIALS AND METHODS

Preparation of the derivatives of 2-aminobenzonorbornene

exo-*2-Aminobenzonorbornene* was prepared by the method of Dominianni & Demarco (1971). The *N*-methyl and *NN*-dimethyl derivatives were synthesized by standard methods. The three drugs were

prepared as the fumarate salts. endo-*2-Aminobenzonorbornene.* To a mixture of formamide (4·5 g, 0·1 mol) and formic acid (11·5 g, 0·25 mol) at 120°C was added a solution of benzonorbornene-2-one (Bartlett & Giddings 1960) (4 g, 0·025 mol) in formamide (4·5 g, 0·1 mol) over 5 min. The resulting mixture was heated at 150°C for 17 h, cooled, and excess formic acid and formamide removed on a rotary evaporator. To the residue was added aqueous hydrochloric acid (60·8 ml, 20%) and the reflux continued for a further 7 h. The cooled acidic mixture was extracted with ether and basified with aqueous sodium hydroxide (20% w/w). The liberated base was extracted with ether, the solvent and water were removed and the product distilled under vacuum to give the title compound as an oil (3g, 75%) b.p. 84–86°C; 1·0 mm Hg; fumarate salt, m.p. 154–158°C, found C, 65·3; H, 6·4; N, 5·0. Calc for $C_{15}H_{17}O_4N$: C, 65·44; H, 6·22; N, 5·08%. The *N*-methyl derivative was prepared as above using the equivalent molar concentration of *N*-methylformamide in place of formamide to provide an oil (3·7 g, 85%) b.p. 66–68°C, 0·1 mm Hg; fumarate salt, m.p. 141–146°C, found C, 66·6; H, 6·4; N, 4·5. Calc. for $C_{16}H_{19}O_4N$: C, 66·42; H, 6·62; N, 4·84%. The *NN*-dimethyl derivative was similarly prepared using *NN*-dimethylformamide providing an oil (3·7 g, 78%) b.p. 72–74°C; 0·01 mm Hg; fumarate salt m.p. 112–114°C, found C, 67·2; H, 7·2; N, 4·5. Calc. for $C_{17}H_{21}O_4N$: C, 67·31; H, 6·98; N, 4·62%.

Rat isolated anococcygeus muscle

The anococcygeus muscles from male Sprague-Dawley rats were dissected out (Gillespie 1972) and mounted under a resting tension of 0·75 g in 20 ml tissue baths in Krebs solution bubbled with 5% CO_2 in O_2 and maintained at 37°C. Changes in tension were recorded from an Ether strain gauge and displayed on a Rikadenki recorder. Drug contact time was such that maximum increases in tension were recorded, the time differing between the directly acting sympathomimetics (usually within 1 min), and the indirectly acting drugs (about 3 min). Results are expressed as the mean increases in tension (g ± s.e.m.). Noradrenaline controls were established on all preparations. In most cases concentration effect lines to (+)-amphetamine and/or 2-aminoindane were also determined. Addition of concentrations of known or anticipated indirectly-acting sympathomimetics were alternated with additions of noradrenaline to minimize the likelihood of tachyphylaxis.

Rat blood pressure and heart rate

Sprague-Dawley rats (340–550 g) were anaesthetized with urethane (250 mg kg^{-1} i.p.), a femoral vein cannulated and heparin injected (2000 U kg^{-1}). Following cannulation of a carotid artery, blood pressure and heart rate were recorded via a pressure transducer and displayed on a Grass polygraph. The trachea was exposed and cannulated. Drugs were injected into the femoral cannula, and washed in with 0·3 ml 0·9% NaCl (saline). Doses of drugs are given as mg kg^{-1} and their effects summarized as the difference in mean arterial blood pressure. A dose interval of 8 min was chosen and injection of indirectly-acting sympathomimetics were interspaced with injections of noradrenaline.

Drugs used

2-Aminoindane hydrochloride (Aldrich), (+)-amphetamine sulphate (B.D.H.), cocaine hydrochloride (M & B), noradrenaline bitartrate (Koch Light), phentolamine mesylate (Ciba), *cis*-2-phenylcyclopropylamine hydrochloride (SKF), *trans*-2-phenylcyclopropylamine hydrochoride (tranylcypromine, Sigma). Doses and concentrations are expressed as the salts.

RESULTS

Rat isolated anococcygeus muscle

Fig. 2 compares the effects of noradrenaline, amphetamine and 2-aminoindane (panel a), and noradrenaline, tranylcypromine and its *cis*-isomer (panel b). The two indirectly-acting sympathomimetics had parallel log-concentration effect slopes.

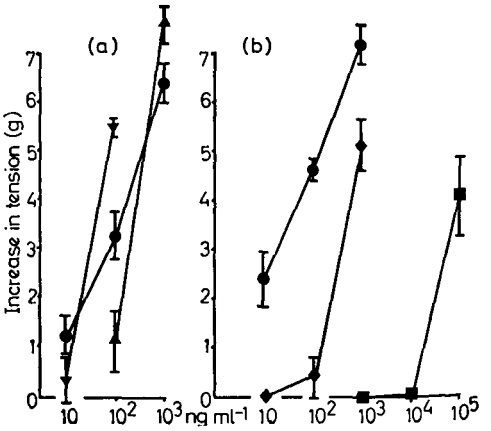

FIG. 2. Mean increases in tension of the rat anococcygeus muscle caused by (a) amphetamine (▼), noradrenaline (●), and 2-aminoindane (▲), and (b) noradrenaline (●), tranylcypromine (◆), and its *cis*-isomer (■). Limits are ± s.e.m.

Noradrenaline was more potent than either, though had a more shallow slope. Tranylcypromine was of similar potency to 2-aminoindane, though its *cis*-isomer was some 100 times less potent.

Fig. 3a compares the effects of the *exo*- and *endo*-isomers of 2-aminobenzonorbornene. The *exo*-isomer was of similar potency to 2-aminoindane, the *endo*-isomer being less potent though the difference was not significant. Fig. 2b shows the stereoselectivity of the *exo*-*N*-methyl derivative. Whilst it was of similar potency to 2-aminoindane (though had a lower maximum effect), the *endo*-isomer was without effect in concentrations up to 10 μg ml^{-1}.

The effects of the two primary amines, and the *exo*-*N*-methyl derivatives were re-examined in the continuous presence of phentolamine (200 ng ml^{-1}) or cocaine (5 μg ml^{-1}). Either of these concentrations of drugs abolished the action of amphetamine (1 μg ml^{-1}) on this preparation. In the same way the actions of the three derivatives of 2-aminobenzonorbornene in concentrations up to 10 μg ml^{-1} were abolished by either phentolamine or cocaine.

Whilst no systematic examination of the effects of the benzonorbornenes on noradrenaline contractions was made, the interspacing with noradrenaline concentrations provided some indication of interactions. During the construction of log concentration effect lines for both amphetamine and 2-aminoindane, the increases in tension caused by noradrenaline (200 ng ml^{-1}) were augmented. The same potentiation was seen during the screening of the *exo*- and *endo*-derivatives. In contrast the *endo*-*N*-methyl derivative had little effect on the responses to noradrenaline, whilst the increases in tension caused by noradrenaline were reduced during examinations of the *exo*-*N*-methyl derivative.

Neither of the *NN*-dimethyl derivatives had any effect on the preparation in concentrations up to 10 μg ml^{-1}, and they were examined no further.

Rat blood pressure and heart rate

Fig. 4 compares the mean increases in arterial blood pressure caused by the *exo*- and *endo*-isomers of 2-aminobenzonorbornene and their *N*-methyl derivatives with those caused by both noradrenaline and amphetamine. The striking feature of the comparison

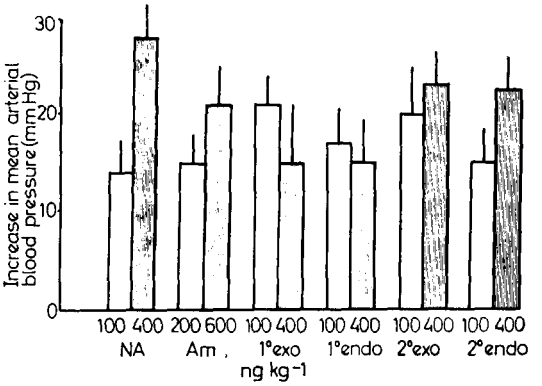

FIG. 4. The increases in mean arterial blood pressure caused by noradrenaline (NA), amphetamine (Am), the *exo*- and *endo*-isomers of 2-aminobenzonorbornene (1° exo, 1° endo), and the *exo*- and *endo*-isomers of *N*-methyl-2-aminobenzonorbornene (2° exo, 2° endo). Means are of groups of between 5 and 20 (± s.e.m.)

is the similarity between the pressor effects of all four derivatives. They were as potent as amphetamine, though in the doses selected the two primary amines appeared to have reached a maximum effect.

In some instances the pressor effect of the derivatives was accompanied by tachycardia, though the change was not easily quantifiable. The effects of noradrenaline and amphetamine on heart rate were unpredictable though there was a tendency for a reflex bradycardia.

During the experiments it was noted that the responses to noradrenaline were progressively reduced following the previous injections of *exo*-2-aminobenzonorbornene or its *N*-methyl derivative. The effects of the four derivatives were further examined using the same time courses described above but with a Latin Square design of dose addition.

Fig. 5 contrasts the antagonism of noradrenaline's pressor effect following injection of *exo*-2-aminobenzonorbornene, with its reproducibility during

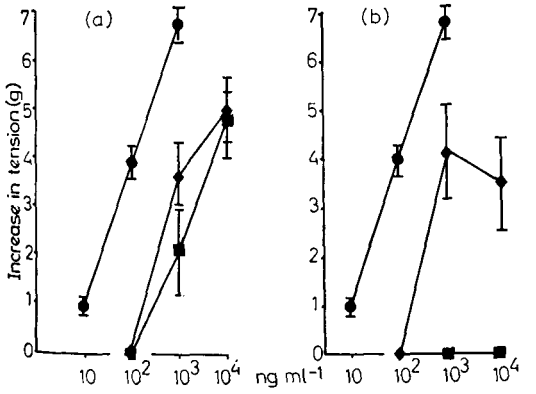

FIG. 3. Mean increases in tension of the rat anococcygeus muscle caused by (a) noradrenaline (●), *exo*-2-aminobenzonorbornene (◆), and its *endo*-isomer (■), and (b) noradrenaline (●), *exo*-*N*-methyl-2-aminobenzonorbornene (◆), and its *endo*-isomer (■). Limits are ± s.e.m.

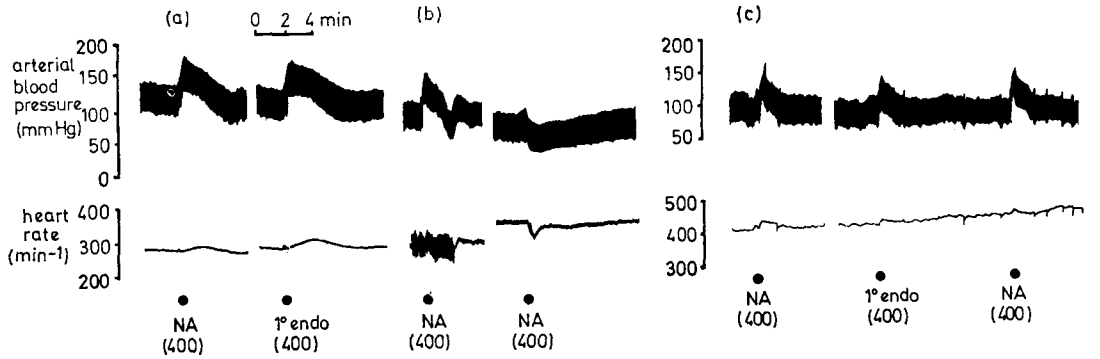

FIG. 5. Changes in blood pressure and heart rate of the anaesthetized rat caused by (a) noradrenaline and *endo*-2-aminobenzonorbornene; (b) two injections of noradrenaline between which were injected two doses of *exo*-2-aminobenzonorbornene (not shown); (c) two injections of noradrenaline separated by an injection of *endo*-2-aminobenzonorbornene. Doses (in brackets) are in ng kg^{-1}.

studies of the equivalent *endo*-derivative. Fig. 6. shows the progressive decrease in noradrenaline's pressor effect when interspaced with injections of *exo*-*N*-methyl-2-aminobenzonorbornene. The equivalent *endo*-isomer did not affect noradrenaline.

DISCUSSION

Patil et al (1974) have reviewed the steric requirements of drugs which interfere with the various components of noradrenergic transmission. It is clear that different components have different steric requirements. Comparisons of stereochemically rigid derivatives of amphetamine have recently concentrated on their differing abilities to inhibit catecholamine uptake either measured directly (e.g. Horn & Snyder 1972), or indirectly by measuring the potentiation of noradrenaline (e.g. Grunewald et al 1976). The latter authors compared derivatives chemically most similar to our own.

Whilst we have measured tissue response in vitro and gross cardiovascular change in vivo, our results are compatible with those above studies which measured the one component of the multiple effects of amphetamine derivatives on noradrenergic transmission.

The derivatives of 2-aminobenzonorbornene have the characteristics of indirectly-acting sympathomimetics. Their actions were similar to those of amphetamine in that they caused a delayed contraction of the anococcygeus muscle which was abolished by either an inhibitior of noradrenaline uptake or by an α-adrenoceptor blocking agent. In vivo, they had marked pressor activity which was accompanied by variable changes in heart rate.

Differences between the *exo*- and *endo*-isomers of 2-aminobenzonorbornene in vitro and in vivo were negligible, though there was a striking difference between the isomers of the *N*-methyl derivatives in

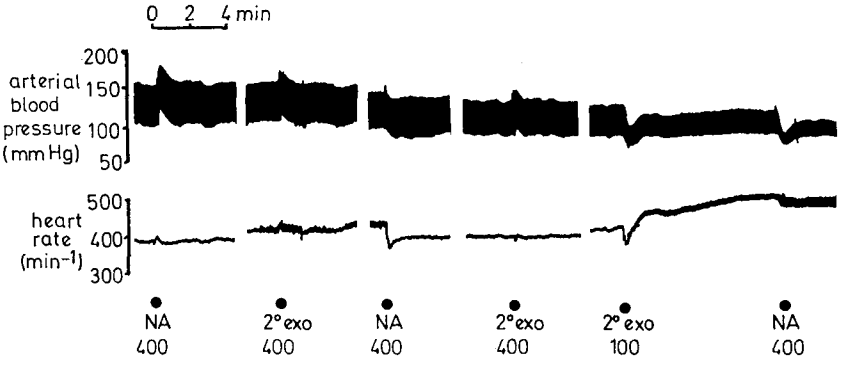

FIG. 6. Changes in blood pressure and heart rate caused by injections of noradrenaline and *exo*-*N*-methyl-2-aminobenzonorbornene. Doses are in ng kg^{-1}.

vitro, activity residing in the *exo*-isomer equivalent to the *anti*-conformation of *N*-methylamphetamine. We can offer no convincing explanation of the relative lack of stereoselectivity between the primary amines. It is not a characteristic common to all primary amines as is apparent in our own striking confirmation of the differences between tranylcypromine and its *cis*-isomer. Horn & Snyder (1972) showed a 600-fold difference between these isomers in their abilities to inhibit catecholamine uptake into the hypothalamus.

Our results in vitro with the derivatives of 2-aminobenzonorbornene are similar to those described by Grunewald et al (1976) for the chemically related sympathomimetics, the *endo*- and *exo*-isomers of 2-aminobenzobicyclo[2.2.2.]octene. They obtained an index of inhibition of noradrenaline uptake in the rat vas deferens. They showed some degree of stereoselectivity of the *exo*-isomer of the primary amine, but greater stereoselectivity when the *N*-methyl derivatives were compared. Inhibition of noradrenaline uptake by competition is one component of the multiple effects of amphetamine on noradrenergic transmission.

The predicted stereoselectivity for the inhibition of noradrenaline uptake compares favourably with the partial stereoselectivity for indirect sympathomimetic activity, but contrasts with the absence of stereoselectivity of the isomers of 2-aminobenzonorbornene on rat blood pressure in vivo. This illustrates only the complexity of mechanisms of action of sympathomimetics. The only points of note were the lower maximum pressor effect compared with that of noradrenaline and amphetamine, and the antagonism of sympathomimetic responses by the *exo*-isomer of *N*-methyl-2-aminobenzonorbornene.

This antagonism had the characteristics of α-adrenoceptor blockade. It was also noted that in the in vitro experiments the responses to the interspacing concentrations of noradrenaline were progressively reduced following additions of this isomer in contrast to its potentiation by amphetamine and 2-aminoindane.

The use of a wide range of modifying agents in pithed rats will elucidate further the mechanisms of action of these new indirectly-acting sympathomimetic amines.

The derivatives of 2-aminobenzonorbornene provide a novel series of structures for examinations of drugs which interfere with noradrenergic transmission. The advantages of the structure are that it is available in two isomeric forms which are structural analogues of two extreme conformations of amphetamine, the molecules are stereochemically rigid, and this rigidity is effected with minimal addition to the molecular weight of amphetamine.

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