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In vitro actions of *NN*-dimethyl-2-aminoindane and related compounds

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The actions of 2-aminoindane, 2-amino-1,2,3,4-tetrahydronaphthalene and amphetamine and their *N*-methyl and *NN*-dimethyl derivatives on the guinea-pig ileum were compared. All, except the tertiary 2-aminoindane, inhibited the responses to electrical stimulation and it is suggested that this is not fully explained by their sympathomimetic properties. *NN*-Dimethyl-2-aminoindane increased these responses and caused contractions of the unstimulated ileum due to a nicotine-like action. The indane series was more effective in producing contractions of the ileum than the other compounds. The three tertiary derivatives antagonized competitively the actions of histamine on the ileum and those of 5-hydroxytryptamine on the rat uterus.

During investigation of the central pharmacological properties of 2-aminoindane (Crooks et al 1973; Little & Rees 1978) and its derivatives (Fig. 1)

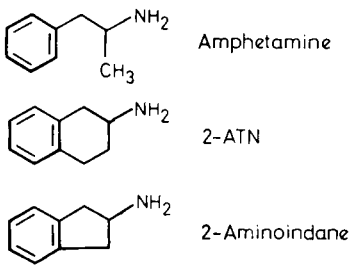

FIG. 1.

certain in vitro actions of these compounds were observed. We were particularly interested in the effects of the restriction of the mobility of the amino group, caused by the formation of the second ring, on the pharmacological properties compared with those of the amphetamines. The addition of one α-methyl group to amphetamine only slightly modifies its action but *NN*-dimethylation considerably decreases its central and sympathomimetic properties. The present results show that in the 2-aminoindane series *NN*-dimethylation changes the pharmacological activity in a different way.

METHODS

(*i*) *The isolated ileum of the guinea-pig*

Segments of ileum (approximately 3 cm in lenght) were suspended in a 10 ml tissue bath in Krebs—Henseleit solution bubbled with 95% O_2, 5% CO_2 at 37 °C. The resting tension was 1 g. Isometric recordings of longitudinal changes in tension were made using a 2 oz Pye Ether tensile/compressive load transducer, the output from which was fed into a Rikadenki pen recorder.

Electrical stimulation of the preparation was applied using two stainless steel electrodes, the cathode being situated in the lumen. Contractions were elicited by rectangular pulses of 0·2 ms duration at 0·2 Hz frequency and just supramaximal voltage (about 30 V).

For the unstimulated preparation there was a 2 min time cycle with a 30 s tissue contact time for the drugs and two washes. This was used for the administration of all agonists except *NN*-dimethyl-2-aminoindane, when it was doubled because of the slower response of the tissue, and 5-hydroxytryptamine (5-HT), when five washes were used to avoid tachyphylaxis.

When the preparations were exposed to modifying agents the following pretreatment times (min) were used: atropine (15) phentolamine and propranolol (30), methysergide (15), pentolinium (15), tetrodotoxin (15) tertiary amphetamine derivatives (15), all other drugs (5). In preliminary experiments the actions of these agents did not increase greatly after these times. The effects of these agents were determined by carrying out concentration-effect curves for the agonist on a pair of tissues, adding the modifying agent to one of them and then repeating the agonist administration. For clarity the control results will only be mentioned where changes in the responses did occur.

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When the effects of morphine were examined a separate piece of ileum was used for each dose addition to avoid problems of tolerance. On each piece a single dose of histamine and then one of *NN*-dimethyl-2-aminoindane was given, electrical stimulation begun, morphine added, stimulation stopped and the former doses repeated.

Unless otherwise stated the results are the mean of 8 determinations.

(ii) Isolated uterus of the rat.

Each horn was suspended in a 25 ml organ bath in de Jalon's solution (as Krebs-Henseleit but with a quarter the concentration of calcium), bubbled with 95% O_2 and 5% CO_2 at 26 °C, one being used as control and the other as test preparation. The resting tension was 1 g and longitudinal tension changes were recorded as for the guinea-pig ileum. A 2 min time cycle for addition of agonists was again used. Pretreatment times for modifying agents were 15 min.

In the experiments determining pA values, the log concentration-effect curves to 5-HT were established in the presence of a minimum of four concentrations of antagonist. ED50 values were derived from these and the dose ratios obtained. The log (dose-ratio—1) was plotted against the negative log of the molar concentration of antagonist. Best fitting straight lines were calculated by the method of least squares.

Throughout the Results section the mean values are shown in the figures with their standard errors. Student's *t*-test was used to determine the significance of the differences.

(iii) Assay of anticholinesterase activity

The continuous titration method used was similar to that described by Alles & Hawes (1940), except that the source of cholinesterase was mouse brain homogenate, and the velocity of reaction was calculated during the 2nd and 3rd min. A Radiometer titration assembly was used and the temperature maintained at 37 °C. The homogenate was made freshly each day using ten brains. These were removed, weighed and added to a cooled solution of Mg Cl_2 (0·04 M) and NaCl (0·05 M), to produce a concentration of 100 mg brain tissue ml^{-1}, homogenized for 30 s, and then maintained at 0 °C. The protein concentration in each day's homogenate was determined by the Biuret method (Gornall et al 1949).

The velocity of reaction was expressed as μmol min^{-1} mg $protein^{-1}$, and the actions of inhibitors as the ratio of velocity in the absence and in the presence of a particular concentration of inhibitor, expressed as a percentage. Results are the means of 5 determinations.

Drugs

Drugs used were: acetylcholine chloride (BDH); amphetamine sulphate (BDH); atropine sulphate (BDH); bradykinin (Sandoz); carbachol chloride (Evans); cyproheptadine hydrochloride (M.S.D.), histamine acid phosphate (BDH); 5-hydroxytryptamine creatinine sulphate (BDH); mepyramine maleate (M & B); methysergide bimalate (Sandoz); morphine hydrochloride (Macfarlan Smith); nicotine hydrogen tartrate (BDH); pentolinium tartrate (M & B); physostigmine salicylate (BDH); propranolol hydrochloride (ICI); tetrodotoxin (Koch-Light); phentolamine mesylate (Ciba); 1-aminoindane hydrochloride, 2-aminoindane hydrochloride and 1,2,3,4-tetrohydroisoquinoline hydrochloride (Aldrick Chemical Co.). The other derivatives of amphetamine used were kindly synthesized by Dr P. A. Crooks of the Department of Pharmacy, University of Manchester. The racemic forms of the 2-amino-1,2,3,4-tetrahydronaphthalene (2-ATN) derivatives were used throughout.

RESULTS

2-Aminoindane, 2-ATN, amphetamine and their *N*-methyl and *NN*-dimethyl derivatives dose-dependently decreased the responses of the electrically stimulated guinea-pig ileum, with the exception of *NN*-dimethyl-2-aminoindane which potentiated the contractions in a concentration-dependent and qualitatively striking manner (Fig 2). The depression of the responses by the other compounds is compared in Table 1. The ATN compounds were slightly more potent than the amphetamines or the indanes but the order of potency in all series was tertiary > secondary > primary. This is complete contrast to their central stimulant and cardiovascular actions (Little 1975).

The contribution of sympathomimetic activity to the inhibition of the responses to electrical stimulation was investigated by testing the effects of a combination of phentolamine and propranolol 1·3 and 1·9 μM respectively. The concentrations used shifted the log concentration to noradrenaline to the right as expected but did not alter the responses to methylamphetamine. The log concentration effect curve to *NN*-dimethyl-2-ATN however was moved significantly to the right (Fig 3).

The resulting effects of the tertiary amine derivatives on the responses of the ileum to other com-

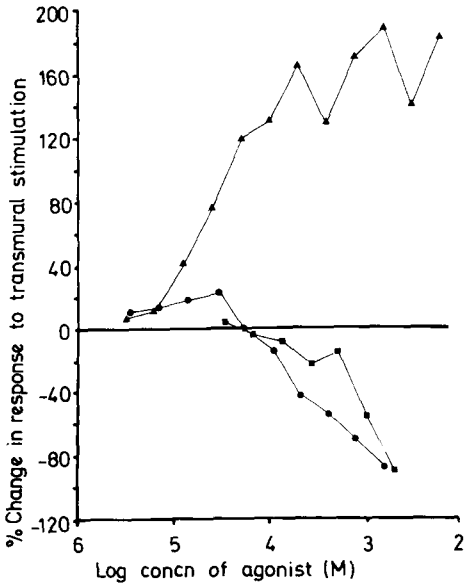

FIG. 2. The effects of the 2-aminoindane (■), *N*-methyl-2-aminoindane (●) and *NN*-dimethyl-2-aminoindane (▲) on electrically induced contractions of the isolated ileum of the guinea-pig.

pounds are summarized in Table 2. A concentration of 25 μM of either *NN*-dimethyl-2-aminoindane or *NN*-dimethyl-2-ATN did not affect the responses of the ileum to acetylcholine, carbachol or bradykinin. This concentration was chosen because it caused small changes in the responses to electrical stimulation and little contraction of the unstimulated ileum.

The log concentration curve of responses of the ileum to nicotine was shifted in a parallel manner to the left by *NN*-dimethyl-2-aminoindane but the second concentration effect curve to nicotine in the control preparations showed a lower maximum than the first. The latter pattern was also seen when *NN*-dimethyl-2-ATN was present.

Table 1. Concentration causing 50% inhibition of the responses of the ileum to electrical stimulation.

	Concn μM
Amphetamine	850
Methylamphetamine	270
Dimethylamphetamine	130
2-ATN	180
Methyl-2-ATN	50
Dimethyl-2-ATN	20
2-Aminoindane	890
Methyl-2-aminoindane	430

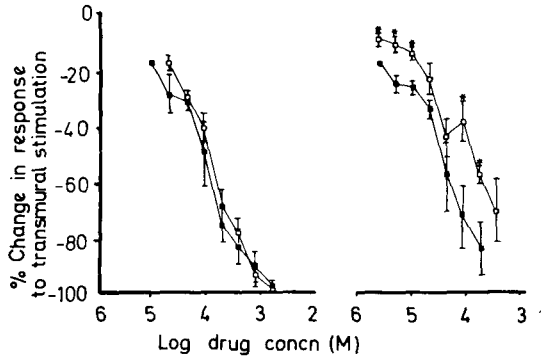

FIG. 3. The effects of phentolamine (1.3 μM) in combination with propranolol (1.9 μM) (open symbols) on the inhibitory effect of methylamphetamine (l.h.) and dimethyl-2-ATN (r.h.) on the electrically stimulated guinea-pig ileum. Solid symbols: methylamphetamine or dimethyl-2-ATN alone. Mean of 8 ± s.e. * indicates $P < 0.05$.

All the tertiary amines antagonized the actions of histamine on the ileum. This antagonism appeared to be competitive as the curves were parallel and it was concentration-dependent and reversible. *NN*-Dimethyl-2-ATN was rather more effective than the other two compounds.

NN-dimethylamphetamine and *NN*-dimethyl-2-ATN, but not *NN*-dimethyl-2-aminoindane decreased the responses of the ileum to 5-HT but this did not appear to be a competitive action as it was not overcome by increasing the 5-HT concentration.

Table 2. The effects of the three tertiary amines dimethylamphetamine (I), dimethyl-2-ATN (II) and dimethyl-2-aminoindane (III) on the responses of the ileum to various agonists.

Tertiary amine concn (μM)	Agonist	Comments
II 23·7	Nicotine	Decreased maximum
III 25·4	Nicotine	Parallel shift of d.-r. curve to left
I 25·0	Histamine	Parallel shift of d.-r. curve to right
II 23·7	Histamine	Parallel shift of d.-r. curve to right
III 25·4	Histamine	Parallel shift of d.-r. curve to right
I 25·0–400·0	5-HT	Decreased maximum
II 25·4–378·0	5-HT	Decreased maximum
III 25·4–407·0	5-HT	No change

None of the three tertiary compounds (at 25 μM) caused any change in the responses of the ileum to acetylcholine, carbachol or bradykinin.

All the compounds when applied alone contracted the ileum. The indanes gave reproducible responses (Fig. 4b) with sigmoid or bell-shaped dose-response curves but the amphetamines and the tetralins gave only small irregular contractions (Fig. 4a). Neither 1-aminoindane nor 1,2,3,4-tetrahydroisoquinoline contracted the ileum but the quaternary derivative of 2-ATN produced small responses.

Table 3 illustrates the effects of various antagonists on the responses of the ileum to *NN*-dimethyl-2-aminoindane. These were clearly inhibited by atropine, potentiated by physostigmine and unaffected by either mepyramine or methysergide. The tryptaminergic antagonist cyproheptadine significantly decreased the responses to *NN*-dimethyl-2-aminoindane but as it also decreased the responses to both 5-HT and to acetylcholine this was clearly a non-specific action.

The combination of phentolamine and propranolol moved the log concentration effect curve to *NN*-dimethyl-2-aminoindane to the right in both the rising and the falling phases. This was significant only at certain concentrations.

Pentolinium (Fig. 4c), tetrodotoxin and morphine each decreased the responses to dimethyl-2-aminoindane, the size of the maximum contractions produced being reduced in each case.

The possible contribution of anticholinesterase activity to the above results were investigated. The tertiary derivatives of 2-aminoindane and 2-ATN were found to possess weak activity, comparable with that of morphine, while *NN*-dimethylamphetamine had little action (Table 4).

Further information on the action of *NN*-dimethyl-2-aminoindane was sought by the use of the isolated rat uterus. However it caused only small contractions of this tissue at high concentrations (1–10 mM) and had no effect at lower doses.

A comparison of the effects of the three tertiary amines on the responses to 5-HT was made on this preparation as they were found to antagonize these while they did not affect responses to acetylcholine. Unlike their actions on the ileum, this antagonism by

Table 3. The effects of antagonists on the responses of the ileum to *NN*-dimethyl-2-aminoindane.

Antagonist	Concn of antagonist	Comments
Atropine	1·4 and 5·8 nM	Decreased maximum
Physostigmine	536 nM	Potentiatim
Mepyramine	6·2 nM	No change
Methysergide	2·1 μM	No change
Cyproheptadine	3·1 nM	Non-specific effect
Phentolamine	1·3 μM	D.-r. curve shifted to
and propranolol	1·9 μM	left
Pentolinium	46·4 μM	Decreased maximum
Tetrodotoxin	3·1 μM	Decreased maximum
Morphine	532 nM	Decreased maximum

Table 4. The effects of the tertiary amines on the cholinesterase activity of mouse brain homogenates, compared with those of physostigmine and of morphine.

Drug	Concn causing a 50% inhibition of activity
Dimethyl-2-aminoindane	447 μM
Dimethyl-2-ATN	562 μM
Dimethylamphetamine	5·6 mM
Morphine	199 μM
Physostigmine	14 nM

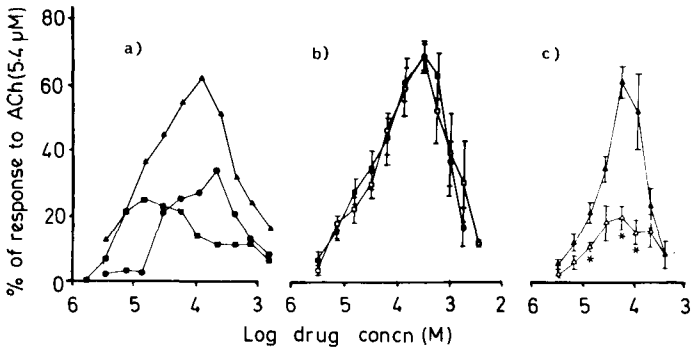

FIG. 4. The contractile effects of amphetamine derivatives on the guinea-pig ileum expressed as a percentage of a standard response to acetylcholine (5·4 μM). Panel a, the effects of dimethylamphetamine (■), dimethyl-2-aminoindane (●), and dimethyl-2-ATN (▲). Panel b, the reproducibility of responses to dimethyl-2-aminoindane, first addition (■), and second addition (□). Panel c, the antagonist action of pentolinium (□ 46·4 μM) on contractions caused by dimethyl-2-aminoindane. Means of 8 (± s.e., where shown) * indicates $P < 0.05$.

NN-dimethyl-2-aminoindane and by *NN*-dimethyl-2-ATN was clearly competitive, causing parallel shifts to the right of the 5-HT concentration effect curves. The pA values for this antagonism are: *NN*-dimethylaminoindane $pA_2 = 6{\cdot}48$, $pA_{10} = 5{\cdot}56$, $pA_2 - pA_{10} = 0{\cdot}92$; *NN*-dimethyl-2-ATN $pA_2 = 6{\cdot}56$, $pA_{10} = 5{\cdot}73$, $pA_2 - pA_{10} = 0{\cdot}83$; *NN*-dimethyl amphetamine $pA_2 = 5{\cdot}23$, $pA_{10} = 4{\cdot}86$, $pA_2 - pA_{10} = 0{\cdot}57$.

DISCUSSION

The results from these experiments may be conveniently divided into three sections; the inhibition of the electrically stimulated ileum, the contractile responses to *NN*-dimethyl-2-aminoindane and the antagonism of 5-HT and histamine by the tertiary amines.

The order of potency of the inhibition of the responses of the ileum to electrical stimulation was tertiary amines > secondary amines > primary amines (excluding *NN*-dimethyl-2-aminoindane). This is different from the order of potency described previously for their sympathomimetic actions. The tertiary derivative of amphetamine is less potent than the primary or secondary amines in noradrenergic receptor stimulation. The 2-ATNs have been shown to possess indirect sympathomimetic activity, causing release of noradrenaline and the order of potency in this action was primary > secondary > tertiary (Cloetta & Waser 1923; Pennefather 1968).

The decrease in the actions of *NN*-dimethyl-2-ATN in inhibiting the responses of the ileum, when phentolamine and propranolol were used suggests that at least part of this action was due to increased noradrenergic activity. That this is not the whole explanation was suggested by the fact that the shift in the log concentration effect curve to the tertiary ATN derivative was less than that seen in the corresponding noradrenaline curve and that the responses to methylamphetamine were unaffected. In support of this are the results of Knoll & Vizi (1971) who showed that, while treatment with reserpine plus α-methyl-*p*-tyrosine abolished the action of amphetamine in decreasing acetylcholine output from the ileum, it only partially decreased the action of methylamphetamine.

The indanes were clearly more effective in causing contractions of the ileum than the other two series of compounds, or 1-aminoindane. A contractile action of amphetamine on the ileum has been described previously (e.g. Vane 1960) but the mechanism has not been clearly explained. (The aboral portion of the ileum, which is known to contract to noradrenaline was not used in the present experiments.) Innes (1963) and Innes & Kohli (1969) suggested that it was due to an action on tryptamine receptors, but Kohli (1968) showed that this had not been conclusively demonstrated.

The results using *NN*-dimethyl-2-aminoindane show it to cause contractions by releasing acetylcholine, as illustrated by the effects of atropine, physostigmine and morphine. The effects of pentolinium and tetrodotoxin suggest that this was due to a nicotine-like action on the ganglion cells of the myenteric plexus.

The effect of phentolamine and propranolol on the log concentration effect curve to *NN*-dimethyl-2-aminoindane can be partially explained by proposing an inhibitory, sympathomimetic, component in its action, but this does not explain why the falling phase was lowered. Many ganglion-stimulating drugs have antagonist actions at high concentrations and this may be the reason for the shape of the curves obtained with the tertiary indane.

The results of the measurement of acetylcholinesterase activity show that the differences between the actions of *NN*-dimethyl-2-aminoindane and the other tertiary amines cannot be explained by their anticholinesterase activity.

That *NN*-dimethyl-2-aminoindane possesses little direct stimulant action on either acetylcholine (muscarinic) or 5-HT receptors was illustrated by its lack of stimulant action on the rat uterus.

The three tertiary amines were competitive antagonists of the action of histamine on ileum. The possession of a tertiary amine group is characteristic of compounds with this action and the structures of the three correspond to the general structure described by Witiak (1970) for this property.

The antagonism of the actions of 5-HT on the ileum by the tertiary amines differed from that on the uterus. The effects on the ileum were not competitive, although the lack of antagonism of all the other agonists tested, except histamine, suggested that they were specific. If the direct component of the action of 5-HT on the ileum were inhibited but not the indirect then a parallel shift in the 5-HT dose-response curve would not be expected.

The effect of *NN*-dimethylamphetamine on the uterus was also non-competitive. Amphetamine and related compounds have been observed to antagonize the action of 5-HT on the uterus (Gaddum & Hameed 1954). The pA_2 values obtained for the tertiary indane and ATN derivatives on this tissue were close to that obtained for dihydroergotamine by

Gaddum et al (1955), suggesting a similar mechanism of action.

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