Method A. Alkylations using benzylic or unsaturated halides (e.g., **39**) were carried out in the presence of anhydrous K_2CO_3 and a suitable solvent: A_1, Me_2CO; A_2, DMF; or A_3, MeCOEt. The preparation of **14** below is a typical procedure.

***N*-(4-Methoxybenzyl)imidazole (14).** Imidazole (3.4 g, 0.05 mol), 4-methoxybenzyl bromide (10.05 g, 0.05 mol), and anhydrous K_2CO_3 (14 g, 0.1 mol) in dry Me_2CO (50 ml) were stirred and heated under reflux for 4 hr. The mixture was allowed to cool, the solids were filtered off, and the filtrate was evaporated. The product **14** was isolated by chromatography on silica with $CHCl_3$–5% MeOH: 4.88 g (52%); mp 59° [EtOAc–petroleum ether (bp 40–60°)].

Method B. *N*-(3-Phenylpropyl)imidazole (38). To a stirred solution of imidazole (6.8 g, 0.1 mol) and finely powdered NaOH)5.0 g, 0.125 mol) in dry *n*-BuOH (100 ml) at 125°, 1-bromo-3-phenylpropane (19.9 g, 0.1 mol) was added dropwise over 20 min. The reaction mixture was maintained at 125° for a further 20 min, allowed to cool, and diluted with water (200 ml). The mixture was extracted with Et_2O and dried ($MgSO_4$) and after removal of the solvent, the residue was distilled yielding **38** (6.4 g, 36%), bp 142–144° (0.5 mm).

***N*-(4-Aminobenzyl)imidazole (28).** A solution of *N*-(4-nitrobenzyl)imidazole (**10**, 2.03 g, 0.01 mol) in MeOH (25 ml) was added dropwise to a stirred suspension of 10% Pd/C (0.5 g) in water (10 ml) containing $NaBH_4$ (0.78 g, 0.02 mol) under nitrogen over a period of 5 min. The mixture was stirred for a further 10 min, allowed to cool, filtered, acidified (2 *N* HCl) to destroy excess $NaBH_4$, and then basified (1 *N* NaOH). The mixture was extracted with $CHCl_3$ and the product isolated by evaporation of the dried ($MgSO_4$) $CHCl_3$ solution, followed by chromatography on silica with $CHCl_3$–5% MeOH to yield **28** (1.34 g, 78%), mp 127° (EtOAc).

Biological Methods. Male mice of the CFLP strain (Carworth Europe) weighing 20–22 g were allocated to experimental groups of ten animals, so that the mean body weight of each group was the same. The compounds to be tested were added to powdered commercial feed at a level of 0.1% (w/w). This is equivalent to a daily dose of ca. 140 mg/kg; for a typical compound such as **14** this is ca. 750 μmol/kg. Animals were allowed food and water ad libitum for 10 days, after which they were killed and bled and their livers removed and frozen pending analysis.

Plasma cholesterol and triglycerides were measured using a Technicon Autoanalyzer (method N24A for cholesterol and method N78 for triglycerides). Treated groups were compared with controls using Student's t test. Liver lipid and cholesterol were determined on $CHCl_3$–MeOH extracts of pooled liver samples.[4]

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Conformational Effects on the Activity of Drugs. 5.[1] Pharmacological Properties of 2-(*p*-Nitrophenyl)-4-isopropylmorpholine, a Cyclic Analog of INPEA

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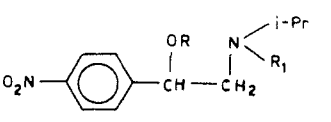
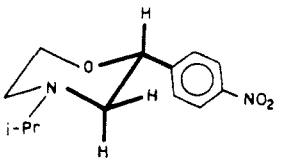
2-(*p*-Nitrophenyl)-4-isopropylmorpholine (V), an analog of 1-(*p*-nitrophenyl)-2-isopropylaminoethanol (INPEA, I) in which the OCHCHN chain of I is locked in a morpholine ring, loses the β-receptor blocking activity of I on various isolated preparations. The same ineffectiveness is observed in the *O*-methyl (II), *N*-methyl (III), and *N,O*-dimethyl analog (IV) of I. However, some other properties which are present in I, such as inhibitory effect on acetylcholine or on 5-HT, intrinsic α-sympathomimetic activity, and potentiation of catecholamines, are maintained; this demonstrates a complete dissociation of these effects from β-receptor blockade. The interactions with the α-adrenoceptors and with the uptake mechanism are discussed on the basis of the structure–activity relationship between I and its analogs II–V.

In addition to blockade of β-receptors[2] INPEA, 1-(*p*-nitrophenyl)-2-isopropylaminoethanol (I), has been shown to exert various effects on the adrenergic effector system. INPEA exhibited sympathomimetic activity on cardiac muscle[3] and on smooth muscle strips of taenia coli.[4] Furthermore, Gulati and others[5] observed that INPEA competitively antagonized the excitatory effects of catecholamines on α-receptors. On the other hand, INPEA appeared to potentiate the effects of exogenous catecholamines.[6] Janiec and Chrusciel[7] observed that INPEA inhibited the uptake of [^{3}H]noradrenaline by the adrenergic nerves of rat heart muscle. Finally, INPEA has been shown to antagonize in various degrees the effects of histamine and 5-HT on rabbit aortic strips.[5]

In a previous paper[1] we observed that 2-(*p*-nitrophenyl)-4-isopropylmorpholine (V), an analog of INPEA in which the chain OCHCHN is incorporated in a morpholine ring, lost the β-receptor blocking activity on isolated preparations of cardiac muscle. However, some other properties observed in the parent compound (INPEA) were still present. The aim of this work was to investigate further the pharmacological properties of the INPEA analog (V) and of *O*-methyl (II), *N*-methyl (III), and *N,O*-dimethyl (IV) derivatives of I, in order to compare their pharmacological effects with those of I.



I, R, R1 = H
II, R = Me; R1 = H
III, R = H; R1 = Me
IV, R = R1 = Me



V

Pharmacology. 1. Methods. (a) Isolated Rat Vas Deferens. Vasa deferentia were obtained by using the method

described by Wakade and Krusz.[8] Field stimulation of vasa deferentia was achieved by two silver electrodes placed alongside the tissue and separated by a distance of 4 mm. The frequency of stimulation varied from 2 to 10 cps and the voltage was adjusted to be supramaximal. A pulse width of 0.5–1 msec was employed throughout and the duration was 30 sec. After thorough washing, the tissues were allowed to stabilize. For experiments on reserpine-pretreated tissues, animals were given reserpine, 5 mg/kg, 24 hr before beginning the experiments, and the drug effects were recorded in the same way as for normal tissues.

(b) Isolated Guinea-Pig Atria. The degree of blockade against isoprenaline-induced positive inotropic activity was examined for INPEA cyclic analog V and for compounds II–IV, with respect to the parent compound (INPEA). Guinea-pig atria were obtained in a way similar to that previously described for rabbit isolated atria.[1]

(c) Isolated Guinea-Pig Colon. The terminal colon supplied with both sympathetic (periarterial) and parasympathetic (pelvic) nerves was obtained from adult female guinea pigs as previously described.[9]

The following drugs were used as salts: adrenaline and noradrenaline as bitartrates; INPEA (I), INPEA analogs II–V, isoprenaline, phenylephrine, methoxamine, tyramine, cocaine, pargyline, histamine, phentolamine, and reserpine as hydrochlorides; 5-hydroxytriptamine–creatinine (5-HT) as sulfate; dihydroergotamine mesylate; acetylcholine chloride.

2. Results. (a) Isolated Rat Vas Deferens. On the isolated rat vasa deferentia the INPEA analog V, when added to the bath at a concentration ranging from 4×10^{-5} to 4×10^{-4} M, exhibited varying degrees of intrinsic effects. These effects were represented at times by slow rhythmic activity, whereas other times they were manifested by a contraction; Patil and others[6] obtained similar results with INPEA itself. Also the reserpine-pretreated tissues showed a similar degree of intrinsic sympathomimetic activity as controls (six experiments). In the presence of α-blocking drugs, i.e., phentolamine and dihydroergotamine 1×10^{-5}–3×10^{-5} M, this activity was not observed (three experiments). It was not possible to obtain the dose–response curve due to the fact that, when employed at concentrations higher than 5×10^{-4} M, the INPEA analog V exhibited α-blocking properties. The same α-blocking effect has also been demonstrated in the case of INPEA itself.[5]

The potentiating effect of INPEA analog V after 10 min of exposure to concentrations of 4×10^{-5} M on the responses to noradrenaline and to transmural stimulation is shown in Table I. In the same conditions the contractions induced by methoxamine, 2×10^{-5} M, were not significantly affected. Moreover, the responses to tyramine, 4×10^{-5} M, were inhibited by INPEA analog V (six experiments). To investigate the nature of the potentiating effect of INPEA analog V and its relationship with uptake and enzymatic destruction, cocaine, 2×10^{-6}–4×10^{-6} M (three experiments), was employed, while MAO and COMT were inhibited with pargyline, 2.5×10^{-5}–5×10^{-5} M, and with tropolone, 4×10^{-4}–8×10^{-4} M, respectively (three experiments). The potentiating effect of INPEA analog V was additive with that of drugs inhibiting MAO and COMT but not with that of cocaine even when MAO and COMT inhibitors and cocaine were employed at doses which produced maximal response.

Similar results were found in a few experiments in which the other INPEA derivatives (II–IV) were used.

(b) Isolated Guinea-Pig Atria. Although prolonged exposure to high concentrations of INPEA analog V reduced the spontaneous frequency of the beat in isolated atria, the drug did not cause an initial sympathomimetic action (five experiments) at variance with that observed for INPEA on the same preparation.[3] As previously observed for the isolated rabbit atria,[1] the INPEA analog V added to the bath at concentrations ranging from 4×10^{-6} to 4×10^{-4} M was unable to block the positive inotropic and chronotropic responses of isolated guinea-pig atria to isoprenaline, 2×10^{-8}–2×10^{-7} M (six experiments). On the contrary, INPEA at a concentration of 2×10^{-5} M abolished the responses of isoprenaline (four experiments). The lack of β-blocking effect was also observed when the other INPEA derivatives were employed (three experiments).

(c) Isolated Guinea-Pig Colon. The INPEA analog V at concentrations of 2×10^{-5}–4×10^{-5} M did not significantly change the spontaneous activity and the regular movements of isolated colon. With these concentrations the response to pelvic and sympathetic stimulation and to exogenous acetylcholine, histamine, and 5-HT was reduced by a mean of 70% (four experiments). At doses of 2×10^{-4}–4×10^{-4} M the responses were further significantly reduced to 5–10% of control responses; the relaxing activity of phenylephrine, 4×10^{-7}–2×10^{-6} M, was abolished, while that of isoprenaline, 2×10^{-7}–4×10^{-7} M, was hardly affected. INPEA at 4×10^{-5} M significantly reduced and at 4×10^{-4} M abolished both the effects of isoprenaline, 2×10^{-7} M, and phenylephrine, 4×10^{-7} M (three experiments). Similar results were observed when compounds II–IV were used (three experiments).

Discussion

When the INPEA molecule is locked in a morpholine cycle (V), it is ineffective in blocking β-adrenergic receptors on different pharmacological preparations. The same ineffectiveness is observed in the *O*-methyl (II), *N*-methyl (III), and *N,O*-dimethyl analog (IV) of INPEA. On the other hand, the failure of INPEA analogs to block β-receptors is accompanied by the maintenance of some other properties of INPEA, demonstrating a complete dissociation of these effects from β-receptor blockade also in this INPEA chemical series.

INPEA analog V, as well as the other derivatives (II–IV), antagonized in various degrees the effects of acetylcholine, histamine, and 5-HT on the isolated guinea-pig intestine and showed α-receptor blocking activity on the same preparation. Moreover, varying degrees of intrinsic sympathomimetic effects on isolated rat vas deferens are present in INPEA analogs similar to those observed for INPEA itself.[6] These effects are present also in reserpinized tissues demonstrating that they are direct and not due to liberation of noradrenaline from sympathetic nerve endings. Unfortunately, it was not possible to draw the dose–response curve for INPEA analog V and the other derivatives (II–IV) because of their α-blocking properties which appear at high concentrations of the drugs, as observed for INPEA.[5]

The fact that in the presence of INPEA derivatives the responses of the isolated vas deferens to noradrenaline were much higher than those to methoxamine suggests that the potentiating action of these drugs could be sustained by a block on the uptake of amines. In fact, INPEA analogs inhibited the contractions of tyramine, confirming that their effect could involve blockade of transport to sites of storage and binding (cocaine-sensitive uptake). This hypothesis is also consistent with the observation that the potentiation induced by INPEA analogs was approximately equivalent to that due to cocaine (300%).[8]

Moreover in vas deferens preparations in which MAO and COMT were inhibited, the INPEA analogs produced a small but significant potentiation of noradrenaline, demonstrating that this effect is partially independent of the inactivation of amines caused by enzyme inhibition.

Table I. Effect of INPEA Analog V and of INPEA (I) on Responses[a] of Isolated Rat Vas Deferens to Transmural Stimulation (TS) and to Noradrenaline (NA)

	Control	In presence of INPEA analog V, 4×10^{-5} *M*	% increase
TS, 2 cps	15.3 ± 3.61 (6)	43.2 ± 1.69 (6)	282
TS, 5 cps	42.5 ± 1.68 (6)	70.2 ± 4.50 (6)	165
TS, 10 cps	63.4 ± 3.11 (5)	85.6 ± 6.35 (5)	135
NA, 2×10^{-5} *M*	8.2 ± 2.41 (6)	26.3 ± 4.52 (6)	320
NA, 4×10^{-5} *M*	16.4 ± 2.80 (6)	36.7 ± 5.61 (6)	223
NA, 2×10^{-4} *M*	24.1 ± 3.62 (6)	48.4 ± 6.29 (6)	200
		In presence of INPEA, 4×10^{-5} *M*	
TS, 2 cps	12.2 ± 2.28 (3)	31.8 ± 1.82 (3)	262
TS, 5 cps	32.5 ± 3.05 (3)	53.5 ± 4.51 (3)	164
TS, 10 cps	56.3 ± 3.22 (3)	70.0 ± 4.83 (3)	124
NA, 2×10^{-5} *M*	11.3 ± 2.15 (4)	35.2 ± 2.91 (4)	314
NA, 4×10^{-5} *M*	21.2 ± 3.89 (4)	44.5 ± 4.65 (4)	209
NA, 2×10^{-4} *M*	29.2 ± 3.00 (4)	57.8 ± 5.88 (4)	197

[a] mm ± standard error; number of experiments are in parentheses. The strong potentiating effect of INPEA analog V on the responses to transmural stimulation and to noradrenaline is not significantly different from that of INPEA. The maximal effect occurs at low frequency of stimulation and at low concentration of noradrenaline, when the uptake processes are not saturated.

The fact that in INPEA analogs the disappearance of β-blocking activity is accompanied by a strong potentiating effect of exogenous and endogenous catecholamines seems to provide another example for differences between affinity to receptors and to the uptake mechanism.

In regard to the structure–activity relationship, on passing from INPEA (I), in which the presence of the *N*-isopropyl group causes a low α-stimulating activity,[10,11] to the corresponding morpholine (V) and to compounds III and IV in which the steric hindrance around nitrogen is enhanced, the weak intrinsic α-sympathomimetic activity observed for INPEA is still present. It may be thought that the additional moiety, which should hinder contact ion pairing, may have a favorable effect on proton mobility.[11,12] Moreover, the fact that II, IV, and V appear to possess approximately a similar degree of a α-sympathomimetic activity as that of INPEA itself, in spite of the absence of hydroxylic hydrogen, could suggest that the hydroxylic proton does not play an essential part in the interaction involving α-activation, as previously observed.[12,13]

The fact that V shows a degree of α-sympathomimetic activity as that of the parent compound (I) and of II, III, and IV seems to indicate that these compounds, and in particular INPEA, could function at the receptor site in a conformation around the C–C bond of the ethyl side chain which can be allowed by the morpholine ring. The values[14] of the vicinal coupling constants of the proton α to the aryl group (2.4 and 10.1 Hz for V and 2.2 and 10.8 Hz for V·HCl) indicate[15] that the morpholine analog of INPEA preferentially exists in solution in the conformation shown in V, in accordance with the large *A* value (3.1 kcal/mol[16]) of the aryl moiety. Although it is not possible to establish whether or not V interacts with the receptor in its preferred conformation, it may be stressed that the mutual orientation in the preferred conformation of V of the centers which should play a role in the interaction of α-sympathomimetic drugs with their receptor[10] is consistent with that calculated[17,18] or found, both on the solid state[19] and in solution,[20] for some α-adrenergic stimulating agents and for INPEA itself.[14,21]

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