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Report

Synthesis and Pharmacological Activity of *N*-Alkyl-1,2-Diphenylethanolamines

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A series of *N*-alkyl-1,2-diphenylethanolamines were synthesized and their pharmacological activities evaluated on various mammalian organs and sytems. All compounds produced a generalized inhibitory effect on smooth and cardiac muscles and an increase in coronary flow as well as a brief reduction in rabbit blood pressure. The latter effect was not prevented by pretreatment of the animals with atropine, propranolol, or metoprolol. The compounds were devoid of local anesthetic activity and their inhibitions of the contraction of the isolated rabbit intestine and perfused heart were reversed by exogenous calcium ions. It is proposed that the compounds produce their effects through calcium-channel blockade. The inhibitory effects of some of these compounds were comparable to those of a known calcium-channel blocker.

KEY WORDS: *N*-alkyl-1,2-diphenylethanolamines—synthesis, pharmacology; calcium-channel blockers—*N*-alkyldiphenylethanolamines, synthesis; phenethylamines: α-phenyl, β-hydroxy—synthesis, pharmacology; ethanolamines: 1,2-diphenyl—synthesis, pharmacology.

INTRODUCTION

1,2-Diphenylethanolamine was reported to produce moderate analgesic activity in mice (1), while only very little such activity was observed in rats (2,3). 1,2-Diphenylethanolamine also showed antispasmodic activity which was about one-tenth that of papaverine (4), and a transient fall in blood pressure was caused by the compound in rats (5) and dogs (6).

In view of such diverse pharmacological properties, it was surprising that the synthesis and pharmacological screening of analogues of 1,2-diphenylethanolamine have not been reported, especially in the light of the close structural relationship between these compounds and β-phenylethanolamines, which constitute the main class of adrenergic agonists. As alkyl substitution at the basic nitrogen usually results in modification of the pharmacological activity, a series of *N*-alkyl-1,2-diphenylethanolamines (Table I) was synthesized and their pharmacological properties were studied.

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MATERIALS AND METHODS

Materials

All chemicals were obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. Melting points were determined using a Mettler FP5 melting-point apparatus and are uncorrected. Analyses of all new compounds for C, H, and N were done by Galbraith Laboratories Inc., Knoxville, Tennessee (Table I). Infrared spectra were recorded on a Perkin–Elmer 580 B infrared spectrometer as KBr disks. NMR spectra were obtained with a Jeol FX100, 100-MHz NMR spectrometer. Both the infrared and the NMR spectra were consistent with the assigned structures.

Methods

Chemistry

The *N*-alkyldiphenylethanolamines were prepared from benzoin (0.05 mol) which was dissolved by refluxing in 200 ml methanol; to this solution were added 0.5 ml glacial acetic acid and 0.5 g sodium acetate dissolved in 2 ml water. The solution was cooled to 30°C and the appropriate amine (0.2 mol), dissolved in methanol, was added. The reaction mixture was stirred for 30 min, sodium borohydride (0.15 mol) was then added, portionwise, and the stirring was continued for a further 6 hr. Methanol was evaporated under reduced pressure, and the excess sodium borohydride decomposed with water. The crude product was filtered and

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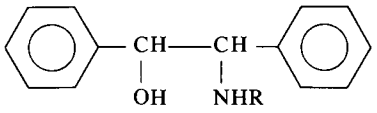
Table I. Elemental Analysis and Physical Constants of *N*-Alkyl-1,2-diphenylethanolamines



Compound	R	mp (°C)	Yield (%)	Elemental analysis
1	CH_3	137.1	27.5	$C_{15}H_{17}NO$. Theoretical: C 79.3%, H 7.5%, N 6.16% Found: C 79.41%, H 7.7%, N 6.14%
2	CH_2CH_3	137.1	25.5	$C_{16}H_{19}NO$. Theoretical: C 79.67%, H 7.88%, N 5.8% Found: C 79.68%, H 7.87%, N 5.78%
3	$CH(CH_3)_2$	133.2	27.5	$C_{17}H_{21}NO$. Theoretical: C 80%, H 8.2%, N 5.5% Found: C 80%, H 8.0%, N 5.5%
4	$CH_2CH_2CH_2CH_3$	125.0	5.5	$C_{18}H_{23}NO$. Theoretical: C 80.3%, H 8.55%, N 5.2% Found: C 80%, H 8.6%, N 5.0%
5	$CH_2CH(CH_3)_2$	123.7	7.3	$C_{18}H_{23}NO$. Theoretical: C 80.3%, H 8.55%, N 5.2% Found: C 80.4%, H 8.67%, N 5.16%
6	CH_2Ph	152.3	29.9	$C_{21}H_{21}NO$. Theoretical: C 83.17%, H 6.93%, N 4.62% Found: C 83.34%, H 7.10%, N 4.63%

recrystallized from ether. Melting points and percentage yields are given in Table I.

The hydrochloride salts were prepared by passing dry hydrogen chloride gas into the solutions of the bases in ether.

Pharmacology

Isolated Tissues. (i) Spontaneously contracting rabbit jejunum: White New Zealand rabbits (2.5 kg) were killed, the abdomens were opened, and pieces of jejunum, 2 cm long, were removed and suspended under a tension of 0.5 g in Krebs' solution (pH 7.4) at 37°C (7). The bathing fluid was oxygenated with a gas mixture of 5% CO_2 in O_2. Each tissue was allowed to equilibrate in the bathing fluid for 30 min, with two changes of bathing fluid before drug addition. Isometric contractions of the tissue were recorded using an isometric Myograph transducer connected to a physiograph recorder (Narco BioSystem). Solutions of the compounds were made by dissolving the hydrochloride salts in water (2 mg/ml). Each dose of the compounds or diltiazem was allowed to contact the tissue for 3 min and then washed off. Each compound was tested on a separate piece of jejunum from the same rabbit. The interval between the doses was 15 min. The percentage inhibition of the spontaneous contractions induced by each dose of the test compounds at the end of the contact time was then calculated. For this purpose the mean amplitude of the last three contractions at the end of the contact time was calculated and compared with the mean of the last three control contractions just before the addition of the compound to the tissue. The ID_{50} value, the molar concentration of compound inhibiting the contractions by 50%, was calculated for individual experiments by linear regression analysis using the log concentration and percentage inhibition relative to the maximum inhibition induced by the compound. When examining the effects of receptor blockers on the compound-induced inhibitions, the antagonist was allowed to contact the tissue for 5 min prior to the addition of the test compound.

(ii) Electrically stimulated rat vas deferens: Male Sprague–Dawley rats (200 g) were killed and the lower abdomen was opened. The vas deferens was dissected out (3 cm long) and suspended in Krebs' solution (pH 7.4) at 37°C under 0.5 g tension (8). Each tissue was allowed to equilibrate in the bathing fluid for 60 min, with three changes of bathing fluid before the induction of electrical stimulation and addition of drugs. The tissue was electrically field-stimulated using two parallel platinum electrodes and a Grass S44 stimulator at a frequency of 0.1 Hz, a 1-msec pulse width, and 40 V. Contractions (twitches) of the tissue were recorded isometrically via an isometric transducer connected to a physiograph recorder (Narco-BioSystem). Drugs were dissolved in water and each dose was allowed to contact the tissue for 10 min and then washed off. The interval between the doses was 20 min. Each compound was tested on a new tissue. The percentage inhibition of the twitches induced by each dose at the end of the contact period as well as the ID_{50} value for each drug in each experiment was calculated as outlined above.

(iii) Isolated perfused rabbit heart: White New Zealand rabbits (2.5 kg) were injected iv via the ear vein with heparin 1000 IU kg^{-1}. Five minutes later, the animals were exsanguinated, and each heart was rapidly excised and placed in ice-cold Ringer–Locke's solution (7). The aorta was cannu-

lated retrogradely and suspended from the perfusion apparatus (Palmer, U.K.). The heart was perfused using Ringer–Locke's solution maintained at 37°C and gassed with 5% CO_2 in O_2. A constant perfusion flow rate of 10 ml min^{-1} was maintained. A resting tension of 3 g was applied to each heart and the developed isometric tension was displayed on the chart. The test compounds were injected into the perfusing fluid just before entry into the aorta, 45 min after commencement of the perfusion. Each drug was tested on a separate heart at a dose interval of 20 min. Coronary flow was quantified by measuring the volume of perfusate leaving the heart over 1 min. The effect of each compound at a fixed dose of 0.5 μmol on coronary flow was examined for 1 min starting from the time of onset of maximum inhibition of contraction force. Heart rate was monitored by increasing the chart speed to count the beats over a 10-sec period. The influence of a fixed dose of 0.5 μmol of each compound on the heart rate was examined when the effect on the force of contraction had stabilized. The ID_{50} values of the compounds on the force of contraction were calculated by a procedure similar to that outlined for the spontaneously contracting rabbit jejunum.

Intact Animals. (i) Rabbit blood pressure: White New Zealand rabbits (2.5 kg) were anaesthetized with urethane, 1 g kg^{-1} iv via the ear vein. The animals were then prepared for recording of arterial blood pressure (9). The left femoral vein was exposed and cannulated. Heparin, 1000 IU kg^{-1}, was injected. The right carotid artery was cannulated and connected to an ITT Cannon blood-pressure transducer attached to a physiograph recorder (MK-l-V-P, Narco-Bio-System). Drugs were administered iv at intervals of 20 min. When examining the influence of receptor blockers on the compound-induced fall in blood pressure, the blockers were administered 15 min prior to injection of the submaximal dose of each compound. Changes in arterial blood pressure (mm Hg) were quantified by the blood-pressure calibration system built in the physiograph recorder.

(ii) Rabbit corneal reflex: To examine the influence of the compounds on the rabbit corneal reflex, the procedure described in Ref. 9 was followed. Five-tenths milliliter of a 0.2% solution (w/v) of each compound in normal saline was instilled into one conjunctival sac of a rabbit over a period of 5 min and the other eye received 0.5 ml of normal saline (control). Corneal reflex was then tested at intervals of 5 min for 2 hr. Each compound was tested on three different animals. The reflex was recorded as either present or absent.

The Influence of Exogenous Calcium Chloride ($CaCl_2$). For studying the influence of exogenous $CaCl_2$ on the compound-induced inhibitions, the chemical was always added at the time when the inhibitory effect had stabilized. In certain cases $CaCl_2$ was added at intervals of 1 min.

Statistical Analyses. Statistical analyses of the pharmacological data for comparing the effects of the six compounds and those of diltiazem were calculated using Student's *t* test or analysis of variance as appropriate.

RESULTS

The synthesis of the title compounds has been accomplished by a one-step reaction involving the reductive amination of benzoin with the appropriate primary amine using sodium borohydride to effect reduction. The reaction was carried out in a slightly acidic medium which was found to be optimal for rapid reduction (10). The reductive amination reaction afforded only one diastereomer of each product as revealed by the ^1H and ^{13}C NMR spectra of compounds 1–6. Further work is being conducted to assign the relative configuration to the products.

Effects on Spontaneously Contracting Rabbit Jejunum

The addition of any of the six test compounds or diltiazem to the fluid bathing the tissue depressed the spontaneous activity in a dose-dependent manner. Complete suppression of activity was observed at a final bath concentration of 70–90 μ*M* for the compounds and 50 μ*M* for diltiazem. Table II shows that the ID_{50} values for compounds 3 and 4 and diltiazem were significantly lower than those of the other compounds ($P < 0.05$; N = 4). The inhibitory effects of the compounds were not prevented by pretreatment of the tissue with propranolol (1 μ*M*) and/or tolazoline (50 μ*M*). However, the depressant effect of the compounds and diltiazem were reversed by the exogenous addition of $CaCl_2$ (5–10 m*M*). Figures 1 and 2 show the inhibitions induced by compounds 3 and 5, respectively, and the reversal of the inhibitions by $CaCl_2$. Complete reversibility of the inhibition induced by the compounds was observed within 0.5–2 min.

Table II. The Effects of *N*-Alkyl-1,2-diphenylethanolamines and Diltiazem on Isolated Organ Preparations

	ID_{50}, μ*M* (mean ± SE; *N* = 4)		Perfused rabbit heart	
			Force of contraction	Coronary flow (0.5 μmol each)
Compound	Rabbit jejunum	Rat vas deferens	ID_{50}, μmol (mean ± SE; *N* = 4)	% increase (mean ± SE; *N* = 4)
Diltiazem	24.7 ± 3.7*	45 ± 2**	0.19 ± 0.01**	185 ± 18**
1	42.1 ± 1.6	80 ± 6.5	0.25 ± 0.01**	165 ± 10**
2	45.0 ± 1.8	75 ± 2	0.5 ± 0.05	90 ± 8
3	35.0 ± 2*	48 ± 1.5**	0.4 ± 0.1	100 ± 10
4	33.0 ± 1.1*	50 ± 2**	0.5 ± 0.03	95 ± 11
5	43.0 ± 2.8	70 ± 1.9	0.7 ± 0.07	50 ± 6
6	45.0 ± 3.4	75 ± 6.1	0.8 ± 0.1	30 ± 5

* Significantly different from the values of the other compounds ($P < 0.05$).
** Significantly different from the values of the other compounds ($P < 0.01$).

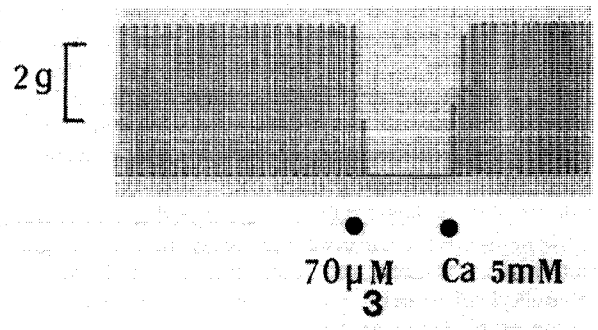


Fig. 1. The inhibitory effect of compound 3 (70 μ*M*) on the spontaneous contractions of the isolated rabbit intestine and the reversal of the inhibition with exogenous calcium chloride solution (5 m*M*).

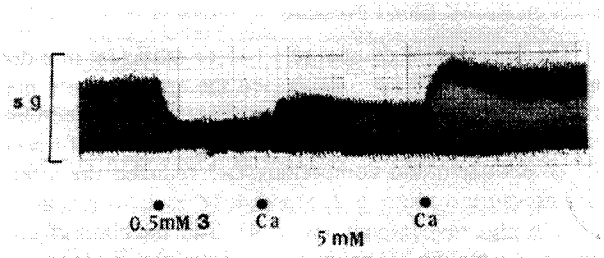


Fig. 3. The depressant effect of compound 3 (0.5 m*M*) on the force of contraction of the isolated perfused rabbit heart and the reversal of the inhibitory effect by exogenous calcium chloride solution (5 m*M*).

The addition of papaverine (40 μ*M*) or isoproterenol (0.5 μ*M*) to the spontaneously contracting jejunum completely suppressed the contractions but the induced inhibitions were not reversed by the addition of $CaCl_2$ (5–20 m*M*).

Effects on Electrically Stimulated Rat Vas Deferens

The addition of any of the six compounds or diltiazem to the stimulated tissue depressed the twitches of the muscle in a dose-dependent fashion. The maximum inhibition was observed 4–6 min after the addition of each compound.

The ID_{50} values for the various compounds and diltiazem are shown in Table II. The ID_{50} values for compounds 3 and 4 and diltiazem were significantly lower than those for the other compounds ($P < 0.01$; $N = 4$). The differences between diltiazem and compounds 3 and 4 were insignificant ($P > 0.05$; $N = 4$).

Effects on Perfused Rabbit Heart

The injection of each of the compounds or diltiazem in bolus doses of 0.1–1 μmol induced dose-dependent negative inotropic effects. All the inhibitory effects were reversed by bolus injections of 5–10 mmol of $CaCl_2$. In all cases the gradual addition of $CaCl_2$ at intervals of 1 min resulted in complete reversal within 3 min. Figures 3 and 4 show the negative inotropic effects of compounds 3 and 5, respectively, and their reversal by the gradual addition of $CaCl_2$.

The ID_{50} values for the compounds and diltiazem are shown in Table II. The ID_{50} values for diltiazem and compound 1 were significantly lower than those for the other compounds ($P < 0.01$; $N = 4$). The difference between diltiazem and compound 1 was insignificant ($P > 0.05$; $N = 4$). Furthermore, the injection of 0.5 μmol of compounds 1, 2, 3, and 5 and diltiazem decreased the heart rate by 17 ± 2, 8 ± 1, 10 ± 2, 8 ± 2, and 10 ± 1.4%, respectively ($N = 4$). Compounds 4 and 6 did not influence the heart rate. The negative chronotropic effect of compound 1 was significantly greater than that of diltiazem or any of the three active compounds ($P < 0.01$; $N = 4$). The differences between diltiazem and compounds 2, 3, and 5 were insignificant ($P > 0.05$; $N = 4$). The injection of 0.5 μmol of diltiazem or any of compounds 1–6 increased the coronary flow (Table II). The difference in the increases induced by compound 1 and diltiazem were insignificant ($P > 0.05$; $N = 4$) but were significantly greater than those induced by compounds 2–6 ($P < 0.01$; $N = 4$). The increases induced by compounds 2–4 were significantly greater than those induced by compounds 5 and 6 ($P < 0.05$; $N = 4$).

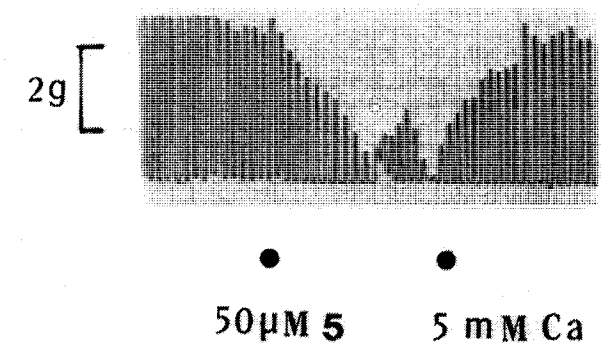


Fig. 2. The inhibitory effect of compound 5 (50 μ*M*) on the spontaneous contractions of the isolated rabbit intestine and the reversal of the inhibition by exogenous calcium chloride solution (5 m*M*).

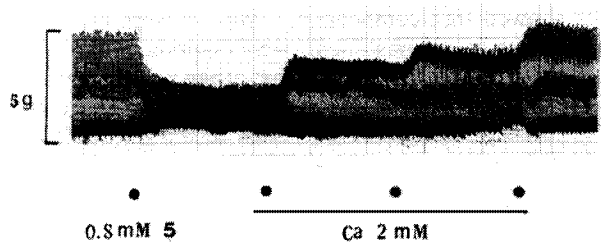


Fig. 4. The depressant effect of compound 5 (0.8m*M*) on the force of contraction of the isolated perfused rabbit heart and the reversal of the inhibitory effect by exogenous calcium chloride solution (2 m*M*).

Effects on Rabbit Blood Pressure

The injection of compounds 1–5 or diltiazem in a dose range of 2–12 μmol kg^{-1} decreased the arterial blood pressure in a dose-dependent manner. The induced decreases were brief, lasting less than 1 min. The injection of 6 μmol kg^{-1} of diltiazem and compounds 1–5 reduced the arterial blood pressure by 16.5 ± 2, 5 ± 0.4, 18 ± 2, 16 ± 3 and 18 ± 3 mm Hg, respectively (N = 4). The injection of compound 6 (6 μmol kg^{-1}) produced an initial fall in blood pressure of 7.1 ± 1 mm Hg followed by a rise of 10 ± 2 mm Hg (N = 4). The decreases in blood pressure were not prevented by pretreatment of the animals with propranolol (20 μmol kg^{-1}), atroprine (20 μmol kg^{-1}, or metroprolol (10 μmol kg^{-1}).

Effects on the Rabbit Corneal Reflex

The instillation of any of the compounds (0.2% solution in saline) into the conjunctival sacs of four rabbits did not depress the corneal reflex during the whole 2-hr test period.

DISCUSSION

A series of N-alkyl-1,2-diphenylethanolamines, differing in the size of the N-alkyl substituents, has been synthesized and their pharmacological activities were evaluated in different *in vivo* and *in vitro* systems. The results (Table II showed that all compounds produced qualitatively similar depressant effects on the various test preparations, with some potency differences. This general depressant action is reminiscent of that of local anesthetics or calcium-channel blockers. However, the failure of these compounds to depress the rabbit corneal reflex shows that they are devoid of local anesthetic activity. The finding that exogenous calcium chloride can reverse the negative inotropic effect and the depressant action on rabbit intestine (Figs. 1–4) suggests that the compounds may have produced their effects by interfering with calcium-ion influx in these tissues and, in this respect, seem to mimick calcium-channel blockers. This suggestion is further supported by the dissimilarity of the effects of the compounds to that of papaverine or isoproterenol on the rabbit jejunum and also by the failure of β-adrenoceptor and muscarinic receptor blockers to prevent the hypotensive effect.

Table II compares the effects of the test compounds and diltiazem, a known calcium-channel blocker (11). The results showed that compounds 3, with N-isopropyl, and 4, with an N-*n*-butyl substituent, were equipotent to diltiazem and were more active than the other compounds with smaller or larger N-substituents in depressing the activities of isolated smooth muscles. When tested on the isolated rabbit heart, compound 1 was the most potent in the series and equipotent to diltiazem in inducing negative inotropic effects. Thus, the negative inotropic activity of the compounds seems to decrease in the presence of large N-alkyl substituents. A similar structure–activity relationship was observed for the effects of the compounds on coronary flow. Many of the compounds lowered the arterial blood pressure and decreased the heart rate. Compounds 4 and 5, although structural isomers, differed markedly in their negative chronotropic activities, and compound 6, which possesses an N-aralkyl substituent, was unique in producing a biphasic effect on rabbit blood pressure.

Compound 1 was more potent in the perfused heart than compounds 3 and 4, which were more active on the isolated smooth muscle preparations. This result may be attributed to differences in the accessibility of the compounds to their sites of action in the different tissues. It may also be due to differences in the contributions of the extracellular and intracellular calcium ions to the contractions in the different tissues. However, other factors like an additional intrinsic activity of compound 1, such as an effect on intracellularly bound calcium or catecholamine release, cannot be ruled out.

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