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Desoxyisoprenaline: an adrenoceptor stimulating and blocking agent*

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Desoxyisoprenaline (*N*-isopropyldopamine, DOI), an optically inactive analogue of isoprenaline, which lacks the alcoholic –OH group, was shown to stimulate β-adrenoceptors in cat papillary muscle, rat aorta and rat trachea, in addition to causing a hypotensive effect in anaesthetized cats. DOI was also shown to stimulate α-adrenoceptors in rat aorta. Furthermore, DOI blocked both α- and β-adrenoceptors in rabbit aortic strips and β-adrenoceptors in rat trachea. These different actions indicate that DOI has both adrenoceptor stimulating and blocking properties.

During the past two decades, the relation between chemical structure and pharmacologic activity of adrenaline-like compounds has been extensively investigated (see Ariëns & Simonis, 1960; Ariëns, 1963; Ariëns, 1967). Most studies were designed to determine whether or not compounds act as α- or β-adrenoceptor stimulants, and to ascertain their potency relative to noradrenaline, adrenaline, or isoprenaline (isoproterenol). The discovery of the β-adrenoceptor blocking agent, dichloroisoprenaline (Powell & Slater, 1958), was an outcome of such investigations and opened up a new concept of intervention in adrenergic transmission.

We report on adrenoceptor interactions of an optically inactive analogue of isoprenaline, 1-(3,4-dihydroxyphenyl)-2-isopropylaminoethane HCl (desoxyisoprenaline, *N*-isopropyldopamine, DOI). This compound differs from isoprenaline only in the lack of the alcoholic hydroxyl group.

METHODS AND MATERIALS

Male, New Zealand rabbits, 1·6 to 2·0 kg, 2 to 4 months old, and male, Sprague-Dawley rats, 160–250 g, 5 to 8 weeks old, were used, as were cats of either sex, 3 to 4 kg. Rabbits and rats were killed by cervical dislocation and cats were anaesthetized with Dial-urethane (0·9 ml/kg) before excising the tissues for examination.

Aortic strip preparation

Spirally cut strips of rabbit and rat thoracic aorta were prepared according to Furchgott & Bhadrakom (1953) and suspended in isolated organ baths containing 10 or 30 ml of a modified Krebs bicarbonate solution of the following composition in mmol/litre: KCl, 4·6; $CaCl_2 \cdot 2H_2O$, 2·5; KH_2PO_4, 1·2; $MgSO_4 \cdot 7H_2O$, 1·2; NaCl, 118·2; $NaHCO_3$, 24·8 and dextrose, 10·0. The bath media were aerated with a mixture of 5% CO_2 in oxygen and the temperature was maintained at 37·5° by means of a constant

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temperature circulating unit. Drugs, dissolved in Krebs bicarbonate solution, were added in volumes of 0·1 to 0·3 ml so that bath fluid dilutions were insignificant. Removal of the test drug from the bath was accomplished by repeated washing.

In experiments designed to show α-adrenoceptor blockade by DOI, responses of aortic strips were measured isotonically with a light frontal writing lever (magnification, 1 : 20) and recorded on a smoked drum kymograph. A mechanical vibrator was used to reduce the friction between the lever and the smoked paper. In all other aortic strip experiments contractions were measured isometrically in g tension with a Grass FT-03 force-displacement transducer and recorded on a Grass polygraph. Aortic strips were subjected to an initial tension of 2·0 to 2·5 g which was maintained for the duration of the experiment according to Wurzel, Pruss & others (1970).

β-Adrenoceptor stimulation was determined by measuring the decrease in tension produced by DOI or (−)-isoprenaline (+)-bitartrate (Isolevin) when administered to either rabbit histamine-contracted or rat 5-HT-contracted aortic strips. In these experiments, the α-adrenoceptor blocking agent, phentolamine (1 μg/ml for rabbit; 0·3 μg/ml for rat tissues), was incorporated in the bathing solution. α-Adrenoceptor stimulation was determined by measuring the increase in tension produced by DOI in the presence of the β-adrenoceptor blocking agent, propranolol (0·03 μg/ml).

Cat papillary muscle preparation

The anaesthetized cat's thoracic cavity was opened and the heart was quickly excised, placed in a beaker containing Krebs bicarbonate solution and immediately washed free of blood. The right ventricle was incised and a papillary muscle removed. One end of the muscle was fastened to a tissue holder containing bipolar stimulating electrodes. The other end was attached to a Statham force-displacement transducer by a silk thread. The papillary muscle was then suspended in a 50 ml organ bath containing Krebs bicarbonate solution maintained at 37·5° and aerated with 5% CO_2 in oxygen. A resting tension of 2 g was applied and the papillary muscle paced, using a Grass Model 4 stimulator, at a frequency of 1 pulse/s and a duration of 1·5 ms at a voltage twice threshold. Recordings were made on a Sanborn 150 polygraph.

Rat trachea preparation

Spirally cut tracheal strips were prepared by the method of Constantine (1965). Contractions were measured isometrically with a Grass FT-03 force-displacement transducer and recorded on a Grass polygraph as changes in g tension. Initially, a tension of 1 g was applied. Before testing for β-adrenoceptor activity, the tissues were made to contract with an effective concentration of acetylcholine.

Cat blood pressure

An attempt was made to correlate the effects of DOI on rabbit aortic strips with the effects of DOI on the animal's blood pressure responses. However, (−)-isoprenaline did not produce consistent vasodepressor effects in this species. Since the cat is the test animal frequently used to evaluate the noradrenaline-like activity of compounds, experiments were conducted in cats anaesthetized with α-chloralose (100 mg/kg, i.v.). The animals were prepared according to standard procedures for femoral arterial pressure recording.

The following drugs were used: (−)-isoprenaline (+)-bitartrate dihydrate (Isolevin, (−)-isoprenaline), (−)-noradrenaline bitartrate monohydrate and adrenaline bitartrate

(Winthrop Laboratories), histamine dihydrochloride (Mann Laboratories), 5-hydroxytryptamine creatinine sulphate (5-HT) and acetylcholine bromide (Sigma Chemical Co.), Glyceryl trinitrate (Eli Lilly Co.), Dial-urethane (Ciba Co.) and α-chloralose (Fisher Scientific Co.). Desoxyisoprenaline HCl was supplied by Dr. F. P. Luduena of the Sterling-Winthrop Research Institute, phentolamine mesylate was supplied by Dr. A. J. Plummer of Ciba Co., propranolol HCl was supplied by Dr. R. O. Davies of Ayerst Co. All salts used for the preparation of the Krebs bicarbonate solution and sodium nitrite were of reagent grade. The concentrations of all compounds are expressed as the free bases with the exception of phentolamine mesylate and propranolol HCl which are in terms of the salt.

RESULTS

Aortic α-adrenoceptor blockade

The effect of DOI on the dose-response curves obtained with noradrenaline on aortic strips is depicted in Fig. 1. In these experiments, noradrenaline and DOI were added to the bath simultaneously. A dose-dependent blockade of the contractile responses to noradrenaline was observed. The inhibitory effects of various concentrations of DOI on the aortic contractile responses to noradrenaline, adrenaline, histamine and (—)-isoprenaline are shown in Fig. 2. DOI was administered simultaneously with concentrations of noradrenaline, adrenaline and histamine that produced approximately 80% of the maximal tissue response and with a concentration of (—)-isoprenaline that produced a response approximately 50% of the maximum. The responses to histamine were not altered, whereas responses to noradrenaline were depressed in a dose-dependent fashion. The effects of various concentrations of DOI on the contractile response to (—)-isoprenaline, however were, biphasic; at concentrations up to 4 μg/ml the response was enhanced and at higher concentrations it was depressed.

Similar results were obtained when DOI was added to the bath 5 min before the agonists. However, results obtained with the simultaneous administration of DOI and (—)-isoprenaline showed a more pronounced enhancement of the contractile response.

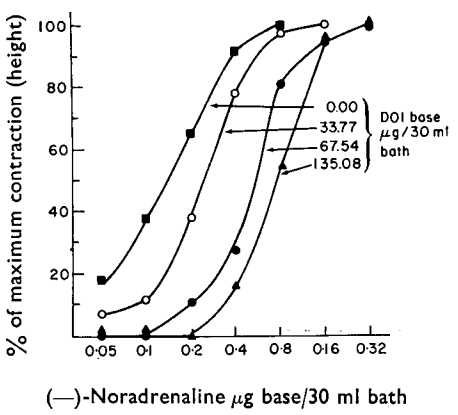

FIG. 1. Antagonism by desoxyisoprenaline (DOI) of (—)-noradrenaline-induced contraction of rabbit aortic strips. Ordinate, contraction of the rabbit aortic strip as % of maximal contraction. Abscissa, concentrations of (—)-noradrenaline in μg base/30 ml of bath fluid. Each point represents the mean of at least 5 experiments.

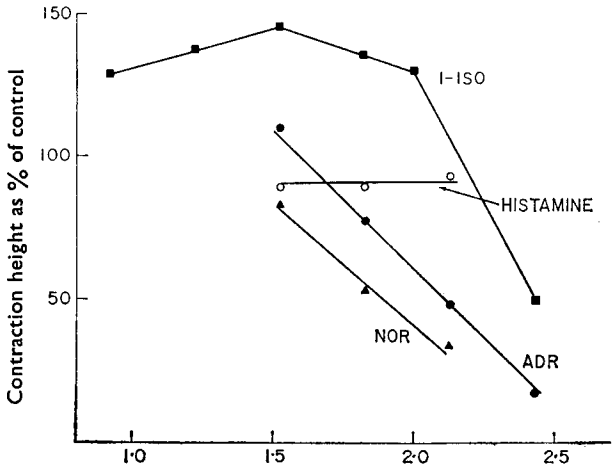

FIG. 2. Antagonism by desoxyisoprenaline (DOI) on (−)-noradrenaline (NOR), (−)-adrenaline (ADR), histamine and (−)-isoprenaline (l-ISO)-induced contractions of rabbit aortic strip. The concentration of (−)-isoprenaline was chosen to produce a contraction of about 50% of the maximal response, whereas the concentrations of the other three agonists used were chosen to produce approximately 80% of the maximal tissue response. Ordinate, response to agonist in presence of DOI expressed as % of control response, i.e., response to the agonist in the absence of DOI. Abscissa, log concentration of DOI in μg base 30/ml bath fluid. Each point represents the mean of at least 4 experiments.

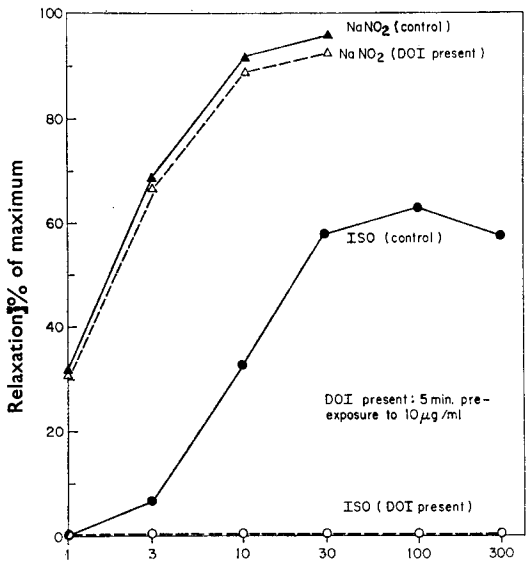

FIG. 3. Effect of desoxyisoprenaline (DOI) on $NaNO_2$ or (−)-isoprenaline-induced relaxation of histamine-contracted rabbit aortic strips. Ordinate, relaxations as % of maximal response. Abscissa, concentrations of (−)-isoprenaline (ng/ml) and $NaNO_2$ (μg/ml). Each point represents the mean of at least 6 experiments.

Aortic β-adrenoceptor blockade

Preliminary experiments demonstrated that in concentrations of 1 and 3 μg/ml DOI caused a surmountable blockade of β-adrenoceptor stimulation induced by (—)-isoprenaline. Ten μg/ml of DOI, administered 5 min before each addition of (—)-isoprenaline, completely abolished the (—)-isoprenaline-induced relaxations of the rabbit aortic strip (Fig. 3). The relaxation caused by $NaNO_2$, however, was not altered by 10 μg/ml DOI, indicating that the blocking effects of DOI on (—)-isoprenaline-induced stimulation was specific (Fig. 3).

Aortic α- and β-adrenoceptor stimulation

Fig. 4, upper panel, showed that, in the presence of β-adrenoceptor blockade produced by propranolol, DOI caused a contraction of rat thoracic aortic strips. These responses were abolished by 0·3 μl/ml phentolamine, whereas KCl-induced contractions were unaffected, In Fig. 4, lower panel, the polygraph tracing shows that after α-adrenoceptor sites had been blocked by phentolamine, DOI produced a relaxation of 5-HT contracted rat thoracic aortic strips. These relaxations, but not those produced by $NaNO_2$, were abolished by 0·03 μg/ml propranolol. The experiment shown in Fig. 4 is typical of 5 replicate experiments.

Myocardial β-adrenoceptor stimulation

The isolated cat papillary muscle, similar to other myocardial preparations, contains mainly β-adrenoceptors. In 4 experiments, concentrations of DOI, ranging from 0·1 to 10 μg/ml, produced a marked positive inotropic response similar in time course to that observed with (—)-isoprenaline; however, DOI was 1/20 to 1/100 times as potent. The threshold concentration of DOI in this system was 0·1 μg/ml. In 4 additional experiments, the effect of propranolol (1 μg/ml) was tested against the positive inotropic actions of 10 μg/ml DOI and 0·6 mg/ml $CaCl_2$. Propranolol abolished the response to DOI, whereas the response to $CaCl_2$ remained unaltered.

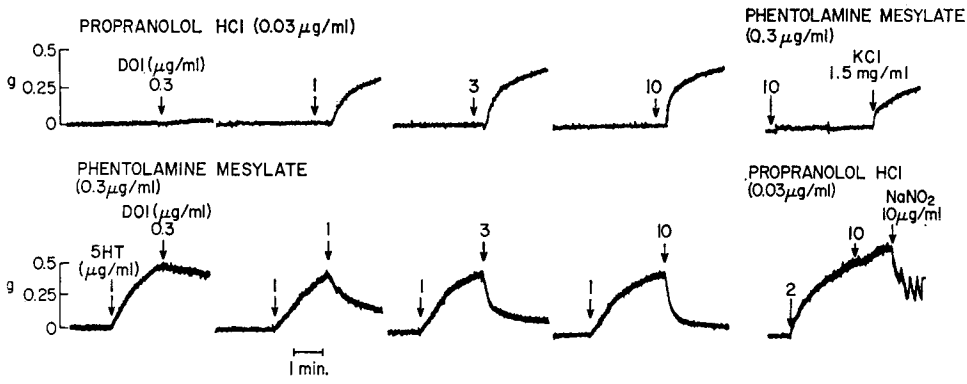

FIG. 4. α- and β-Adrenoceptor stimulating properties of desoxyisoprenaline (DOI) on rat aortic strips. Upper panel: DOI-induced contractions in the presence of the β-adrenoceptor blocking agent, propranolol HCl. These contractions were blocked by the α-adrenoceptor blocking agent, phentolamine mesylate. KCl-induced contractions were unaffected by both β- and α-adrenoceptor blockade. Lower panel: DOI-induced relaxations of 5-HT-contracted rat aortic strips in the presence of phentolamine. These relaxations were blocked by propranolol; in contrast, relaxations to $NaNO_2$ could be elicited.

Tracheal β-adrenoceptor stimulation and blockade

Similar to its action on aorta, DOI had a dual effect on β-adrenoceptors of tracheal smooth muscle. In 7 experiments, DOI relaxed acetylcholine-contracted rat tracheal strips in a dose-dependent fashion; its threshold concentration was 0·3 μg/ml, and a maximal relaxation was observed with 30 μg/ml. The relaxation to DOI was blocked by 0·06 μg/ml propranolol, whereas the relaxation caused by $1{\cdot}2 \times 10^{-4}$ M glyceryl trinitrate was unimpaired (2 experiments). With acetylcholine-contracted tracheal strips, (−)-isoprenaline caused a relaxation which was decreased by pretreatment of the strips for 5 min with 10 μg/ml of DOI (2 experiments).

Cat blood pressure

The effect of DOI on the blood pressure of the anaesthetized cat was examined in 3 experiments. Intravenous doses of DOI up to 100 μg/kg, administered 2 to 4 min before injection of (−)-isoprenaline (1 μg/kg, i.v.), did not alter the vasodepressor response to (−)-isoprenaline. Moreover, 10 and 100 μg/kg DOI by itself caused significant hypotensive response of -35 ± 3 and -43 ± 3 mm Hg, respectively.

DISCUSSION

Since Ahlquist's (1948) classification of catecholamines as either α- or β-receptor stimulants, many studies have been undertaken to determine which substances possessed the optimal chemical configuration necessary for specific interaction with these receptors (see Ariëns & Simonis, 1960; Ariëns, 1963; Ariëns, 1967). A wealth of information on structure-activity relations has emerged from these studies and many new compounds were synthesized which were either adrenoceptor agonists or antagonists. Over the past few years, reports from various laboratories indicated that β-adrenoceptors in different tissues might have different structural characteristics (Furchgott, 1967; Lands, Arnold & others, 1967; Takagi & Takayanagi, 1970). Furthermore, β-adrenoceptor activity of rabbit and rat aorta decreases with increasing age (Fleisch, Maling & Brodie, 1970).

The present study reports on desoxyisoprenaline (1-(3,4-dihydroxyphenyl)-2-isopropylaminoethane HCl, *N*-isopropyldopamine, DOI), an optically inactive analogue of (−)-isoprenaline, a prototype β-adrenoceptor stimulant. Lands, Luduena & Tuller (1954) have reported that although DOI (WIN 5571) had only about half the activity of (+)-isoprenaline it was more toxic. The compound lowered cat and dog arterial pressure, produced a positive inotropic effect on the perfused rabbit heart, and relaxed the cat and rat uterus, Ariëns (1963) pointed out that elimination of the alcoholic OH-group leads to a greater loss of affinity for β- than for α-adrenoceptors. We found DOI to stimulate as well as to block α- and β-adrenoceptors in rabbit and rat aortic strips. Furthermore, it exhibited both β-adrenoceptor agonist and antagonist effects in the rat tracheal preparation. In the cat isolated papillary muscle, DOI caused a positive inotropic response that was blocked by propranolol, a β-adrenoceptor antagonist. In addition, DOI induced a fall in arterial pressure in anaesthetized cats. Some of these effects are similar to those reported for (+)-isoprenaline (Lands, Luduena & Tullar, 1954). This would be expected from the Easson-Stedman hypothesis (1933), which states that the dextro-rotatory form of a catecholamine will behave pharmacologically like its desoxy-isomer. The unexpected finding that low doses of DOI potentiated the aortic contractile responses to (−)-isoprenaline suggested

to us that DOI might be an effective β-adrenoceptor antagonist. However, despite the fact that DOI blocked β-adrenoceptors in aorta and trachea, it was ineffective in blocking the vasodepressor response to (−)-isoprenaline in the cat and on the isolated cat papillary muscle. Propranolol blocked all of these responses. It could be suggested that DOI did not block (−)-isoprenaline in the intact cat because this analogue might have been quickly inactivated by catechol-*O*-methyltransferase (Axelrod, 1960; Hertting, 1964). Thus, the fleeting action of DOI in the intact animal would only permit the observation of the initial agonist and not of the later antagonist effects. The observation that DOI blocked the response to (−)-isoprenaline in vascular and tracheal tissue, but not in cardiac muscle, is in accord with recent reports describing agents which selectively block β-adrenoceptor function in one organ but not in another (Levy, 1966; Dunlop & Shanks, 1968). The β-adrenoceptor blocking activity of DOI probably occurs in tissues in which the compound either has little intrinsic stimulant effects or in which it interacts with a receptor for which it has a relatively low affinity.

Perhaps the most significant finding in this communication deals with the structure-activity relations between DOI and (−)-isoprenaline on aorta and trachea. (−)-Isoprenaline is a β-adrenoceptor stimulant in these tissues. Removal of the alcoholic-OH group resulted in a compound, DOI, that proved to be both a β-adrenoceptor agonist and antagonist. This denotes the extreme importance of the substituent on the β-carbon of the side chain. Various substitutions on this carbon atom could conceivably result in a new series of both β-adrenoceptor agonists and antagonists. Finally, the fact that DOI blocks both α- and β-aortic adrenoceptors, although unusual, is not surprising, since isoprenaline, the parent compound, stimulates both of these receptors.

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