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β-Adrenoceptor stimulating properties of *para*-dimethylaminobenzaldehyde

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Summary

1. Studies on the various isolated tissues indicate that *para*-dimethylaminobenzaldehyde (DMAB) is a β-adrenoceptor stimulant. DMAB is antagonized by the β-adrenoceptor blocking agent, MJ 1999, but not by the α-adrenoceptor blocking agent, phentolamine.

2. A study of dose-response relationships suggests a competitive interaction between MJ 1999 and DMAB.

3. DMAB was about 122 times less potent than isoprenaline on the isolated guinea-pig tracheal preparation. The effects of DMAB on isolated rabbit atria were not only very weak, but were also very brief. On this tissue, DMAB was respectively 72,000 and 55,400 times less active than isoprenaline in producing positive chronotropic and inotropic effects. DMAB caused the relaxation of the guinea-pig taenia coli and the rabbit ileum. These actions were very weak in comparison with those of isoprenaline.

4. These results suggest that a compound (DMAB) structurally different from isoprenaline may mimic isoprenaline responses by stimulating β-adrenoceptors through different mechanisms.

Introduction

During the past few years studies have been undertaken to elucidate the cardiovascular actions of simple aldehydes. Among the aliphatic and aromatic aldehydes, acetaldehyde has been examined in greater detail and has been shown to be the most active pressor agent, although depressor and biphasic responses have also been produced by other aldehydes (Wingard, Hitchcock & Teague, 1955). The sympathomimetic actions of acetaldehyde have been ascribed to the stimulation of both α- and β-adrenoceptors (James & Bear, 1968) and to the release of catecholamines (Eade, 1959). The majority of these aldehydes, however, have been found to have a depressant action, but their mechanism of action has not been sufficiently investigated. The responses of other tissues to these compounds are also unknown. This has led to the study of the effects of *para*-dimethylaminobenzaldehyde on various isolated tissues. The following are the results of this study.

Methods

The following tissues were isolated from animals which had been stunned by a blow on the head.

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Ileum

The last 25 cm portion of the ileum of the rabbit (3–4 kg) was flushed several times with Ringer solution (NaCl, 9 g ; KCl, 0·4 g ; $CaCl_2$, 0·24 g ; $NaHCO_3$, 0·5 g ; dextrose, 2 g/litre). Segments 1 cm in length were suspended in the tissue baths containing Ringer solution thermostatically regulated at 37·5° C. One end of the segment was tied to an anchoring glass rod in the bath solution and the other end was attached to a Grass force-displacement transducer by means of a thread. The initial tension on the tissue was set at 1 g, allowing the tissue to equilibrate for about 30 min or until uniform rhythmic contractions were obtained. Control responses to individual drugs were recorded before testing antagonism between drugs. A dose of one of the antagonists was added to the bath 2 min before applying a dose of the agonist, which was allowed to act until a steady response was obtained. The tissue was washed several times and allowed to attain uniform rhythmic contractions before applying another dose.

Taenia coli

Strips of taenia coli (1 cm long) were isolated from guinea-pigs (400–600 g) and suspended in tissue baths. The experiment was started when the spontaneous activity of the tissue reduced to a minimum after about 90 min under a tension of 1 g.

Trachea

The guinea-pig tracheal preparation was obtained according to the method of Timmerman & Scheffer (1968). The trachea was cut in such a way as to leave one cartilage ring between the scissor cuts, the number of cuts being eight to ten. The trachea was transferred to the tissue bath. Contractions were induced by methacholine (5×10^{-7}M) and the ability of the β-adrenoceptor agonists to reduce these contractions was assessed in the absence and presence of the antagonist. Before recording tissue responses to drugs, the tissue was allowed to equilibrate for about 60 min.

Atria

The atria were dissected out from the hearts of freshly killed rabbits and were set up as described for the ileum. After applying 1 g tension the atria were allowed to equilibrate for 60–90 min. The preparation was washed repeatedly and allowed to recover for at least 30 min before adding the next dose. When the effects of increasing concentrations of a drug were studied, no control observations were made between successive doses of the same drug. Before studying the actions of another drug, however, the control responses to the test agent were always obtained. A dose of the antagonist was added to the bath 15 min before adding a dose of an agonist, which was allowed to act until a steady response was produced. Spontaneous rate and contractile force of atria were measured as beats per minute and tension in grams, respectively, and expressed as the % change in the corresponding control value.

Aortic strip

Rabbit aortic strips were set up according to the method of Furchgott & Bhadrakom (1953). The aorta obtained from freshly killed rabbit was cut trans-

versely into spirals (4–5 mm wide and 60 mm long) which were mounted vertically in an organ bath as mentioned above. The tissue was allowed to equilibrate under 1·5 g tension for about 2 h.

All experiments were carried out using 15 ml baths containing Ringer solution (pH 7·3) at 37·5° C, which was continuously aerated with a mixture of oxygen (95%) and carbon dioxide (5%). Isometric tensions were recorded using a Grass model 7 polygraph and force displacement transducers (Grass FT .03). The solutions were made in distilled de-ionized water. The α- and β-adrenoceptor blocking agents were applied to the bath 15 min before the exposure of the test agent. The following drugs were administered: isoprenaline sulphate, MJ 1999 [4-(2-isopropylamino-1-hydroxyethyl) methanesulphonanilide], DMAB (*para*-dimethylaminobenzaldehyde), methacholine (acetyl-beta-methylcholine chloride) and reserpine.

For each dose at least five observations were made on the tissues from different animals, and at least five different doses were used to draw dose-response curves. The results were averaged and molar doses were plotted against percentage effects on logarithmic-probability paper. From the line on the graph the doses for 50% effects (ED50) were read, and statistically analysed by the Litchfield-Wilcoxon (1949) method.

The dose-response curves for isoprenaline and DMAB were drawn both in the absence and in the presence of MJ 1999 on the rabbit ileum, and on the taenia coli and tracheal preparations of the guinea-pig. The ED50 values for isoprenaline and DMAB on these tissues were obtained from those curves which were drawn in the presence of a constant dose of MJ 1999.

Cumulative dose-response curves for isoprenaline and DMAB were also drawn only for the guinea-pig trachea but were not used for calculating ED50.

On the atrial preparation, dose-response curves for isoprenaline and DMAB were obtained only in the absence of MJ 1999, and were used for ED50 determinations.

Results

Ileum

Figure 1 shows the inhibitory effects of isoprenaline and *para*-dimethylaminobenzaldehyde (DMAB) on the isolated rabbit ileum and the guinea-pig taenia coli. In the ileum, isoprenaline ($3{\cdot}1 \times 10^{-9}$M) and DMAB ($1{\cdot}2 \times 10^{-6}$M) produced complete relaxation which was inhibited by MJ 1999 (10^{-5}M). By increasing the dose of DMAB it was possible to reverse the blockade produced by pretreatment with MJ 1999 (10^{-5}M). One or two washings were sufficient to remove the drugs, with rapid and complete restoration of the spontaneous activity of the ileum. Isoprenaline and DMAB had nearly identical rates of onset of action and produced 100% relaxation in nearly the same time. These characteristics were maintained even in the presence of MJ 1999. Pretreatment with phentolamine ($1{\cdot}4 \times 10^{-8}$M–10^{-6}M) did not modify the response of ileum to DMAB ($1{\cdot}2 \times 10^{-6}$M) or to isoprenaline ($3{\cdot}1 \times 10^{-9}$M).

Taenia coli

Isoprenaline ($1{\cdot}2 \times 10^{-10}$M) and DMAB ($1{\cdot}8 \times 10^{-7}$M) induced maximal relaxation of the guinea-pig taenia coli. This effect was inhibited by pretreatment with

MJ 1999 (10^{-5}M) as shown in Fig. 1 (E, F). This preparation had very little spontaneous activity and responded slowly to isoprenaline and DMAB. Under our recording set-up, the relaxant effect of isoprenaline and DMAB was recorded by that part of the curve below the base line. The effects of the drugs were reversible, as shown by the rapid return of the pen to the base line position on washing. Phentolamine ($1{\cdot}4 \times 10^{-8}$–10^{-6}M) did not modify the response of taenia coli to DMAB ($1{\cdot}8 \times 10^{-7}$M) or isoprenaline ($1{\cdot}2 \times 10^{-10}$M).

Trachea

When methacholine (5×10^{-7}M) caused the full scale contraction of guinea-pig tracheal muscle, the application of DMAB (10^{-7}M) and isoprenaline (10^{-9}M) almost completely relaxed the increased tension (Fig. 2). In the presence of MJ 1999 (10^{-7}M) the relaxation rate was much slower than with preparations not exposed to MJ 1999 (Fig. 2).

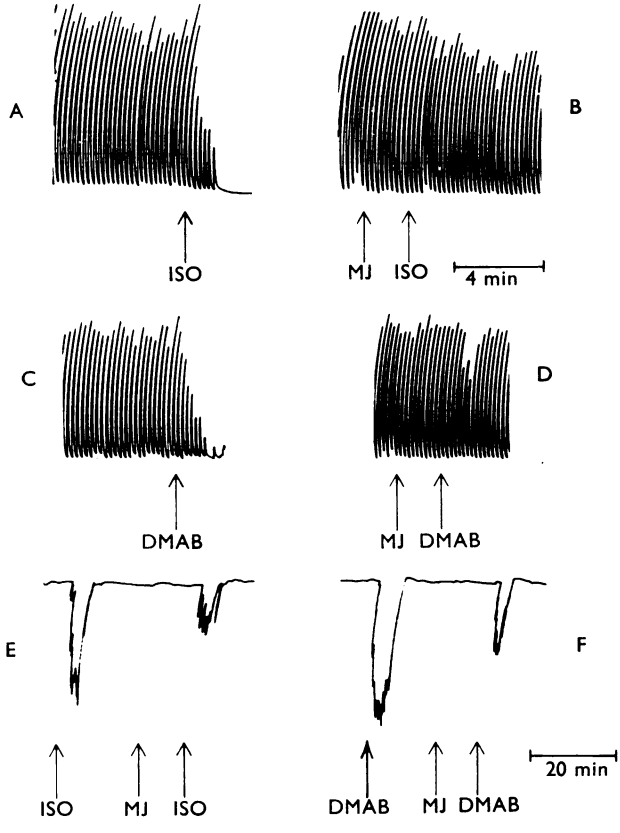

FIG. 1. Inhibitory effects of isoprenaline (ISO) and *para*-dimethylaminobenzaldehyde (DMAB) and the blockade by MJ 1999 on the isolated rabbit ileum (A, B, C and D) and the guinea-pig taenia coli (E and F). The drugs were added at the arrows. A, ISO ($3{\cdot}1 \times 10^{-9}$M); B, MJ 1999 (10^{-6}M) plus ISO ($3{\cdot}1 \times 10^{-9}$M); C, DMAB ($1{\cdot}2 \times 10^{-6}$M); D, MJ 1999 (10^{-6}M) plus DMAB ($1{\cdot}2 \times 10^{-6}$M); E, ISO ($1{\cdot}2 \times 10^{-10}$M), MJ 1999 ($10^{-6}$M) plus ISO ($1{\cdot}2 \times 10^{-10}$M); F, DMAB ($1{\cdot}8 \times 10^{-7}$M), MJ 1999 ($10^{-6}$M) plus DMAB ($1{\cdot}8 \times 10^{-7}$M).

Figure 3 shows the cumulative dose-response curves for isoprenaline and DMAB on the guinea-pig tracheal preparations in the presence and absence of MJ 1999 (10^{-7}M). After pretreatment with MJ 1999, the dose-response curves of isoprenaline and DMAB were shifted to the right. However, phentolamine ($1{\cdot}4 \times 10^{-8}$M) had no effect on tracheal relaxation produced by DMAB ($1{\cdot}7 \times 10^{-6}$M) and isoprenaline ($2{\cdot}8 \times 10^{-9}$M).

Table 1 gives the ED50 values and potency comparison of isoprenaline and DMAB on the rabbit ileum and guinea-pig taenia coli and trachea. The ED50 indicates the mean molar concentration obtained in at least five preparations which would produce a 50% of the maximum possible response in the presence of a constant dose of MJ 1999. It can be seen that the ease with which isoprenaline

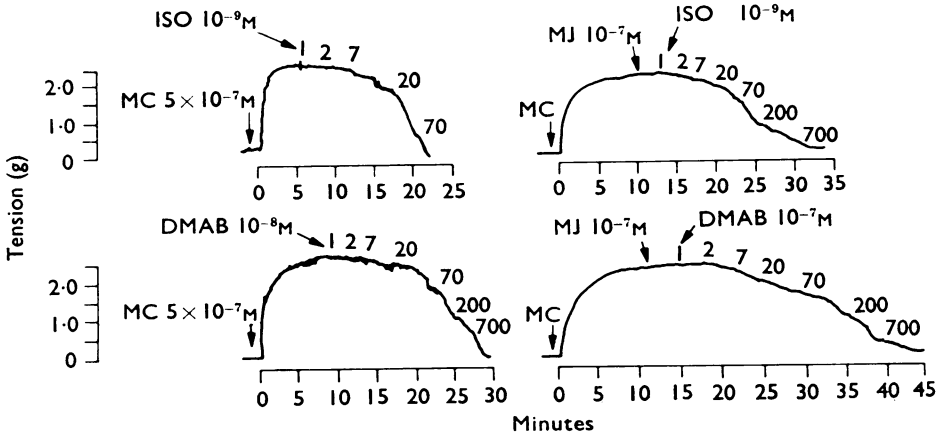

FIG. 2. Cumulative dose effects of isoprenaline (ISO) and *para*-dimethylaminobenzaldehyde (DMAB) in the absence and presence of MJ 1999 (MJ) on the guinea-pig trachea. Contractions were produced by methacholine (MC ; 5×10^{-7}M). The figures above the responses are multiples of the indicated concentrations of isoprenaline and DMAB.

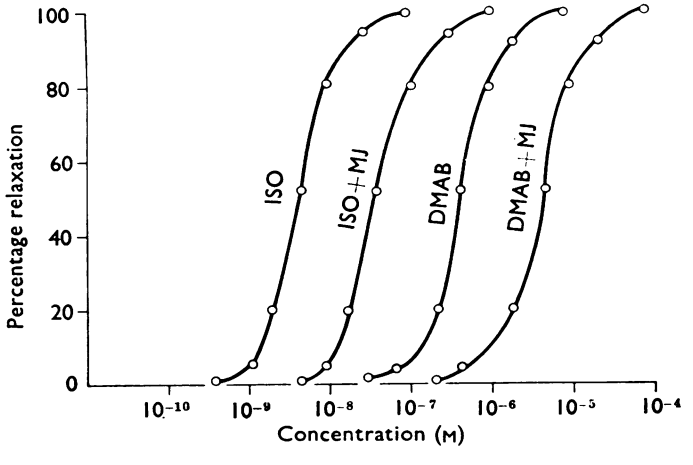

FIG. 3. Cumulative dose-response curves for isoprenaline (ISO) and *para*-dimethylaminobenzaldehyde (DMAB) in the absence and in the presence of MJ 1999 (10^{-7}M) on the guinea-pig trachea. Methacholine (5×10^{-7}M) was used to induce contraction against which the activity of the agonists was measured.

and DMAB produced their relaxing effects is in the order guinea-pig taenia coli ; rabbit ileum ; guinea-pig trachea, although their relative potencies follow in exactly the reversed order.

Atria

Isoprenaline ($6{\cdot}2 \times 10^{-11}$M to 4×10^{-9}M) and DMAB ($9{\cdot}6 \times 10^{-7}$M to $1{\cdot}2 \times 10^{-5}$M) produced positive inotropic and chronotropic effects on the isolated rabbit atria (Fig. 4). The effects of different doses of these drugs are summarized in Table 2. In terms of dose required for a 50% increase in the control atrial rate and atrial force of contraction, DMAB was respectively about 72,000 and 55,400 times less potent than isoprenaline Since DMAB is only slightly soluble in water, it was not possible to use higher doses to produce maximum effects. Our observations, however, indicate that with the dose range studied DMAB has a lesser effect on the atrial rate than on the atrial force of contraction, whereas isoprenaline has almost the same effect on the rate and the force of contraction of the rabbit atria. The effects of DMAB on the atria were brief, lasting 1–3 min, whereas the effects of isoprenaline were long, lasting 30–50 min. These effects were readily removed by washing. Pre-

TABLE 1. *Comparison of β-adrenoceptor stimulating properties of isoprenaline (ISO) and* para*dimethylaminobenzaldehyde (DMAB) in the presence of MJ* 1999 (10^{-6}M)

Tissue	Drug	ED50 and range (M)	Slope and range*	Potency
Rabbit ileum	ISO	4×10^{-9} ($2{\cdot}5 \times 10^{-9}$–$7{\cdot}3 \times 10^{-9}$)	2·59 (0·74–9·08)	400
	DMAB	$1{\cdot}6 \times 10^{-6}$ ($7{\cdot}5 \times 10^{-7}$–$3{\cdot}6 \times 10^{-6}$)	3·99 (0·57–7·13)	1
Guinea-pig taenia coli	ISO	$9{\cdot}6 \times 10^{-10}$) ($6 \times 10^{-10}$–$1{\cdot}53 \times 10^{-9}$)	2·26 (1·14–3·17)	687
	DMAB	$6{\cdot}6 \times 10^{-7}$ ($4{\cdot}10 \times 10^{-7}$–$8{\cdot}8 \times 10^{-7}$)	2·02 (1·23–3·31)	1
Guinea-pig trachea	ISO	$4{\cdot}5 \times 10^{-8}$ ($3{\cdot}6 \times 10^{-8}$–$5{\cdot}1 \times 10^{-8}$)	2·15 (1·22–3·76)	122
	DMAB	$5{\cdot}5 \times 10^{-6}$ ($3{\cdot}6 \times 10^{-6}$–$8{\cdot}1 \times 10^{-6}$)	3·16 (1·15–3·78)	1

* 95% confidence limits.

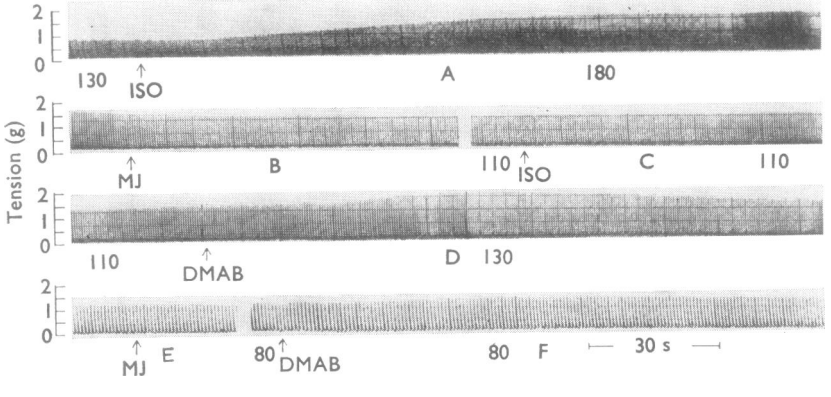

FIG. 4. Effects of isoprenaline (ISO) and *para*-dimethylaminobenzaldehyde (DMAB) in the absence and in the presence of MJ 1999 on the rate and force of contraction of the isolated rabbit atria. The drugs were added at the arrows. The numbers indicate atrial rate. A, ISO ($1{\cdot}2 \times 10^{-10}$M) ; B, MJ 1999 ($10^{-6}$M) ; C, ISO ($1{\cdot}2 \times 10^{-10}$M) added 5 min after adding MJ 1999 ; D, DMAB ($1{\cdot}2 \times 10^{-6}$M) ; E, MJ 1999 (10^{-6}M) ; F, DMAB ($1{\cdot}2 \times 10^{-6}$M) added 15 min after adding MJ 1999.

treatment with phentolamine ($1{\cdot}4 \times 10^{-8}$M) did not modify the responses of atria to these drugs. In five experiments the atria from rabbits, previously treated with reserpine to deplete the catecholamines in the heart, were used. Reserpine (5 mg/ml in 20% ascorbic acid) was injected in 1·5 mg/kg intravenously on the first day, 5 mg/kg on the second day, the animal being killed on the third day. The responses of the atria from these animals to DMAB ($9{\cdot}6 \times 10^{-7}$M to $1{\cdot}2 \times 10^{-5}$M) and isoprenaline ($6{\cdot}2 \times 10^{-11}$M to 4×10^{-9}M) were not significantly different from those of untreated atria.

Aortic strip

On the rabbit aortic strip, isoprenaline (10^{-7}M) and DMAB ($6{\cdot}6 \times 10^{-6}$M) produced only slight relaxation which could be blocked by MJ 1999 (10^{-6}M) but not by phentolamine ($1{\cdot}4 \times 10^{-8}$ and 10^{-6}M). In view of the very small magnitude of the inhibitory effects of isoprenaline and DMAB, we did not determine their ED50 values in this preparation.

Discussion

The results show that DMAB produced sympathomimetic effects that can be compared with those produced by the β-adrenoceptor agonist, isoprenaline, on the rabbit intestine (Furchgott, 1960), guinea-pig taenia coli (Brody & Diamond, 1967), guinea-pig trachea (Foster, 1966), rabbit aortic strip (Furchgott, 1955) and rabbit atria (Lands & Howard, 1952). The parallel shift in the dose-response curves for isoprenaline and DMAB by the β-adrenoceptor blocking agent, MJ 1999 and the inability of phentolamine to antagonize DMAB and isoprenaline on any of these preparations suggest that DMAB has a specific β-adrenoceptor stimulating action.

In terms of percentage change in the inotropic and chronotropic effects, DMAB seems to have a greater effect on the atrial force of contraction than on the atrial rate. Isoprenaline, on the other hand, produces almost the same percentage increase in the atrial rate and force of contraction. DMAB differs from acetaldehyde in that it does not release catecholamines in the isolated rabbit atria, for reserpinization does not modify its responses whereas acetaldehyde has been shown to have no effect on the reserpinized atria (Kumar & Sheth, 1962).

The potency ratio between isoprenaline and DMAB varied from one tissue to another. There is a great potency difference between the two drugs with regard

TABLE 2. *Effects of isoprenaline (ISO) and* para-*dimethylaminobenzaldehyde (DMAB) on the isolated rabbit atria*

		Percentage increase in the atrial rate and force	
Drug	Dose	Rate/min (mean±S.E.M.)	Force (g) (mean±S.E.M.)
ISO	$6{\cdot}2 \times 10^{-11}$M	22±1·6	29±2·6
	$2{\cdot}5 \times 10^{-10}$M	59±2·9	57±5·8
	10^{-9}M	99±2·1	94±4·2
	$4{\cdot}0 \times 10^{-9}$M	124±0·1	115±1·3
DMAB	$9{\cdot}6 \times 10^{-7}$M	15±1·5	20±4·2
	$4{\cdot}8 \times 10^{-6}$M	35±1·9	49±2·9
	$7{\cdot}2 \times 10^{-6}$M	52±2·9	66±2·4
	$1{\cdot}2 \times 10^{-5}$M	67±4·1	80±3·5

to their effects on the isolated rabbit atria. This difference is less pronounced on other tissues, and is least on the guinea-pig trachea.

The lack of structural resemblance between these β-adrenoceptor agonists and DMAB, and the inability of aldehydes derived from adrenaline, 5-hydroxytryptamine, histamine and tryptamine to stimulate adrenoceptors in the isolated tissues (Renson, Weissbach & Udenfriend, 1964) suggest that a physiological response is the result of a series of favourable interactions among the various cellular components and the drug molecule. Thus the ability of structurally different drugs to produce the same physiological response may be due to their effectiveness in modifying the interactions among the receptors, allosteric sites and the receptor environments (Ghouri & Haley, 1969). In the absence of conclusive evidence to support this hypothesis, the extent to which these factors are involved in the mechanism of action of DMAB is as speculative as any other reasonable explanation for our observations with DMAB.

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