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The effects of diethylcarbamazine citrate on smooth muscle

A. K. ABAITEY* AND J. R. PARRATT†

The Department of Physiology and Pharmacology, Royal College, University of Strathclyde, Glasgow G1 1XW, U.K.

The effect of the anthelmintic drug diethylcarbamazine citrate (DECC) was examined on the guinea-pig isolated ileum, rabbit duodenum, chick oesophagus, rat portal vein and pig coronary artery. DECC contracted all the gastrointestinal smooth muscle preparations. The contractions were antagonized by hexamethonium and atropine but they were not affected by mepyramine or methysergide in concentrations that abolished, or markedly reduced, responses to histamine and 5-hydroxytryptamine. DECC inhibited the responses of the guinea-pig ileum to other spasmogens, namely, acetylcholine, histamine and nicotine. Physostigmine markedly potentiated the responses of the chick oesophagus and the rabbit duodenum to DECC. DECC relaxed the potassium chloride-induced contractions of the pig coronary artery strips; these relaxations were not modified by propranolol or calcium chloride. There was no evidence that DECC released histamine from skin or muscle.

Diethylcarbamazine (DECC) is a widely used antifilarial drug with outstanding filaricidal action against *Wuchereria bancrofti*, *Brugia malayi* and *Loa loa* of man (Rollo, 1965) and *Dirofilaria immitis* of the dog. It is also the drug of choice in destroying the microfilariae of *Onchocerca volvulus* in man. More recently, the drug has been used, with varying results, in the treatment of bronchial asthma (Mallen, 1965; Benner & Lowell, 1970; Srinivas & Antani, 1971; Koivikko, 1973; Sly & Matzen, 1974).

In a recent study of the cardiovascular effects of DECC in anaesthetized cats (Abaitey & Parratt, 1976) we concluded that the drug has marked effects on sympathetic ganglia. The present study was designed to examine the effects of DECC on a variety of isolated smooth muscle preparations, including some which contain parasympathetic ganglia. Apart from the initial study by Harned, Cunningham & others, 1948), there have been no studies of the effects of diethylcarbamazine on smooth muscle.

METHODS

Guinea-pig ileum. Pieces of ileum were set up in a 10 ml organ bath containing Tyrode solution (composition, g litre^{-1}: NaCl 8·0, KCl 0·2, $MgCl_2$ 0·1, $CaCl_2$ 0·2, NaH_2PO_4 0·05, $NaHCO_3$ 1·0, glucose 1·0) at 32°. The solution was bubbled with 5% carbon dioxide in oxygen. Contractions were recorded isotonically on smoked paper by means of an isotonic frontal writing lever. The resting tension was 0·5 g.

Chick oesophagus (Bowman & Everett, 1964) and rat fundus strips. These smooth muscle preparations were suspended in a 10 ml organ bath containing Krebs solution (composition, g litre^{-1}: NaCl 6·92, KCl 0·35, $MgSO_4$ 0·29, $CaCl_2$ 0·28, KH_2PO_4 0·16, $NaHCO_3$ 2·1, glucose 1·0) at 32° (for oesophagus) and 37° (for fundus strips) bubbled with a mixture of 5% carbon dioxide in oxygen. The Krebs solution for the fundus strips contained indomethacin ($2·8 \times 10^{-6}$ M), mepyramine ($2·5 \times 10^{-6}$ M) and atropine ($2·9 \times 10^{-6}$ M). The resting tensions for the oesophagus and the fundus strips were 2 and 1 g respectively.

Rabbit duodenum, rat portal vein and pig coronary artery strips. These smooth muscle preparations were set up in Krebs solution bubbled with 5%CO_2 in oxygen at 37°. Contractions were recorded isometrically with Devices force-displacement transducers and a two-channel pen recorder. The resting tensions for duodenum, portal vein and coronary artery strips were 3, 0·5 and 1 g respectively.

Rat hindquarters perfusion. The method of Feldberg & Mongar (1954) was used. The venous effluent was collected 3, 5 and 10 min after the administration of either DECC or compound 48/80 and assayed for histamine (on guinea-pig ileum) and for 5-hydroxytryptamine (5-HT) (on rat fundus strips).

Drugs. Drugs used were barium chloride, calcium chloride, potassium chloride (Analar); atropine sulphate, histamine dihydrochloride, nicotine hydrogen tartrate, physostigmine sulphate (BDH); al-

* Present address: Faculty of Pharmacy, University of Science and Technology, Kumasi, Ghana.
† Correspondence.

prenolol hydrochloride (Hässle); propranolol hydrochloride (ICI); 5-hydroxytryptamine (Koch-Light); hexamethonium bromide, mepyramine maleate (May and Baker); quazodine (Mead Johnson); Ro 20–1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone, Roche); methysergide bimaleate (Sandoz); acetylcholine chloride (Sigma); diethylcarbamazine citrate (Burroughs Wellcome); (–)-isoprenaline bitartrate (Wyeth).

RESULTS

Effects of DECC on intestinal and oesophageal smooth muscle. DECC, in concentrations between $6{\cdot}5 \times 10^{-5}$ and $2{\cdot}1 \times 10^{-3}$ M, caused dose-dependent contractions of the guinea-pig ileum (eight preparations). rabbit duodenum (nine preparations) and the chick oesophagus (15 preparations). These responses were similar to those produced by nicotine (6×10^{-6} — 2×10^{-5} M) in that they were abolished, or markedly reduced, by hexamethonium ($2{\cdot}8 \times 10^{-6}$ to $0{\cdot}6 \times 10^{-3}$ M; Fig. 1) and slightly inhibited by atropine at $5{\cdot}8 - 8{\cdot}7 \times 10^{-9}$ M, which prevented the effects of acetylcholine ($1{\cdot}1 \times 10^{-8}$ to $2{\cdot}75 \times 10^{-6}$ M). The responses to acetylcholine, nicotine and DECC on the rabbit duodenum and chick oesophagus (Fig. 2) were potentiated by physostigmine ($6{\cdot}2 \times 10^{-8}$ to

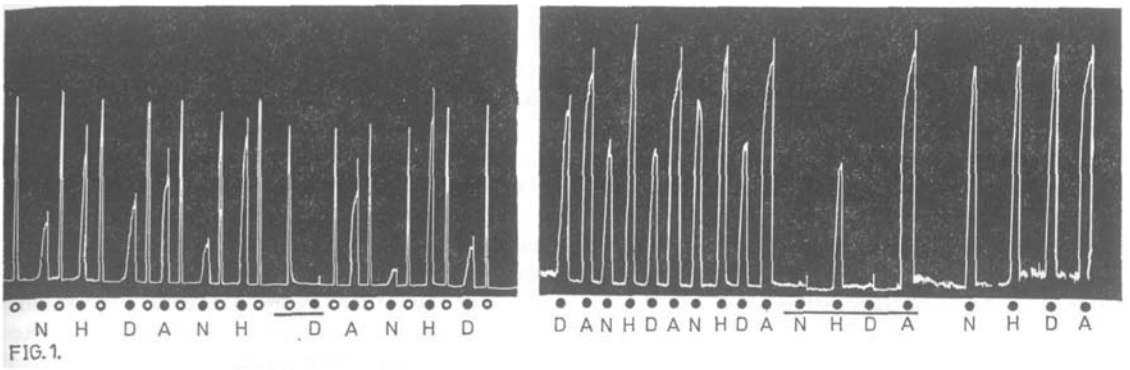

FIG. 1.

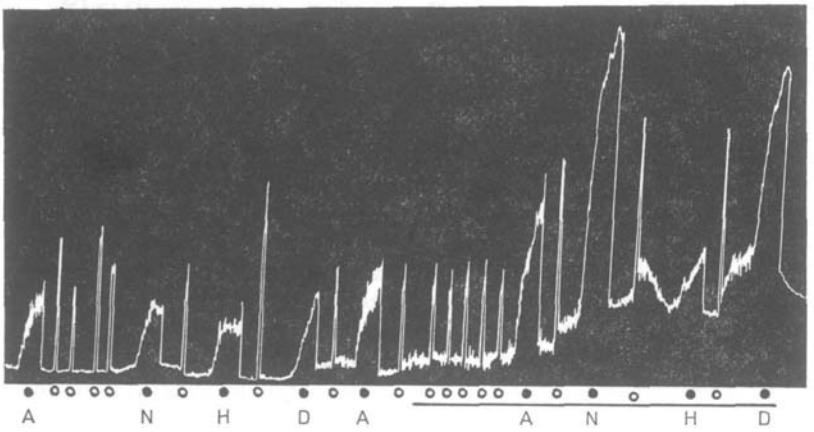

FIG. 2.

FIG. 1. Chick oesophagus; two separate experiments. Left hd trace, the effects of nicotine (N, $1{\cdot}2 \times 10^{-5}$ M), histamine (H, $2{\cdot}7 \times 10^{-6}$ M), diethylcarbamazine (D, $1{\cdot}02 \times 10^{-5}$ M) and acetylcholine (A, $8{\cdot}25 \times 10^{-7}$ M). The corresponding concentrations in the rt hd trace are nicotine ($2{\cdot}0 \times 10^{-5}$ M), histamine ($5{\cdot}4 \times 10^{-6}$ M), diethylcarbamazine ($6{\cdot}37 \times 10^{-5}$ M) and acetylcholine ($2{\cdot}75 \times 10^{-6}$ M). The open circles denote electrical stimulation and the continuous lines represent the presence of hexamethonium ($2{\cdot}76 \times 10^{-5}$ M, left hd trace; $4{\cdot}14 \times 10^{-5}$ M, rt hd trace). Hexamethonium abolished the response to nicotine and diethylcarbamazine but did not prevent the responses to electrical stimulation (compare Bowman & Everett, 1964) or the effects of histamine and acetylcholine.

FIG. 2. The effects of acetylcholine (A, $2{\cdot}75 \times 10^{-7}$ M), nicotine (N, $9{\cdot}0 \times 10^{-6}$ M), histamine (H, $2{\cdot}4 \times 10^{-6}$ M) and diethylcarbamazine (D, $3{\cdot}9 \times 10^{-5}$ M) on the chick oesophagus. The continuous line denotes the presence of physostigmine ($1{\cdot}24 \times 10^{-7}$ M) and the open circles represent electrical stimulation. Physostigmine markedly potentiated the responses to electrical stimulation, acetylcholine, nicotine and diethylcarbamazine, but not that of histamine and itself induced a gradually developing muscle spasm.

$1{\cdot}2 \times 10^{-7}$ M). On the oesophagus (five preparations) the effects of electrical stimulation were also potentiated (Fig. 2).

There was no evidence that DECC contracted smooth muscle through interactions with histamine or 5-HT receptors. DECC-induced contractions of the guinea-pig ileum (six preparations) and chick oesophagus (four preparations) were unaffected by mepyramine at concentrations $1{\cdot}25 \times 10^{-8}$ M that inhibited the responses to histamine by more than 90%, or by methysergide ($1{\cdot}05 \times 10^{-7}$ M) that inhibited the responses of 5-HT by more than 50%. On the guinea-pig ileum desensitization of 5-HT receptors by a large dose of 5-HT ($5{\cdot}7 \times 10^{-5}$ M) abolished the effects of this spasmogen without altering the responses to DECC ($2{\cdot}08 \times 10^{-3}$ M), barium chloride ($1{\cdot}35 \times 10^{-3}$ M), histamine ($2{\cdot}16 \times 10^{-7}$ M), acetylcholine ($5{\cdot}5 \times 10^{-8}$ M), or nicotine (8×10^{-6} M).

Apart from contracting the guinea-pig ileum, DECC also inhibited the response of the tissue to nicotine, acetylcholine and histamine, although not to barium chloride. With acetylcholine and histamine (Fig. 3) the dose-response curves were shifted in a parallel manner; the calculated pA_2 values were 4·1 for acetylcholine (two estimations) and 4·8 for histamine (mean of three estimations).

Rat portal vein. In each of eight preparations, DECC initially produced a dose-dependent enhancement of both the amplitude and the frequency of the spontaneous myogenic contractions of the portal vein. This initial phase was followed by decrease in both the amplitude and the frequency of the spontaneous activity (Fig. 4). In some preparations the initial stimulatory effect of DECC was absent. Similar results were obtained with nicotine. Hexamethonium had no effect on the responses of the portal vein to DECC or nicotine.

Isoprenaline inhibited the amplitude without affecting the frequency of the contractions of the portal vein. Alprenolol ($8{\cdot}75 \times 10^{-7}$ M) abolished the effect of isoprenaline ($9{\cdot}4 \times 10^{-8}$ M) but did not alter the response to DECC ($1{\cdot}3 \times 10^{-3}$ M).

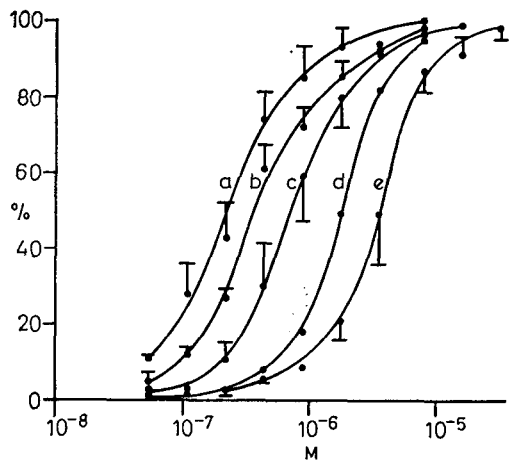

FIG. 3. Guinea-pig ileum. Log dose/response curves to histamine (M) in the presence of diethylcarbamazine. The concentrations of diethylcarbamazine used are represented as follows:—a, control; b, $1{\cdot}3 \times 10^{-5}$ M; c, $2{\cdot}6 \times 10^{-5}$ M; d, $1{\cdot}3 \times 10^{-4}$ M; e, $2{\cdot}6 \times 10^{-4}$ M. Each point is the mean of at least three observations. Ordinate—% maximal contraction. Abscissa—Histamine (M).

Pig coronary artery strips. In view of the increases in local myocardial blood flow observed when DECC was administered to anaesthetized cats (Abaitey & Parratt, 1976), experiments were made to see whether the drug relaxed isolated coronary arteries *in vitro*. The effect of DECC on coronary artery strips contracted with potassium chloride was investigated in ten preparations. The mean ED25 and ED50 values were 0·13 and $2{\cdot}0 \times 10^{-3}$ M respectively. Relaxations produced by DECC were not enhanced by the phosphodiesterase inhibitors, quazodine or Ro 20–1724. Propranolol and calcium chloride also had no effect on the responses to DECC.

Rat hindquarters perfusion. The perfusate collected from two rats was assayed for histamine on the guinea-pig isolated ileum. No histamine could be detected (i.e. $< 0{\cdot}001$ μg ml^{-1}) in the perfusate collected 10 min before, and up to 28 min after, the injection of DECC (5 mg) intravenously into the hindquarters. In the same rat, the perfusate collected over the first 3 min after the injection of compound 48/80 (100 μg) contained $0{\cdot}26$ μg ml^{-1} of histamine (i.e. $0{\cdot}88$ μg total). Similar results were obtained from a second rat.

The perfusate collected from one rat was assayed for 5-HT on the rat fundus strip preparation. DECC (5 mg) failed to release 5-HT (i.e. $< 0{\cdot}007$ μg ml^{-1}) whereas compound 48/80 (100 μg) released $0{\cdot}027$ μg ml^{-1} of 5-HT within the first 3 min (i.e. $0{\cdot}073$ μg total) and $0{\cdot}009$ μg ml^{-1} (i.e. $0{\cdot}041$ μg total) during the next 6 min of injection.

DISCUSSION

DECC contracted all the gastrointestinal smooth muscle preparations. These contractions were readily antagonized by hexamethonium and slightly inhibited by atropine. Mepyramine and methysergide,

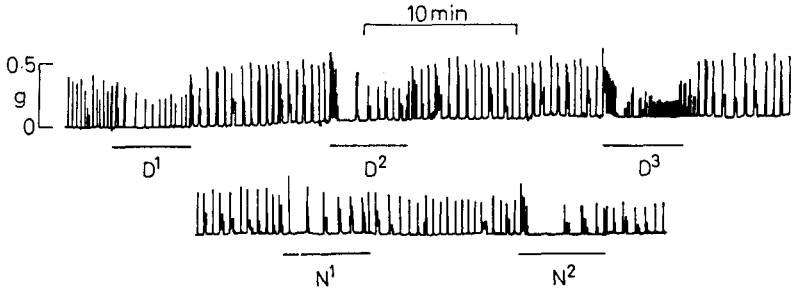

FIG. 4. The effects of diethylcarbamazine (D, upper trace) and nicotine (N, lower trace) on rat portal vein. The concentrations of drugs used were as follows:— D^1, 1·02 mM; D^2, 2·04 mM; D^3, 4·08 mM; N^1, 0·8 mM; N^2, 1·6 mM. The continuous lines denote the presence of the drug in the organ bath.

in concentrations that abolished or markedly reduced the responses to histamine and 5-HT, had no effect on the responses of these preparations to DECC. On the chick oesophagus and the rabbit duodenum, the responses to DECC were markedly potentiated by physostigmine. The similarity betweeen the effects of DECC and those of nicotine on the gastrointestinal smooth muscle preparations studied suggests that DECC possesses nicotine-like activity and supports the results of the *in vivo* investigations of Forbes (1972) and of ourselves (Abaitey & Parratt, 1976). DECC was from 2 to 100 times less active than nicotine, depending upon the preparation. The results obtained in the present work differ from the findings of Harned & others (1948) who have reported that DECC has no affect on the guinea-pig intestine and that the drug relaxes the rabbit ileum.

Apart from producing contraction of intestinal smooth muscle, DECC antagonized the contractile responses to other spasmogenic agents and, on the guinea-pig ileum, this resulted in a shift of the log dose/response curves in a parallel fashion. The pA_2 values obtained for DECC against acetylcholine and histamine on the guinea-pig ileum were 4·1 and 4·8 respectively. The similarity of the pA_2 values suggests that DECC is a non-specific antagonist. This suggestion is supported by the findings of Burka & Eyre (1974) that DECC inhibits histamine, prostaglandins E_2 and F_2 and 5-HT on the bovine pulmonary vein *in vitro*.

DECC relaxed the pig coronary artery strips contracted with potassium chloride. The relaxations of the artery strips produced by DECC were not affected by propranolol, calcium chloride, quazodine or Ro 20–1724. The relaxations may therefore be due to a direct spasmolytic effect of DECC. It is worth noting that Orange, Austen & Austen (1971) have demonstrated that DECC acts synergistically with β-adrenoceptor agents in the inhibition of antigen-induced release of slow-reacting substance of anaphylaxis and histamine from human lung fragments and that its inhibitory activity is not prevented by the β-adrenoceptor blocking agent, propranolol. These authors could not establish any effect of DECC on cytoplasmic phosphodiesterase. Furthermore, preliminary studies have not demonstrated that DECC increases tissue concentrations of cyclic AMP (Orange & Austen, 1971).

DECC has been claimed to be a histamine liberator (Deline, Eyre & Wells, 1973). Nevertheless, most of the evidence in the literature from immunological studies points to the fact that DECC inhibits antigen-induced release of both histamine and slow-reacting substances of anaphylaxis (Orange & Austen, 1968; Orange, Valentine & Austen, 1968; Orange, Stechschulte & Austen 1970; Ishizaka, Ishizaka & others, 1971). The results obtained from the rat hindquarters perfusion experiments show that DECC does not liberate either histamine or 5-HT from this preparation. Release of histamine and 5-HT could be demonstrated in the same rats using compound 48/80.

These results, from a variety of isolated preparations, indicate that DECC contracts extravascular smooth muscle by a mechanism that involves stimulation of parasympathetic ganglia. The effects of DECC were similar to those induced by nicotine in that they were slightly reduced by atropine, abolished by hexamethonium and potentiated by physostigmine. These results may explain the fact that, in a dose of 10–20 mg base kg^{-1}, DECC may cause gastrointestinal disturbances (nausea, vomiting) even in persons not infected with filaria (Hawk-

ing, 1966). There was no evidence that the smooth muscle effects of DECC are mediated through effects on histamine or 5-HT receptors or through histamine release. Vascular smooth muscle (portal vein, coronary arteries) was however inhibited by DECC. The mechanism of this effect is obscure but there is no evidence (Abaitey & Parratt, 1976) that it contributes to the haemodynamic effects of the drug *in vivo*.

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