THE EFFECT OF SOME ANTI-ACETYLCHOLINE DRUGS ON THE RESPONSES OF THE ISOLATED RABBIT INTESTINE TO SYMPATHETIC NERVE STIMULATION AND TO THE ACTION OF ADRENERGIC NEURONE BLOCKING AGENTS

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The effects of atropine and some quaternary derivatives on the inhibitory responses of the Finkleman preparation to stimulation of the peri-arterial sympathetic nerves and to added adrenaline were examined. None of the compounds tested appeared to affect the inhibitory responses with the exception of *N*-diphenylmethylatropine, which much enhanced the inhibitory effect of the electrical stimulation. In addition, this compound antagonised *in vitro*, but not *in vivo*, the activity of several adrenergic neurone blocking compounds, but not guanethidine.

ACCORDING to some recent work (Burn and Rand, 1959; Burn, 1961, 1962), a cholinergic mechanism is believed to play a role in the liberation of the sympathetic mediator at postganglionic sympathetic nerve endings. We thought it of interest to use the isolated rabbit intestine as described by Finkleman (1930), to find out whether the responses of this preparation would be influenced by agents known to interfere with the mechanisms of cholinergic mediation.

In the experiments to be described we investigated the influence of anti-acetylcholine drugs on the inhibition of intestinal motility, either by electrical stimulation of the sympathetic postganglionic fibres or by adrenaline. In addition, the possible alterations induced by these drugs on the adrenergic neurone blocking effects of bretylium, guanethidine, xylocholine, hemicholinium and dimethylphenylpiperazinium (DMPP) were sought. According to Wilson (1962) and Bentley (1962), DMPP seems to possess adrenergic neurone blocking properties, besides the known nicotinic ones.

EXPERIMENTAL

Methods

Most of our experiments were made on the isolated intestine segments of the rabbit, prepared with the extrinsic sympathetic supply intact as described by Finkleman (1930). Rabbits of either sex, weighing 2–3 kg., provided isolated intestinal loops of 10–12 cm. which were suspended in 100 ml. baths (Zamboni, 1940), filled with Tyrode solution at a constant temperature of 32° and connected to an isotonic lever (ratio 1 : 4; load 2 g.). The neurovascular hilum was placed on platinum electrodes immersed in the perfusion fluid and connected to an electronic stimulator. Rectangular pulses of 1 msec. duration were applied at a frequency of 20–25/sec. for 30 sec.

A few experiments were also made in the heparinised rat, by recording the arterial pressure through a mercury manometer connected to the cannulated carotid artery and estimating the antagonistic effect exerted by

bretylium against the hypertensive responses seen after intravenous administration of eserine (150–200 μg./kg.). Rats were anaesthetised with urethane given intraperitoneally (1·5 g./kg.), since the typical pressor response to eserine is partially suppressed by barbiturate anaesthesia (Della Bella and Gandini, 1962).

The following drugs were used: atropine sulphate, atropine *N*-methylbromide, scopolamine *N*-butylbromide, *N*-diphenylmethylatropine bromide, adrenaline chloride, bretylium tosylate, guanethidine sulphate, dimethylphenylpiperazinium iodide, xylocholine bromide, hemicholinium bromide, eserine sulphate, cocaine hydrochloride. The concentrations used are expressed as salts.

RESULTS

Influence of Atropine and its Derivatives on the Responses of the Finkleman Preparation to Adrenaline and Electrical Stimulation

Atropine concentrations from 0·1 to 10–20 μg./ml. were used. We observed somewhat unexpectedly in about ten preparations that whereas the first application of atropine at, say, 0·1–0·25 μg./ml. induced the typical

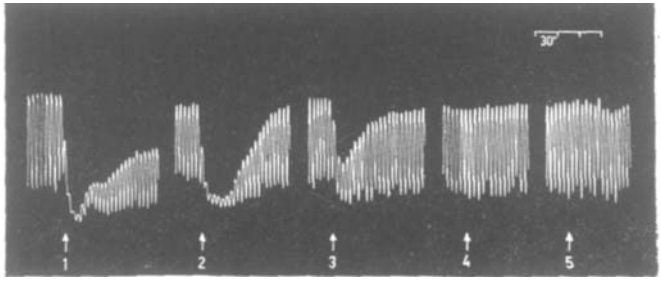

FIG. 1. Isolated rabbit intestine. At 1, 2, 3 and 4, atropine, 0·2, 0·5, 2 and 10 μg./ml. respectively, for 5–10 min. After each dose a prolonged washout. Interval between each dose, 10 min. The desensitisation to atropine motor inhibitory effects is progressive and complete and is followed by unresponsiveness to the diphenyl derivative (10 μg./ml.) (at 5).

marked reduction in spontaneous activity, a second application, after washing and recovery, at a concentration of 3–5 times gave a much smaller inhibition. In the course of 4 or 5 administrations made at intervals, such as to allow the reversion of the effects of each, concentrations of 20–30 μg./ml. may be attained without any apparent effect on the motor activity of the preparations (Fig. 1).

Once unresponsiveness to atropine had developed, the isolated rabbit intestine reacted differently to acetylcholine compared to nicotine. Whereas at the beginning of three experiments the intestine reacted to acetylcholine 0·005 μg./ml. or to nicotine 0·0025 μg./ml. with a strong contraction and a marked increase in tone, after repeated atropine treatment at increasing concentrations and in the presence of atropine at 20 μg./ml., the intestine reacted to nicotine only at 0·5 μg./ml. or more, and did not react to acetylcholine even at 5–10 μg./ml.

A similar rapid loss of responsiveness to the inhibition of spontaneous contractions induced by the quaternary nitrogen derivatives of atropine and scopolamine was observed. The phenomenon appears to be a cross-desensitisation: in fact the intestine no longer sensitive to atropine is

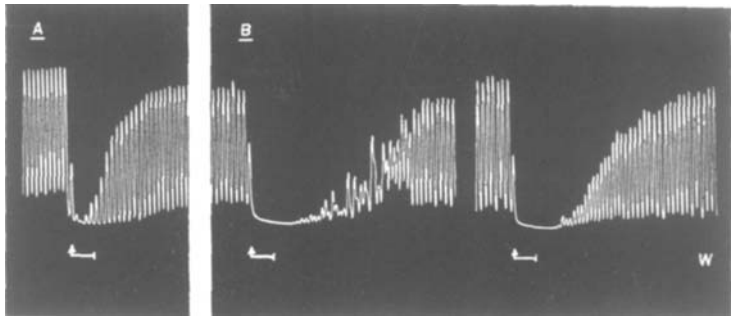

FIG. 2. Isolated rabbit intestine prepared according to Finkleman. In A: response to 30 sec. electrical stimulation of sympathetic postganglionic fibres (at arrow), before *N*-diphenylmethylatropine. In B: after desensitisation to *N*-diphenylmethylatropine: responses to sympathetic stimulation (at arrows) in the presence of *N*-diphenylmethylatropine (15 μg./ml.). Interval between each stimulation period, 15 min. At W: prolonged washing.

unresponsive to quaternary compounds (see Fig. 1); similarly the responses to atropine disappear after the desensitisation to quaternary derivatives.

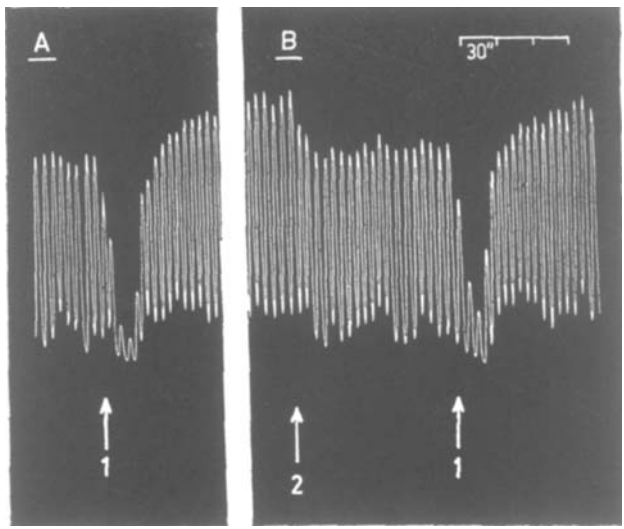

FIG. 3. Isolated rabbit intestine. In A: inhibitory response following adrenaline (0·01 μg./ml.) (at 1). The drug is removed after 30 sec. contact, by thorough washing. In B: after progressive desensitisation, the presence of *N*-diphenylmethylatropine (15 μg./ml.) (at 2) does not practically modify the inhibitory response to adrenaline (0·01 μg./ml.) (at 1).

The influence of the same anti-acetylcholine agents on the motility inhibition due to sympathetic electrical stimulation or adrenaline administration, was also investigated. The compounds under test, at varying concentrations, did not modify the intestine responses to chemical or electrical stimulation. *N*-Diphenylmethylatropine was the single exception and it enhanced significantly the responses to electrical stimulation, in both intensity and duration (Fig. 2), while being completely ineffective on the adrenaline-induced responses (Fig. 3). This enhancement, which is illustrated in Fig. 2, subsides, after the drug is removed by washing, in the course of 3–5 responses. Once the responses have returned to normal, a further treatment with *N*-diphenylmethylatropine appears to be ineffective.

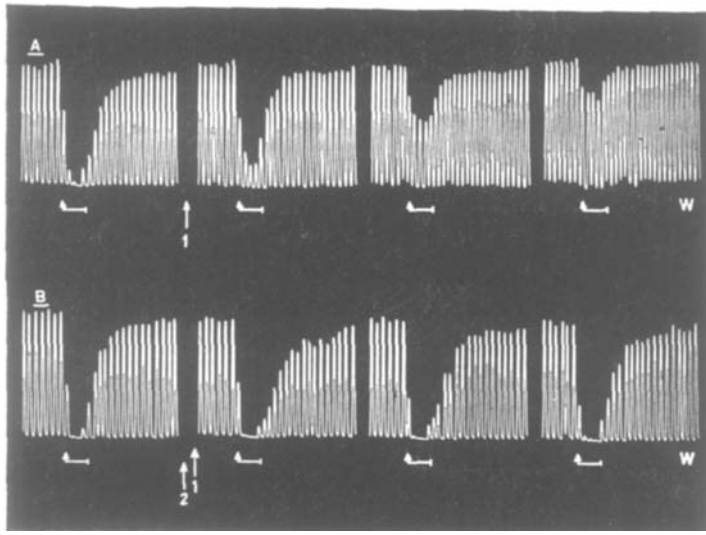

FIG. 4. Isolated rabbit intestine prepared according to Finkleman. In A and in B: responses of two preparations from the same animal to 30 sec. electrical stimulation of sympathetic postganglionic fibres (at arrows). Interval between each stimulation period, 15 min. In A: bretylium 10 μg./ml. (at 1) is left in contact with the drug until washing (at W). In B: same treatment as in A. At 2: progressive desensitisation to *N*-diphenylmethylatropine is developed and the preparation is left in contact with this drug at 15 μg./ml. Under such conditions, the effect of bretylium (10 μg./ml.) (at 1) fails to develop.

Interaction of Atropine and Adrenergic Neurone Blocking Agents in the Finkleman Preparation

The finding that the *N*-diphenyl derivative affected the responses to the sympathetic electrical stimulation made it the more interesting to investigate whether it or the other compounds under study altered the suppressive effect exerted by the adrenergic neurone blocking agents on the inhibition of spontaneous contractions induced by sympathetic stimulation. To elucidate this point two groups of experiments were made.

In the first, the Finkleman preparation was exposed to increasing concentrations of atropine or quaternary compounds, care being taken that the motor activity was no longer affected; then the responses to the appropriate stimulation were standardised and, in the presence of the anti-acetylcholine drugs, the treatment with the adrenergic neurone blocking agent was applied at such a concentration as to reduce the response by 80 per cent or more within 30–45 min. The agents used and their concentrations, μg./ml., were as follows: bretylium, 10–15; guanethidine, 5–7·5; xylocholine, 20; DMPP, 2·5–5; hemicholinium, 20–40.

The results obtained in about thirty preparations showed that among the derivatives under test, only *N*-diphenylmethylatropine antagonised the adrenergic neurone blocking effect; with atropine, in some experiments, an earlier onset of such an effect was observed, whereas *N*-methylatropine and the *N*-butylscopolamine derivative were completely ineffective. Fig. 4 illustrates the antagonistic activity of *N*-diphenylmethylatropine towards bretylium. In particular, it may be seen that the *N*-diphenyl derivative in the presence of bretylium, unlike the response previously demonstrated

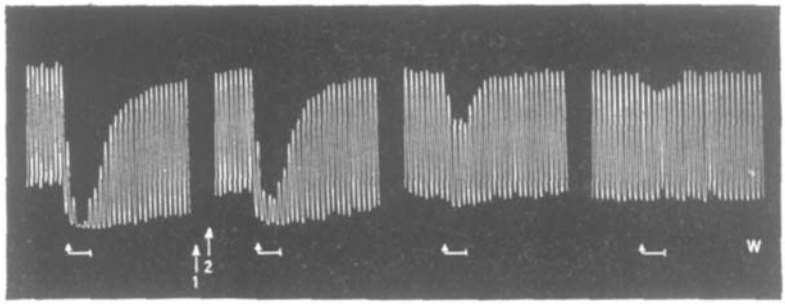

FIG. 5. Isolated rabbit intestine prepared according to Finkleman. Responses to 30 sec. electrical stimulation of sympathetic postganglionic fibres (at arrows). After desensitisation to *N*-diphenylmethylatropine, the preparation is treated with the same drug at 15 μg./ml. (at 1) and subsequently with 5 μg./ml. guanethidine (at 2). At W: prolonged washing. The guanethidine effect is not affected by the presence of *N*-diphenylmethylatropine.

in Fig. 2, does not enhance the postganglionic electrical stimulation effects. Furthermore we observed that the antagonism by *N*-diphenylmethylatropine of a nerve blockage does not persist on washing. In addition, guanethidine was the only adrenergic neurone blocking agent that was not antagonised (Fig. 5).

With the second group of experiments we wanted to investigate whether the adrenergic neurone-blocking effect would be more easily reversed on addition of the anti-acetylcholine drugs. Again, it was observed that *N*-diphenylmethylatropine was the only compound able to enhance the reversion of the sympathetic block, with the exception of the sympathetic paralysis caused by guanethidine.

Influence of N-*Diphenylmethylatropine on the Suppression by Adrenergic Neurone Blocking Agents of the Eserine Hypertensive Responses in the Rat*

Some experiments were performed in the intact rat to determine if *N*-diphenylmethylatropine, as demonstrated for cocaine (Della Bella and Gandini, 1962; Lešić and Varagić, 1961) (Fig. 6), could modify the inhibition by adrenergic neurone blocking agents of the eserine hypertensive responses (Varagić and Vojvodić, 1962). Atropine was not tested, since it is known to possess a direct antagonistic activity (Dirnhuber and Cullumbine, 1955), while, in our hands, *N*-diphenylmethylatropine, in doses up to 4–6 mg./kg. intravenously, does not significantly modify the hypertensive responses.

On the other hand, as previously described, the responses to eserine are much reduced after bretylium or guanethidine administration: this effect occurs in a few minutes and generally increases in the subsequent responses.

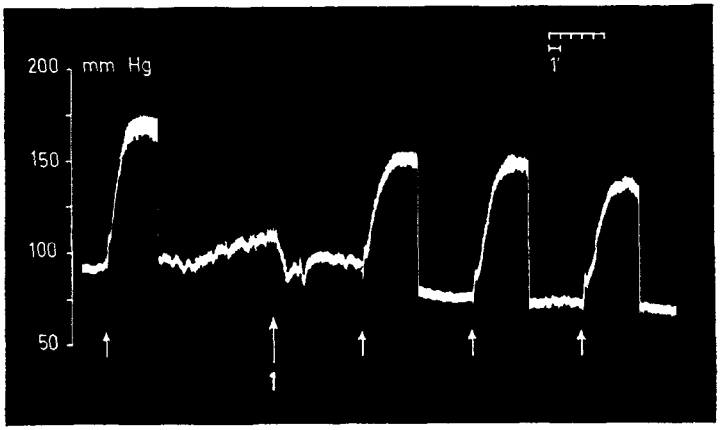

FIG. 6. Rat, 270 g. Recording of the carotid blood pressure. Pretreatment with 5 mg./kg. of cocaine given subcutaneously. At the arrows: eserine (150 μg./kg.) is injected into the jugular vein. At 1: bretylium (4 mg./kg.) i.v. Cocaine pretreatment counteracts the reduction of hypertensive responses by bretylium.

In contrast to the *in vitro* observations, no antagonism towards the adrenergic neurone blocking effect of bretylium was exhibited by *N*-diphenylmethylatropine (Fig. 7).

DISCUSSION

Our results clearly indicate that the effect of adrenergic neurone blocking agents can be effectively antagonised by a compound known to possess anti-acetylcholine properties.

Among the compounds tested, *N*-diphenylmethylatropine, a drug possessing atropine-like as well as ganglion-blocking activity on the peripheral, vagal and sympathetic synapses but devoid of direct adrenaline potentiating activity, when applied to the Finkleman preparation, appeared to be able to potentiate the effects of the electrical stimulation of the sympathetic postganglionic fibres, and also to prevent the sympathetic paralysis caused by adrenergic neurone blocking agents.

The lack of antagonism towards the suppression by bretylium of the eserine hypertensive responses *in vivo* might be ascribed either to the different animal species, or to a different body distribution of the two drugs, preventing *N*-diphenylmethylatropine from reaching effective concentrations at the sympathetic postganglionic neurone, where bretylium, on the contrary, is known to be selectively concentrated (Boura, Copp, Duncombe, Green and McCoubrey, 1960).

It is interesting to note that *N*-diphenylmethylatropine *in vitro* was able to antagonise only those compounds possessing a quaternary nitrogen, namely xylocholine, bretylium, hemicholinium, DMPP, whereas no antagonism towards guanethidine was observed. It has been already suggested that guanethidine may differ from the other agents in mechanism and site of action (Cass and Spriggs, 1961), and our experiments support this.

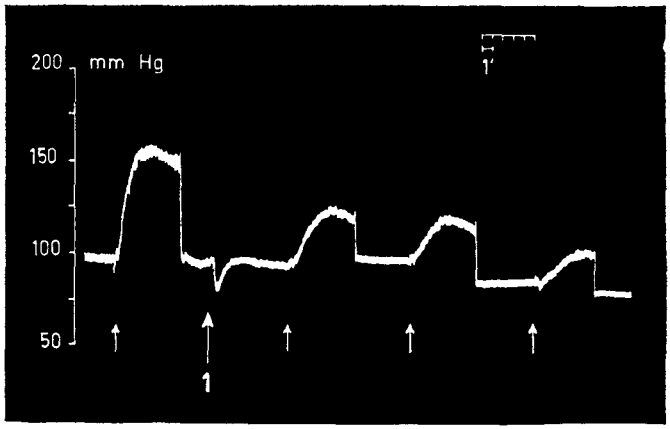

FIG. 7. Rat, 310 g. Recording of the carotid blood pressure. Pretreatment with 6 mg./kg. of *N*-diphenylmethylatropine, i.v. in three divided doses. At arrows: eserine (180 μg./kg.) is injected into the jugular vein. At 1: bretylium (4 mg./kg.) i.v. A considerable reduction of the hypertensive responses occurs, in spite of the *N*-diphenylmethylatropine pretreatment.

Perhaps the most interesting point emerging from our experiments is that it is possible to antagonise the adrenergic neurone blocking effect with a compound whose structure and properties are clearly different from those possessed by the antagonists considered so far and represented, as it is known, by sympathomimetic amines, or by drugs interfering with their metabolic fate such as the monoamine oxidase inhibitors (Day, 1962; Bain, 1960; Boura and Green, 1959; Wilson and Long, 1960).

On the basis of current structure-activity considerations and of available data, *N*-diphenylmethylatropine is a drug prevailingly anti-acetylcholine in its action, and this may represent an indirect argument in favour of the hypothesis of a cholinergic mechanism being involved in the liberation of the sympathetic mediator at the sympathetic postganglionic neurone endings.

By analogy to that suggested by Day (1962) for sympathomimetic amines, a possible explanation of the antagonism towards the adrenergic nerve blocking agents shown by *N*-diphenylmethylatropine might be found in the work of Stephenson (1956) on the mechanism at play in drug antagonism: i.e. it might be that both the agents and *N*-diphenylmethylatropine have an affinity for the sympathetic postganglionic nerve endings and that only their efficacy is different.

Finally we would like to call the attention to the rapid decrease in sensitivity of the rabbit ileum to the direct motor inhibitory effects of atropine. The fact that such desensitisation appeared also towards the quaternary nitrogen derivatives of atropine and scopolamine, despite their different pharmacological properties, thus behaving like a cross-desensitisation, raises the problem of a common denominator in the phenomenon, the meaning of which is worth investigating.

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