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THE ACTION OF NICOTINE ON THE CIRCULAR MUSCLE OF THE HUMAN ILEUM AND COLON *IN VITRO*

BY

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Nicotine is known to cause contraction of isolated gut preparations and it is believed that this action is the result of stimulation of cholinergic ganglion cells in the myenteric plexus. However, there have been various reports of an inhibitory action of nicotine. Magnus (1905) reported a transient inhibition of the small intestine of the cat which was not followed by contraction. Rabbit small intestine was inhibited by nicotine when the cholinergic fibres had been inactivated by botulinum toxin and this was attributed to the presence of inhibitory cells in the myenteric plexus (Ambache, 1951). The same response was observed when the effects of the cholinergic nerves had been blocked by atropine (Lévy & Michel-Ber, 1953). However, Ambache & Edwards (1951) failed to demonstrate the inhibitory action of nicotine in atropinized rabbit intestine.

Gillespie & MacKenna (1960), using a rabbit colon preparation, reported that nicotine in low doses evoked an inhibitory response which was enhanced after atropine. They attributed this to the release of catechol amines either from the extrinsic sympathetic nerves or from some structure associated with them, and they suggested the possibility that nicotine had acted on some form of terminal sympathetic nerve network intervening between the sympathetic nerves and the smooth muscle.

The longitudinal muscle of the rabbit muscular organ (ileo-colic sphincter) has also been shown to relax in the presence of nicotine (Jarrett, 1962). It was thought that this effect was brought about by the release of catechol amines, the site of action being a cholinergic junction between sympathetic nerve and catechol amine store.

The effect of nicotine on the small intestine of the rat was investigated by Lévy & Michel-Ber (1953). An inhibitory response was demonstrated in most experiments, sometimes without using atropine. However, they were unable to obtain this response in the guinea-pig ileum even when atropinized.

The inhibitory action of nicotine on the circular muscle of the human colon was first described by Fishlock & Parks (1963). It has also been reported that the longitudinal muscle of this region relaxes in the presence of nicotine and dimethylphenylpiperazinium (Bucknell & Whitney, 1964). This paper describes the further investigation of this response of colonic circular muscle and includes a description of the action of nicotine on the circular muscle of the human ileum.

METHODS

Circular muscle strips about 20 mm long by 2 mm wide were taken from the human sigmoid colon and the terminal ileum (last 0.6 m). Two methods of obtaining strips were used. Firstly, small segments of bowel were taken from operation specimens, as soon as possible after resection, and immersed in a modified Krebs solution (described below) at room temperature. The strips were dissected from the segments in the laboratory. In this method there was some delay in setting up the strips in the organ-bath, which varied from 10 to 60 min. However, throughout most of the delay the gut had been kept in a modified Krebs solution. Secondly, to avoid the delays involved, muscle strips were dissected from the human bowel at operation and put directly into the modified Krebs solution by the surgeon before the resection had commenced. Using this method most strips were set up in the organ-bath in under 10 min from removal from the patient.

The colonic strips consisted of full thickness of bowel wall taken from between the taeniae coli of the sigmoid colon. Some strips had the mucosa and submucosa dissected off, others did not. The thickness of the strips without the mucosa was about 2 mm. These strips were taken from patients who were undergoing bowel resection for carcinoma of the colon or rectum. The strips from the terminal ileum also consisted of full thickness of bowel wall, some of which had the mucosa removed, and these were taken from patients with carcinoma of the right side of the colon, polyposis coli or Crohn's disease, not affecting the region used. The muscle was regarded as healthy if there was no obstruction to the bowel and if the region from which the strip was taken was both macroscopically and histologically normal.

The strips were set up in an isolated-organ bath in modified Krebs solution equilibrated with 95% oxygen and 5% carbon dioxide at 37° C and placed under a tension of about 2 g. In order to obtain a steady baseline it was found necessary to vary slightly the tension from strip to strip, the range of variation being 1 to 3 g. The preparation was left for about 30 min by which time spontaneous activity had developed. Recording was made on a smoked drum with a direct-writing isotonic lever and a vibrator was used to overcome friction (Bülbring, Crema & Saxby, 1958).

The solution used contained (mM): Na 140, K 5.9, Ca 2.5, Mg 1.2, Cl 122, HCO_3 25, H_2PO_4 1.2, SO_4 1.2 and dextrose 11.5.

The following drugs were used: acetylcholine chloride, adrenaline acid tartrate (–)-noradrenaline bitartrate, nicotine hydrogen tartrate, choline phenyl ether bromide, ββ-dimethylacryloylcholine iodide, hexamethonium bromide, dichloroisoprenaline, pronethalol, bretylium tosylate (Darenthin ampoules, Burroughs Wellcome), guanethidine hemisulphate (Ismelin ampoules, Ciba), procaine hydrochloride and physostigmine sulphate. Concentrations refer to the salts. All solutions were made with physiological 0.9% saline except for the solutions of adrenaline and noradrenaline, which were made with acidified (hydrochloric acid) 0.9% saline.

Premedication and anaesthesia of patients

The preoperative medication given to the patients varied. The majority received a combination of atropine (0.6 mg), or scopolamine (0.5 mg) with Omnopon (20 mg) or morphine (16 mg). Induction of anaesthesia was carried out with intravenous thiopentone sodium (300 to 600 mg) and sometimes succinylcholine chloride (30 to 50 mg) was also given. Anaesthesia was maintained with nitrous oxide, sometimes with the addition of halothane, and relaxation was obtained with 25 to 50 mg of tubocurarine chloride given in divided doses. The premedication was given about 1 hr before the start of the operation and the muscle strips were obtained 2 to 3 hr after the premedication.

Several patients had epidural anaesthesia without atropine.

RESULTS

Muscle strips with mucosa responded in the same way as those without. The sensitivity and behaviour of the strips did not vary with the different premedications and anaesthetic agents used.

Colon

Ninety strips from sixty-one patients were used. Acetylcholine, 50 ng/ml. and above,

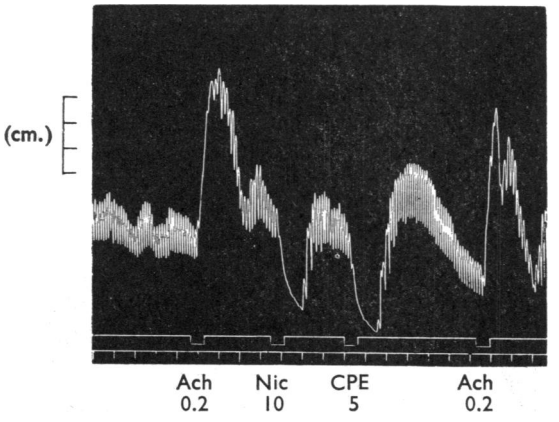

Fig. 1. Circular muscle sigmoid colon preparation: nicotine and choline phenyl ether cause relaxation. Ach = acetylcholine; Nic = nicotine; CPE = choline phenyl ether; concentrations in μg/ml. Strip length 22 mm; lever magnification $\times 7$; tension 2 g. Time marks, 1 min.

caused contraction but occasionally the preparation was more sensitive (10 ng/ml.). Physostigmine (0.5 μg/ml.) potentiated this contraction approximately tenfold.

Nicotine, 2 μg/ml. and above, regularly caused relaxation and inhibition of spontaneous activity (Fig. 1). Concentrations below this had no effect, whilst those above 20 μg/ml. produced a maximal response. Nicotine, 50 μg/ml., blocked the response to further doses of nicotine. No strip contracted during exposure to nicotine, 0.01 to 100 μg/ml., but an increase in tone and spontaneous activity after removal of the drug did occur sometimes. It should be pointed out that drugs are eliminated from the organ-bath by overflow wash-out and that by this method there is very little disturbance to the muscle strip. A contraction following wash-out has been described after the inhibitory action of histamine on the human colon, but not after the inhibitory action of 5-hydroxytryptamine (Fishlock & Parks, 1963).

Choline phenyl ether is one of the most active nicotinic substances known (Hey, 1952). It regularly caused relaxation of the preparation in concentrations of 1 μg/ml. and above (Fig. 1). Below this it had no effect. No contraction on exposure was obtained in any experiment, although an after-contraction was observed sometimes. Choline phenyl ether appeared to be about twice as active as nicotine. $\beta\beta$-Dimethylacryloylcholine has also been described as having nicotinic properties (Holmstedt & Whittaker, 1958). It caused relaxation in concentrations above 10 μg/ml. Again, no response was elicited below this concentration and no contraction was obtained in any experiment. A feeble after-contraction occurred sometimes.

Adrenaline and noradrenaline (50 ng/ml. and above), caused relaxation and inhibition of spontaneous activity. Dichloroisoprenaline, 25 μg/ml. for 2 min, effectively blocked this response. Pronethalol, 25 μg/ml. for 5 min or 10 μg/ml. for 30 min, also blocked this response. The inhibitory actions of nicotine, choline phenyl ether and $\beta\beta$-dimethylacryloylcholine were abolished by the same substances without unmasking any contraction response. Occasionally dichloroisoprenaline and pronethalol themselves caused inhibition of the preparation.

Hexamethonium, 30 to 50 μg/ml. for 5 min, completely blocked the inhibitory effect of

nicotine, choline phenyl ether and ββ-dimethylacryloylcholine. A concentration of 10 μg/ml. for 15 min only partially blocked the response. Hexamethonium did not affect the tone of the preparation.

Bretylium 40 μg/ml. for 30 min, completely blocked the response to nicotine (10 μg/ml.) without affecting the action of catechol amines. Concentrations of 25 to 40 μg/ml. produced a partial block only. In one experiment 100 μg/ml. failed to abolish completely the effect of nicotine. Bretylium tended to increase the tone of the preparation.

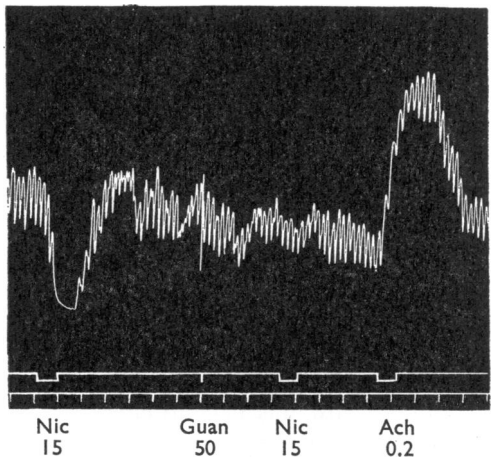

Fig. 2. Circular muscle sigmoid colon preparation: the relaxation caused by nicotine is blocked by guanethidine. Nic = Nicotine; Ach = acetylcholine; Guan = guanethidine; concentrations in μg/ml. Strip length 20 mm; lever magnification ×7; tension 2 g. Time marks, 1 min.

Guanethidine, 10 μg/ml. for 20 min, abolished the response to nicotine, 6 to 9 μg/ml. (Fig. 2). Concentrations below this gave only partial block. Guanethidine, 50 μg/ml. for 15 min, completely blocked nicotine, 10 to 15 μg/ml. It frequently reduced the tone of the preparation.

Procaine, 30 μg/ml. for 30 min, abolished the inhibitory action of nicotine. Several experiments had to be abandoned because of the prolonged inhibition caused by procaine.

Physostigmine, 0.5 μg/ml., although potentiating acetylcholine tenfold did not reveal any contraction in the presence of nicotine, 10 ng/ml. and above. Neither was the inhibitory effect of nicotine altered in any way.

Ileum

Fifteen strips from ten patients were investigated. The ileum was usually more sensitive to acetylcholine than the colon and contracted in the presence of 1 to 10 ng/ml. Several strips were similar to the colon in sensitivity.

Nicotine (2 μg/ml. and above) (Fig. 3), choline phenyl ether (0.5 μg/ml. and above) and ββ-dimethylacryloylcholine (5 μg/ml. and above) all caused relaxation and inhibition of spontaneous activity. No contraction was obtained with nicotine (0.01 to 100 μg/ml.). Nicotine, 50 μg/ml. blocked further responses to nicotine.

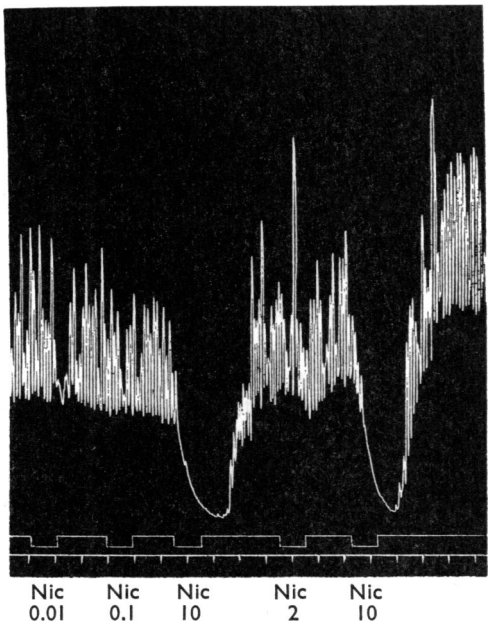

Fig. 3. Circular muscle terminal ileum preparation: nicotine causes relaxation. Nic = Nicotine; concentrations in μg/ml. Strip length 23 mm; lever magnification ×7; tension 1.5 g. Time marks, 1 min. The small response following 0.01 μg/ml. of nicotine occurred immediately on removal of the drug from the bath and is almost certainly a wash-out disturbance. The sensitivity of the preparation does vary slightly and this may account for the absence of a response to 2 μg/ml.; it may be, however, that the higher concentration of 10 μg/ml. has inhibited the response.

The sensitivity of the ileum to adrenaline and noradrenaline was the same as the colon. Pronethalol, 30 μg/ml. for 5 min, blocked the inhibitory action of the catechol amines, nicotine, choline phenyl ether and ββ-dimethylacryloylcholine. Pronethalol did not unmask any parasympathetic action of the nicotinic substances.

Hexamethonium, 25 μg/ml. for 2 min, blocked nicotine (10 μg/ml.), choline phenyl ether (5 μg/ml.) and ββ-dimethylacryloylcholine (10 μg/ml.).

Procaine, 30 μg/ml. for 30 min, blocked nicotine (10 μg/ml.).

Although physostigmine, 1 μg/ml., greatly potentiated the effect of acetylcholine, no contraction was obtained with nicotine (0.1 to 5 μg/ml.). The inhibitory effect of nicotine was unaffected by the presence of physostigmine.

DISCUSSION

The structure and functional status of muscle strips

In this investigation strips of full thickness of bowel wall have been used, as well as strips without mucosa and submucosa. The myenteric plexus is situated between the longitudinal and circular muscle layers. Even in the colon, where most of the longitudinal muscle is concentrated into the taeniae, there is a thin investing layer of longitudinal muscle between. Histological examination of the muscle strips used has clearly demonstrated the presence of ganglion cells.

Strips of alimentary muscle have been used previously by many workers (Young, 1914; Evans & Underhill, 1923; Gasser, 1926). Harry (1963) described experiments on strip preparations of circular muscle of the guinea-pig ileum. Later, Brownlee & Harry (1963) compared the behaviour of circular and longitudinal muscle strips. They described the main difference of response in these two layers and concluded that the circular muscle strip was a useful preparation for studying the action of drugs on the nervous plexuses of the guinea-pig ileum.

The effects of premedication and anaesthesia

The most obvious criticism that can be made of this work relates to the unknown effect of the premedication and anaesthesia. Some of the agents used may cause a partial cholinergic or ganglionic blockade. Despite the variety of drugs used, the sensitivity of the strips to acetylcholine remained remarkably constant. In general, strips from all regions contracted to acetylcholine in doses of 50 ng/ml. and above; occasionally strips were even more sensitive (10 ng/ml.). The concentration of atropine in the tissues 2 or 3 hr after a total body dose of 0.6 mg must be very small, much smaller in fact than the concentration found necessary by Gillespie & MacKenna (1960) to block the parasympathetic effect of nicotine in the rabbit colon (0.1 μg/ml.).

Strips from patients anaesthetized spinally and not given atropine preoperatively responded to acetylcholine and nicotine in the same way as strips from patients fully premedicated and anaesthetized generally. We think it unlikely, therefore, that our preparation has been affected by the preoperative medication or the anaesthetic agents used.

The site of action of nicotine

It is of interest that nicotine, choline phenyl ether and $\beta\beta$-dimethylacryloylcholine cause relaxation of the circular muscle of the terminal ileum and colon; none of these drugs evokes a parasympathetic effect, even when there is adequate adrenergic blockade. Bucknell & Whitney (1964), using longitudinal muscle from the human colon, have found the same phenomenon.

Most authors are agreed that nicotine relaxes smooth muscle by causing the release of a catechol amine and our own experiments support this view. The site of action of nicotine, however, is in doubt. It is possible that nicotine is acting directly on sympathetic ganglion cells or on the peripheral nervous network or on both. Several agents antagonize the nicotine effect, for example hexamethonium, procaine, bretylium and guanethidine. The exact site of action of some of these substances is still in doubt, but it is unlikely that they all antagonize nicotine at the same point. Hexamethonium blocks ganglionic transmission. Procaine prevents conduction in nerve fibres. The precise action of bretylium and guanethidine is difficult to assess. Boura, Copp, Duncombe, Green & McCoubrey (1960) suggested that bretylium specifically anaesthetizes sympathetic postganglionic fibres. Cass & Spriggs (1961) reported that guanethidine inhibited the effect of sympathetic nerve stimulation. They suggested that the primary action of guanethidine is to produce a bretylium-like adrenergic block.

The nicotine substances choline phenyl ether and $\beta\beta$-dimethylacryloylcholine produced the same response as nicotine except that choline phenyl ether was approximately twice as powerful. Loewe & Puttock (1950) described the nicotinic action of choline phenyl ether

and claimed that the threshold dose for sympathetic ganglion stimulation is lower than that of nicotine. Holmstedt & Whittaker (1958) described the ganglion stimulating action of $\beta\beta$-dimethylacryloylcholine and stated that it had only a feeble activity on the guinea-pig ileum.

Our experiments suggest, therefore, the possibility that nicotine is stimulating sympathetic ganglion cells in the colon and ileum. Ganglion cells have been clearly demonstrated in the strip preparations and no parasympathetic effect has been seen in these organs. It may be argued that premedication and anaesthesia induce a parasympathetic blockade accounting for our results. However, in a preliminary investigation of the longitudinal muscle strip of the human jejunum we have found that nicotinic compounds do cause contraction which appears to be cholinergic. If for any reason, such as premedication or anaesthesia, the parasympathetic ganglion cells in the colon and ileum have been rendered insensitive it is necessary to explain why the jejunum has not been affected in a similar way.

The nerve plexuses of the human colon and terminal ileum

No pharmacological evidence for the presence of parasympathetic ganglion cells has been obtained in the colon. Bucknell & Whitney (1964) came to a similar conclusion, using a taenia coli preparation. The same may be true for the terminal ileum but investigation of the longitudinal muscle has not yet been reported.

If there are no parasympathetic cells in the intrinsic plexuses of the human colon the problem arises as to where they are situated. It is possible that they are in the extrinsic nerves themselves. Recently, Vogt (1963) has demonstrated ganglion cells in the core of the hypogastric nerve of the dog. Further investigation of the pathways of the parasympathetic supply to the human colon is required. If our interpretation is correct it seems likely that the composition of the myenteric plexus varies from one region of the alimentary tract to another.

SUMMARY

1. Circular muscle strips were taken at operation from the human terminal ileum and sigmoid colon. They were immersed in modified Krebs solution in an isolated-organ bath at 37°C.

2. The response of these strips to nicotine, choline phenyl ether and $\beta\beta$-dimethylacryloylcholine was recorded.

3. Strips from both regions relaxed in the presence of these substances. No cholinergic response was observed even when physostigmine was present.

4. The relaxation of the colonic strips was antagonized by β-receptor blocking agents (dichloroisoprenaline and pronethalol), hexamethonium, bretylium, guanethidine and procaine.

5. The relaxation of the strips of ileum was antagonized by β-receptor blocking agents, hexamethonium and procaine.

6. The structure and function of the muscle strips are discussed.

7. The possible effects of premedication and anaesthesia are also discussed.

8. It is concluded that nicotine causes the release of a catechol amine and that the probable site of its action is on the sympathetic ganglion cells in the myenteric plexus.

9. The apparent absence of parasympathetic cells in the wall of the colon is discussed.

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