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ACTION OF NICOTINE ON THE RABBIT MUSCULAR ORGAN (ILEO-COLIC SPHINCTER)

BY

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The effects of nicotine on the longitudinal muscle of the rabbit muscular organ (ileo-colic sphincter) have been studied. Nicotine in doses from 1 to 10 μg/ml. inhibits the pendular movements of the muscle. This inhibition can also be produced by acetylcholine in the presence of atropine. In both instances, the inhibitory effect can be blocked by ephedrine, hexamethonium and bretylium. Doses of hexamethonium which block the inhibitory action of nicotine reveal an excitatory action, which itself can be blocked by higher doses of hexamethonium. Bretylium blocks both actions of nicotine at the same dose level. It is suggested that nicotine produces inhibition by releasing catechol amines, the site of action being a cholinergic junction between sympathetic nerve and catechol amine store, and further that bretylium blocks the action of acetylcholine and nicotine at this site as well as at the parasympathetic ganglia.

In a study of the ileo-caeco-colic region in various animals it was noted that longitudinal muscle from the rabbit muscular organ, which is the equivalent of the ileo-colic sphincter in other mammals, was consistently inhibited by nicotine. This is in contrast to most isolated intestinal preparations, in which the response to nicotine is one of muscle contraction. However, in 1905, Magnus noted a transient inhibition not followed by contraction, and a number of authors have noted muscular paralysis following the initial stimulation (Feldberg & Lin, 1949; Emmelin & Feldberg, 1947; Ambache & Rocha é Silva, 1951). The contraction produced is generally held to be due to stimulation of the ganglion cells of Auerbach's plexus, although Evans & Schild (1953) did obtain contractions using preparations, free from ganglion cells, of the circular muscle from the cat duodenum. They also observed paralysis following contraction in these preparations. These authors cited the findings of Langley (1905) and Bauer (1928), who found that nicotine could affect the rhythmic activity of chick amnion, which contains no nervous tissue, in support of the theory that nicotine may have a direct action on the muscle cell. Evans & Schild, however, did not state whether the effect of nicotine which they observed could be blocked by hexamethonium or by large doses of nicotine.

Ambache (1951) and Ambache & Edwards (1951) demonstrated an inhibitory effect of nicotine on rabbit small intestine, in which the cholinergic fibres had been inactivated by botulinum toxin, and on strips of kitten small intestine and stomach after cholinergic block by atropine. The latter drug did not, however, block the stimulant effect of nicotine upon the rabbit ileum. Gillespie & MacKenna (1960)

demonstrated an inhibitory effect of nicotine on the rabbit colon when used in small doses or in atropinized preparations. Larger doses of nicotine produced a biphasic reaction or stimulation alone.

Denys, Levy & Michel-Ber (1960), using isolated rat duodenum, demonstrated that nicotine caused a biphasic reaction of initial inhibition followed by stimulation.

In all of these preparations, the effect of nicotine could be diminished or prevented by previous treatment with drugs which also antagonized the effect of noradrenaline and adrenaline, for example, ephedrine and dihydroergotamine, or by previous treatment of the whole animal with reserpine.

The purpose of this investigation was to study, in more detail, the effect of nicotine on the rabbit muscular organ.

METHOD

Most of the specimens were taken from animals under pentobarbitone anaesthesia. A few were taken from animals killed by a blow on the neck. No difference was observed between the behaviour of preparations taken in either of these ways. Immediately on removal the organ was placed in Tyrode solution, opened and washed, and strips cut in a longitudinal direction. Usually the mucosa was left, but in a number of experiments it was scraped off with a sharp scalpel. The strips were then suspended in a 50 ml. organ bath in Tyrode solution at 37° C and a mixture of 95% oxygen and 5% carbon dioxide bubbled through. The composition of the Tyrode solution was, per litre: sodium chloride 8 g ; potassium chloride 0.2 g ; calcium chloride 0.2 g ; magnesium chloride 0.1 g ; sodium bicarbonate 1 g ; sodium dihydrogen or the phosphate 0.05 g ; glucose 1 g (Na 149 mM, K 2.7 mM, Ca 1.8 mM, Cl 145.2 mM, HCO_3 11.9 mM, H_2PO_4 0.3 mM, glucose 5.6 mM).

Preparations were stained for chromaffin tissue using the Sevki method, described by Adams-Ray & Nordenstam (1956).

RESULTS

Nicotine

Nicotine in concentrations from 1 to 10 μg/ml. caused inhibition of the pendular movements of the longitudinal muscle with little or no perceptible decrease in tone. The effect was similar to that occurring after the administration of adrenaline or noradrenaline, except that, in the case of nicotine, pendular movements would begin again even before washing out the nicotine, whereas, with both adrenaline and noradrenaline, washing was necessary before movements began again. This effect is in contrast to that on rabbit ileum, where nicotine normally produces a contraction, and rabbit colon, where nicotine produces inhibition only in low doses, larger doses resulting in a contraction (Gillespie & MacKenna, 1960). The effect of nicotine was identical in mucosa-free preparations.

When repeated small doses (2 μg/ml.) of nicotine were given, however, with thorough washing between each administration, the response of the preparation gradually changed. At first there was inhibition followed by contraction, and, as further doses were given, the height of the contraction increased, until eventually only a contraction was evoked (Fig. 1). When large doses (more than 10 μg/ml.) of nicotine were given, subsequent doses of nicotine had no effect.

In the study of the interaction of nicotine with other drugs, the dose of nicotine used, except where otherwise stated, was 2 μg/ml.

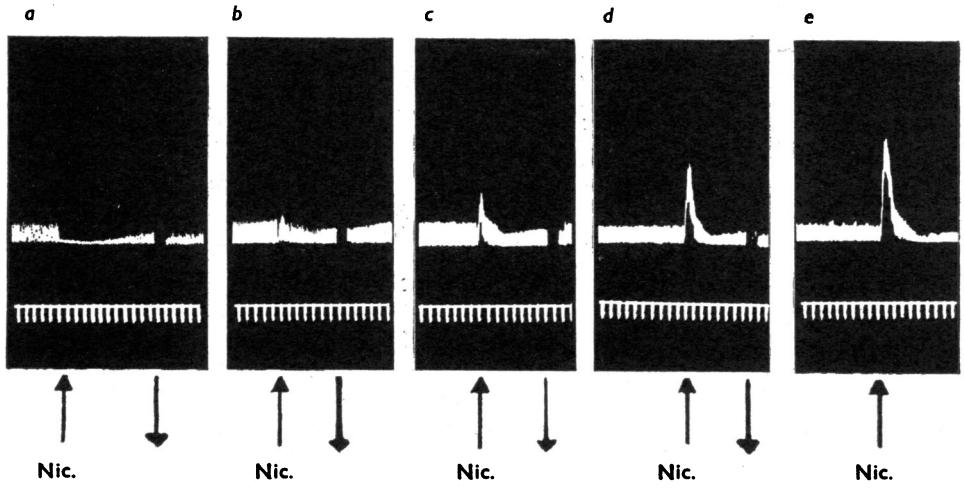

Fig. 1. Showing the change in response of the preparation to repeated doses (2 μg/ml.) of nicotine. *a*—The initial inhibitory response. *b*—At the fifth administration, showing inhibition followed by a small contraction. *c*—At the tenth administration, showing transient inhibition followed by a contraction. *d* and *e*—With further doses, the contractions become greater and are not preceded by inhibition. Time, 30 sec.

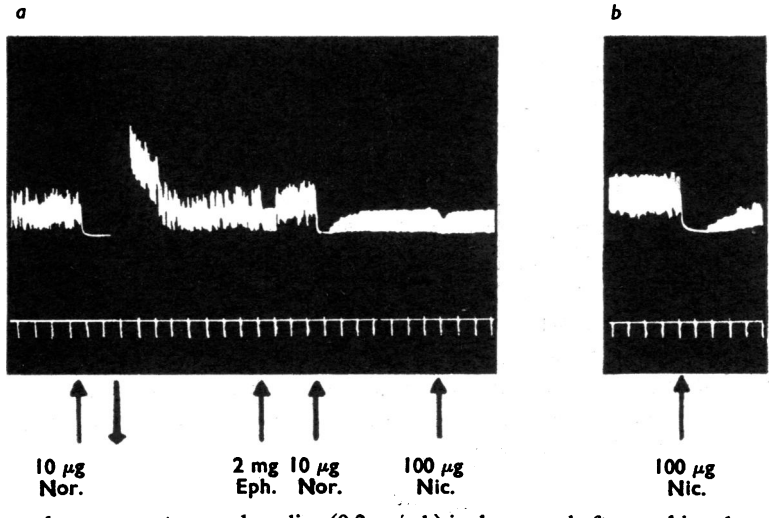

Fig. 2. In *a* the response to noradrenaline (0.2 μg/ml.) is shown and after washing the response to noradrenaline (0.2 μg/ml.) and nicotine (2 μg/ml.) after the administration of ephedrine (40 μg/ml.); *b* shows the response to nicotine in the same preparation after washing. In *a*, a considerable increase in tone is seen following the washing out of noradrenaline. Time, min.

Ephedrine

Curtis (1929) showed that ephedrine could be used *in vitro* as an antagonist of adrenaline. Ambache (1951) showed that the inhibitory action of nicotine on the

isolated intestine could be prevented by pretreatment with large doses of ephedrine. The response of the rabbit muscular organ to large doses of ephedrine (more than 40 μg/ml.) was variable. Usually there was no response, but occasionally inhibition occurred. In either case, the inhibitory effect of subsequent administration of nicotine was diminished or abolished (Fig. 2).

Similarly, ephedrine blocked or diminished the inhibitory actions of noradrenaline and adrenaline and the inhibitory action of acetylcholine in the presence of atropine.

Bretylium

Boura & Green (1959) showed that bretylium blocked the effects normally seen following stimulation of post-ganglionic nerves of the sympathetic nervous system, an effect which persisted after washing the preparation several times. They also demonstrated a readily reversible blockade of the superior cervical ganglion of the cat. The dose of bretylium required to abolish the inhibition produced by nicotine on the rabbit muscular organ was high, 30 to 40 μg/ml. However, three changes of the bath fluid were sufficient to remove immediately the blocking action of bretylium. A similar dose of bretylium also blocked the excitatory effect of nicotine in those preparations where the response to nicotine had changed to contraction (Fig. 3).

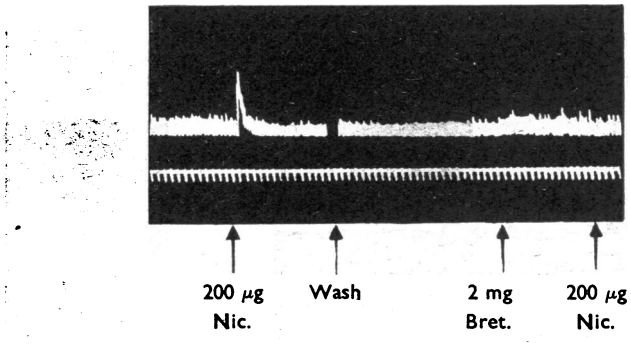

Fig. 3. In this preparation the response to nicotine has changed to contraction. Following the administration of bretylium (40 μg/ml.) nicotine no longer evokes a response. Time, 30 sec.

Hexamethonium

Hexamethonium decreased the inhibitory effect of nicotine and in a dose varying from 30 to 40 μg/ml. blocked it completely. Further increases in the dose of hexamethonium resulted in a change in the response to nicotine, a contraction occurring instead of inhibition. On washing out the hexamethonium, the inhibitory response could again be obtained. When the dose of hexamethonium was again increased, the height of the nicotine-induced contraction also increased to a peak, following which further increases in the dose of hexamethonium diminished the contraction produced by nicotine and eventually abolished it. In order to achieve this latter effect, much higher doses of hexamethonium, from 100 to 200 μg/ml., were required (Figs. 4 and 5).

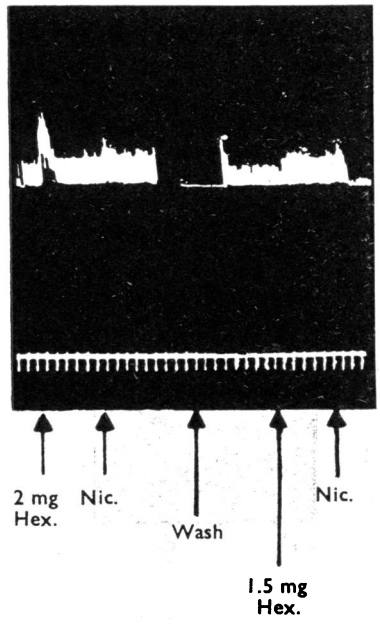

Fig. 4. After the administration of hexamethonium (40 μg/ml.), nicotine evokes a small contraction. After washing, hexamethonium (30 μg/ml.) is added and, following this smaller dose, nicotine still produces inhibition. Time, 30 sec.

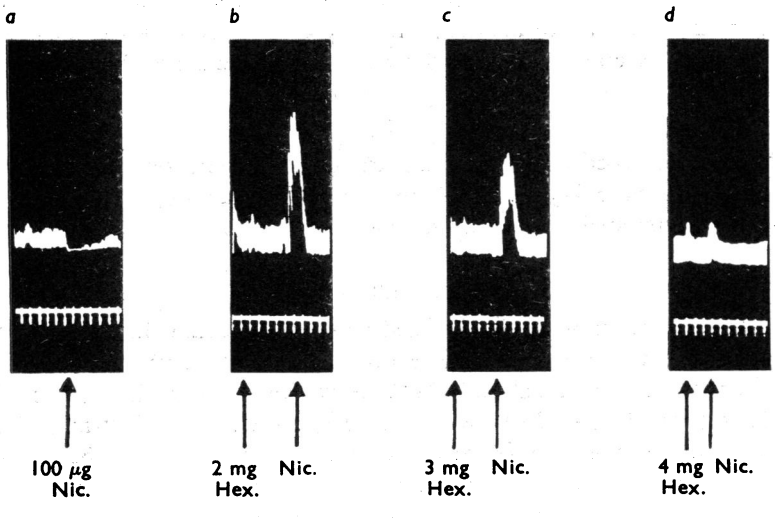

Fig. 5. In *a*, the normal response to nicotine. In *b*, nicotine following the administration of hexamethonium (40 μg/ml.) evokes a contraction. In *c* and *d*, larger doses of hexamethonium diminish the excitatory effect of nicotine. Time, 30 sec.

Atropine

Atropine in doses of 1 to 6 μg/ml. did not affect the inhibitory response to nicotine. However, the atropinized preparation responded to large doses (10 to 20 μg/ml.) of acetylcholine in the same way as to nicotine (see Fig. 6). This inhibitory effect of acetylcholine could be blocked by doses of ephedrine, hexamethonium and bretylium of the same order as those which blocked the inhibitory

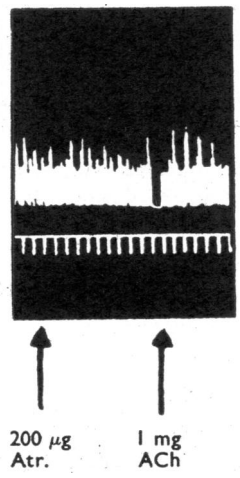

Fig. 6. After the administration of atropine (4 μg/ml.), acetylcholine (20 μg/ml.) produces an inhibitory effect. Time, 30 sec.

effect of nicotine. In preparations in which the response to nicotine had become a contraction, the response to acetylcholine in the presence of atropine was also a contraction, and this could be blocked by hexamethonium and bretylium.

Histology

Careful search of sections stained for chromaffin tissue revealed none in the vicinity of the muscular layers of Auerbach's plexus, although chromaffin tissue was readily demonstrated in colonic mucosa.

DISCUSSION

In addition to inhibition of the isolated intestine, nicotine has been shown to produce effects due to the release of catechol amines in a number of organs (Hoffman, Hoffman, Middleton & Talesnik, 1945 ; Hawkins & Paton, 1958 ; Burn, Leach, Rand & Thompson, 1959). In several instances, the effect of nicotine and that of acetylcholine, in the presence of atropine, was the same. This has now been shown to be true of the isolated rabbit muscular organ. In this preparation the inhibitory effect of both nicotine and of acetylcholine in the presence of atropine can be blocked by hexamethonium and bretylium. These drugs also block the stimulant effect of nicotine and acetylcholine in those preparations where the response has changed to contraction.

The possible sites of the inhibitory action of nicotine are (1) directly on the muscle cell; (2) on ganglion cells of short adrenergic neurones; (3) on the terminations of sympathetic nerves; (4) on the postulated junction between sympathetic nerve ending and noradrenaline store (Burn, 1961); and (5) directly on chromaffin tissue. As no chromaffin tissue could be demonstrated in, or adjacent to, the muscle layers, the latter explanation is unlikely. As acetylcholine, in the presence of atropine, has the same effect as nicotine, a direct action on the muscle cell also seems unlikely, unless one is prepared to postulate both a stimulant and inhibitory effect of acetylcholine on the muscle cell. It is more reasonable to assume that the drugs are acting on nervous tissue.

If there were ganglion cells of adrenergic neurones in the gut wall, it would be reasonable to expect that they would exhibit a similar sensitivity towards the blocking action of hexamethonium and repeated doses of nicotine in the ganglion cells of the cholinergic neurones, at which the stimulant action of nicotine is presumably initiated. That this is not so argues against, but does not of course exclude, this explanation. However, the results can most readily be explained in the light of the hypothesis of Burn & Rand (see Burn, 1961, for fuller discussion) of a junction between adrenergic nerve and noradrenaline store, with acetylcholine as the transmitter.

If this theory is correct, this junction has many of the properties of a ganglion, in that it is stimulated by nicotine and by acetylcholine in the presence of atropine and this stimulation can be blocked by hexamethonium but not by atropine. In the isolated guinea-pig auricle (Giotti, 1954) and the isolated rabbit muscular organ, the sympathetic junction is more sensitive to the blockade by hexamethonium of nicotine stimulation than the parasympathetic ganglion.

Bretylium, however, in the rabbit preparation, blocked both actions of nicotine in similar concentration. Huković (1960) showed that bretylium could block the effects of both vagal and sympathetic nerve stimulation on isolated atria and also that it could block the constrictor action of acetylcholine, in the presence of atropine, on the vessels of the perfused rabbit ear. Kosterlitz & Lees (1961) demonstrated a reversible inhibitory effect of low concentrations of bretylium on the peristaltic reflex of the isolated guinea-pig ileum similar to that seen with ganglion blocking drugs. Boura & Green (1959) had originally demonstrated that bretylium could block the superior cervical ganglion of the cat, an effect which was also readily reversible. It appears, therefore, that bretylium blocks the effects of acetylcholine and nicotine both at ganglia and the hypothetical junction at the termination of the sympathetic nerves. The fundamental action of bretylium may be akin to that of hexamethonium, its selective action in the intact animal being due to its selective distribution, which Boura, Copp, Duncombe, Green & McCoubrey (1960) demonstrated. Boura & Green (1959) did, in fact, note some block of parasympathetic effects in the intact animal, but only in high dosage. However, if the distribution is so unequal, it is hardly surprising that the blockade of the parasympathetic is relatively weak. Bretylium thus becomes a further antagonist of acetylcholine, its distinctive effect in the intact animal being due to its selective distribution.

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