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INTERACTION OF NICOTINE AND ESERINE, EPHEDRINE, ATROPINE, HEXAMETHONIUM, AND ADRENALINE IN ISOLATED GUINEA-PIG AURICLES

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The mixed effect (inhibition and stimulation) of nicotine on isolated hearts is well known. That nicotine acted on nervous tissue was shown by the antagonistic effect of apocodeine (Dixon, 1920), and confirmed by experiments on isolated rabbit auricles (Gronchi, 1939). During experiments planned to study the action of various drugs on the mechanical and electrical behaviour of isolated guinea-pig auricles, we observed that hexamethonium completely antagonized nicotine ; this was a further confirmation of Dixon's interpretation. There were, however, some minor points which seemed to deserve further investigation, such as the specificity of the action of hexamethonium on nervous tissues in the concentrations which antagonize nicotine ; whether chemically different ganglionic blocking agents also antagonize nicotine ; the effect on the action of nicotine of pretreatment with anticholinesterase drugs (which should potentiate the inhibitory action) and with ephedrine (which should antagonize the stimulant action) ; and the mechanism of the progressive change of auricular response to nicotine in relation to the duration of survival of the preparation. During these studies nicotine revealed itself as an excellent compound for investigating whether the autonomic nervous system participates, as was presumed, in the progressive exhaustion of the auricles, and whether there exist compounds able to restore its excitability. Adrenaline proved very effective in restoring the inhibitory action of nicotine.

In the meantime a paper was published on the stimulation of isolated rabbit auricles by substances which stimulate ganglia (Kottegoda, 1953), in which the antagonistic action of hexamethonium to nicotine stimulation was clearly illustrated and its mechanism discussed. A comparison of Kottegoda's results and mine showed that rabbit and guinea-pig auricles react very similarly to nicotine and hexamethonium. The reader is therefore referred to Kottegoda's paper for qualitative information about the hexamethonium - nicotine antagonism.

METHODS

Auricles were isolated from guinea-pigs weighing 300–400 g. and suspended horizontally in a bath containing well-oxygenated Ringer solution, at 29° C. (Giotti, 1953). The contractions were recorded isotonically under slight tension. Although the auricles contract vigorously immediately after immersion in the bath, experiments were not usually started until one hour later. The measurement of drug effects required that the preparation should be in a steady state ; this was maintained, despite frequent washings, for many hours. Guinea-pig auricles were selected, instead of the more commonly used rabbit auricles, on the assumption that the smaller wall thickness would allow better diffusion of drugs and metabolites. Diffusion is better (Schmid, Siess, and Bühler, 1952) if auricles of young guinea-pigs are used. No differences were observed between the actions of freshly prepared solutions of " Nicotinum purissimum Merck " neutralized with 0.01 N HCl and dissolved in the buffered perfusion fluid, of the same solution purified according to Forst's procedure (1943), and of freshly prepared solutions of nicotine acid tartrate whether treated or not treated with charcoal. This agrees with the observations of Larson, Finnegan, Van Slyke, and Haag (1950). Doses are expressed as the final concentration in g./ml. in the bath fluid.

RESULTS

Action of Nicotine on Untreated Fresh Auricles. —Nicotine 2×10^{-8} has no effect on the rate and increases slightly, but inconstantly, the amplitude of contraction, as observed over a period up to 20 min. Effective concentrations lie between 2×10^{-7} and 2×10^{-6}. The action appears with a short latency (8–10 sec.) ; reduction of rate and amplitude predominate at first, but later there is usually an increase of amplitude. Nicotine 2×10^{-6} admin-

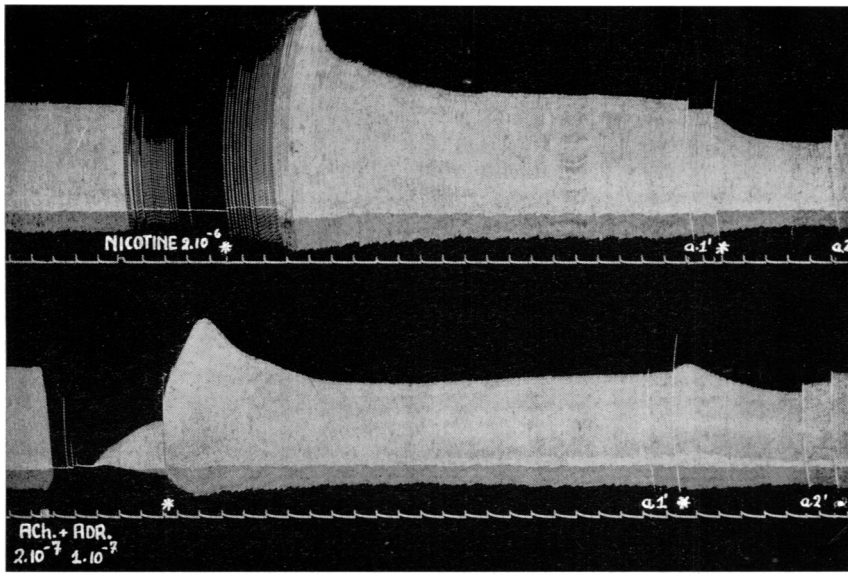

FIG. 1.—Isolated guinea-pig auricles. Similarity in the effects of nicotine (upper tracing) and acetylcholine+adrenaline (lower tracing). Washings are indicated by *. Time, 20 sec. a.1, recording stopped for 1 min.

istered repeatedly gives reproducible results if the administrations are interrupted by washings, and exposure is not prolonged beyond 10 min. If the auricles are left in contact with higher concentrations, and for longer periods, the response may become progressively smaller. The effect of washing differs according to the phase of nicotine action. During inhibition, washing produces a large increase of rate and amplitude; during stimulation it causes a decrease of amplitude, and an increase, or no change, of rate. The course and quality of the action of nicotine, and of subsequent washing, may be almost exactly duplicated by the administration of an appropriate combination of ACh and adrenaline (Fig. 1).

Action of Nicotine on Fresh Auricles After Treatment with Other Drugs.—The unmasking or potentiation of the stimulant action of nicotine by atropine is well known. Fig. 2 shows the potentiation by eserine of the decrease of rate and amplitude caused by nicotine. This potentiation and its antagonism by atropine point to an effect mediated through ACh.

The possibility that the stimulant effect of a drug on an isolated heart may be mediated through the liberation of sympathin-like substances has been substantiated by much experimental evidence. Fig. 3 shows that ephedrine antagonizes the stimulant effect of nicotine after atropine, just as it antagonizes the similar effect of adrenaline. Competition between ephedrine and sympathin for common receptors seems an adequate explanation.

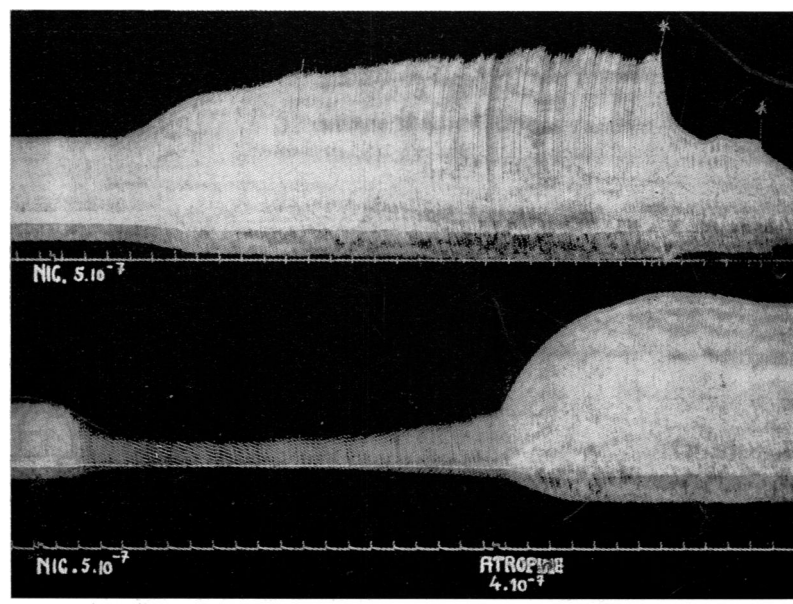

FIG. 2.—Isolated guinea-pig auricles. Interaction between nicotine, eserine, and atropine. The upper record shows the action of nicotine on untreated auricles; the lower record shows that nicotine after eserinization (eserine sulphate 2×10^{-8} in the bath) has an inhibitory action which is reversed by atropine. Interval between eserine and nicotine administration was 45 min. Time, 20 sec. * indicates washing.

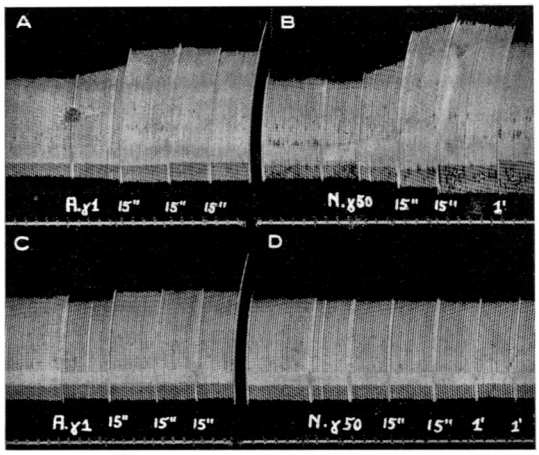

FIG. 3.—Isolated guinea-pig auricles in atropine sulphate 2×10^{-7}. Antagonistic action of ephedrine towards nicotine. A shows the effect of adrenaline 1 μg.; B that of nicotine 50 μg. In C and D adrenaline and nicotine were repeated after ephedrine hydrochloride 2.5 mg. Washings at the end of A, B, and C. Time, 2 sec.

A direct action of nicotine on muscular effectors (Gronchi, 1939), or at a postganglionic level (Tripod, 1949), has also been claimed to explain peculiarities of its action. Kottegoda (1953) reported that hexamethonium (1.25×10^{-4}) inhibited the stimulation of rabbit auricles by nicotine. According to Kottegoda, hexamethonium does not modify the auricular response to adrenaline. In our conditions, hexamethonium 2×10^{-5} fully antagonized the stimulating action of nicotine 2×10^{-6} (after atropine 2×10^{-7}) without changing the response to injected adrenaline ($1–2 \times 10^{-7}$) or to $CaCl_2$ (twofold increase of the normal content of the bath fluid). Hexamethonium 2×10^{-5} also antagonized the inhibitory phase of nicotine action ($2 \times 10^{-7}–2 \times 10^{-6}$) which is evident in fresh untreated preparations. This concentration of hexamethonium did not modify either the response of the auricles to injected ACh (2×10^{-8}) or the negative inotropic effect of KCl (twofold increase of the normal content of the bath fluid), although it decreased the negative chronotropic action of KCl. Hexamethonium, in the range of concentrations ($0.2–2 \times 10^{-5}$) which partially or fully antagonized nicotine, had no direct action on either the mechanogram or the electrogram of the auricles. The partial antagonism between hexamethonium and KCl may be attributed to stimulation of ganglia by KCl (Feldberg and Vartiainen, 1935).

Atropine is known to have ganglionic blocking properties (Feldberg and Vartiainen, 1935; Marrazzi, 1939; Konzett and Rothlin, 1949, and many others). Atropine sulphate 1×10^{-5} completely prevents the action of nicotine 1×10^{-6}. A typical experiment is shown in Fig. 4; it is representative of many others performed with different auricles at various times after completing the preparation. This concentration of atropine is larger than that (2×10^{-7}) necessary to block the muscarine-like effect of a dose of ACh (2×10^{-8}) the action of which on rate and amplitude is similar to that produced by nicotine. The hexamethonium-like activity of atropine is graded according to the dose; 4×10^{-6} does not always completely prevent the action of nicotine 1×10^{-6}; when prevention is

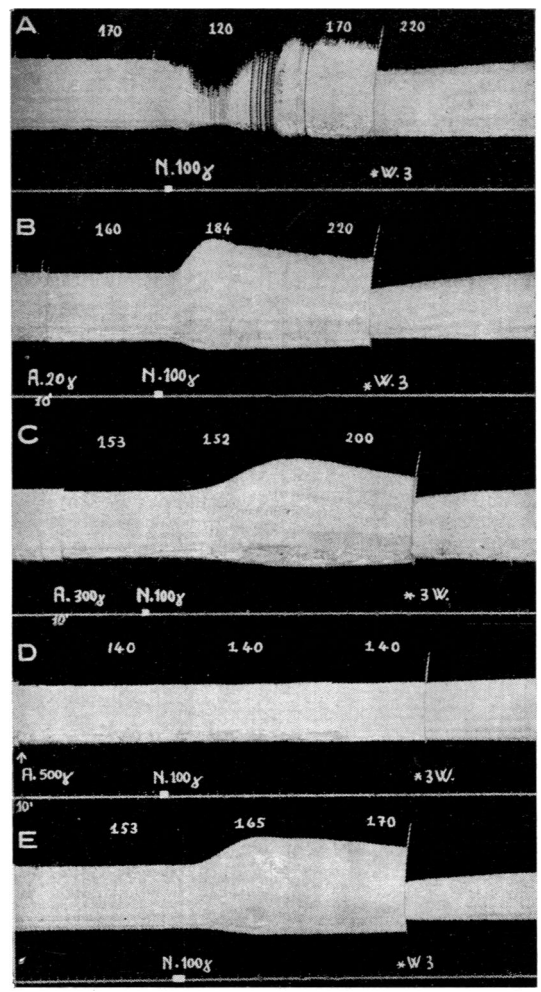

FIG. 4.—Isolated guinea-pig auricles. Influence of different doses of atropine on nicotine action. N, nicotine (as base); A, atropine sulphate; W.3, 3 washings. Numbers on the upper part of each record indicate auricular frequency determined from electrograms. Record A shows effect of nicotine (N), 100 μg. (as base); records B, C, and D show effects of the same dose of N after atropine sulphate (A) 20 μg., 300 μg., and 500 μg. respectively. Record E shows that the auricles still respond to nicotine despite the absence of effect after 500 μg. atropine.

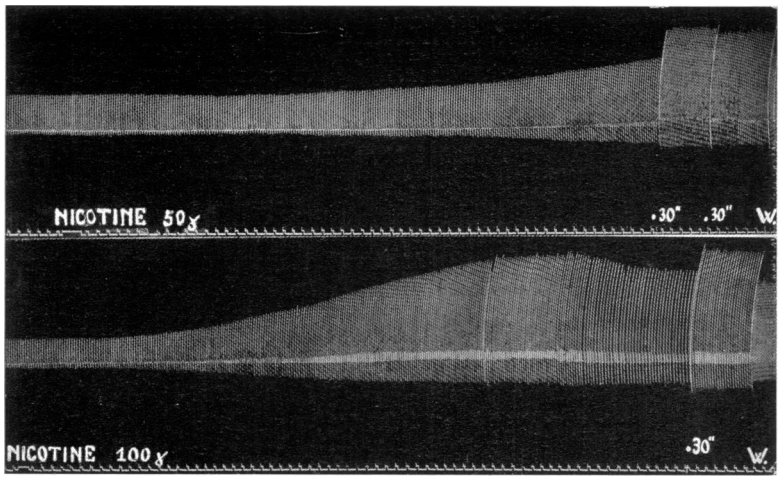

FIG. 5.—Hypodynamic isolated guinea-pig auricles (10 hours of work, several washings). Action of nicotine acid tartrate. W, washings; time, 2 sec. The upper tracing shows the effect of 50 μg. nicotine (as base) and the lower that of 100 μg. nicotine.

not complete there is a delay in the onset of nicotine action; protection is more pronounced towards the chronotropic than the inotropic action (see records C and E of Fig. 4). The action of atropine in antagonizing the chronotropic action of nicotine is also less reversible by washing than is that on the inotropic action. An interaction between atropine, and the sympathin liberated by nicotine, does not seem to play a role in these experiments: the inotropic and chronotropic responses of the auricles to adrenaline are not significantly changed after concentrations of atropine which act like hexamethonium.

Spadolini and Giachetti (1953) claim that the site of action of atropine, perfused in high concentration (1×10^{-5}) through Langendorff hearts, is upon excitatory ACh receptors, which they suppose to be present on myocardial effector cells and to be sensitive to very low concentrations of ACh (see also Spadolini and Domini, 1940). However, in our experiments, ACh added to the bath never stimulated guinea-pig auricles. ACh action was purposely investigated on 10 different preparations in concentrations from 1×10^{-15} to 1×10^{-7}; concentrations between 1×10^{-15} and 1×10^{-10} were ineffective; 1×10^{-9} and larger concentrations always gave the familiar muscarine-like effects. Eserinization did not alter the action qualitatively. Our results agree with Webb's (1950) on isolated rabbit auricles. The fact that nicotine stimulation is also prevented by ephedrine and hexamethonium is a further indication that its mechanism of action differs from that suggested by Spadolini and Domini for ACh. The results of experiments with ACh show furthermore that nicotine stimulation is not mediated through an effect of liberated ACh on the synthesis of ACh, such as happens when ACh causes stimulation of exhausted auricles (Bülbring and Burn, 1949; Burn, 1950).

Action of Nicotine on Hypodynamic Auricles.—In auricles exhausted by frequent washings and many hours of work the inhibitory actions of nicotine (1×10^{-6}), which may be predominant in fresh auricles, are less pronounced, or are replaced by a stimulant action (Fig. 5). This fact contrasts with the unchanged response to the minimal effective concentrations of ACh (1×10^{-9}–1×10^{-8}). Hexamethonium fully antagonizes stimulation by nicotine, as Kottegoda (1953) demonstrated in hypo-

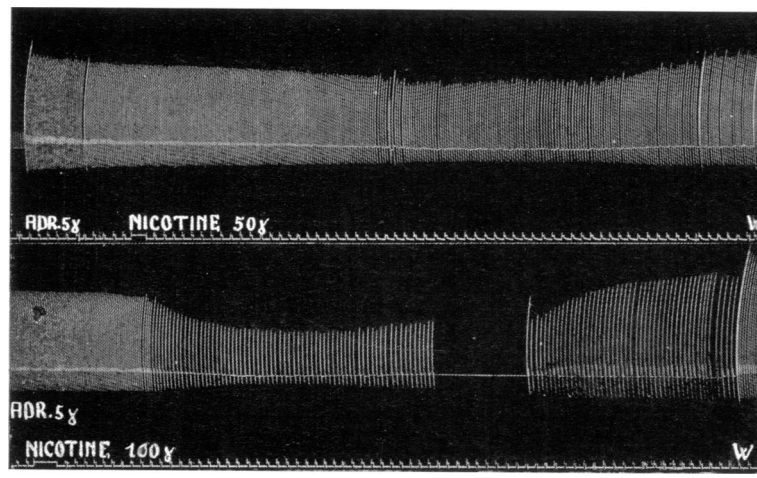

FIG. 6.—Hypodynamic isolated guinea-pig auricles (continuation of experiment of Fig. 5). The action of nicotine after adrenaline (ADR). The upper tracing shows the effect of 50 μg. nicotine after 5 μg. adrenaline, and the lower the effect of 100 μg. nicotine after 5 μg. adrenaline. Cf. with Fig. 5.

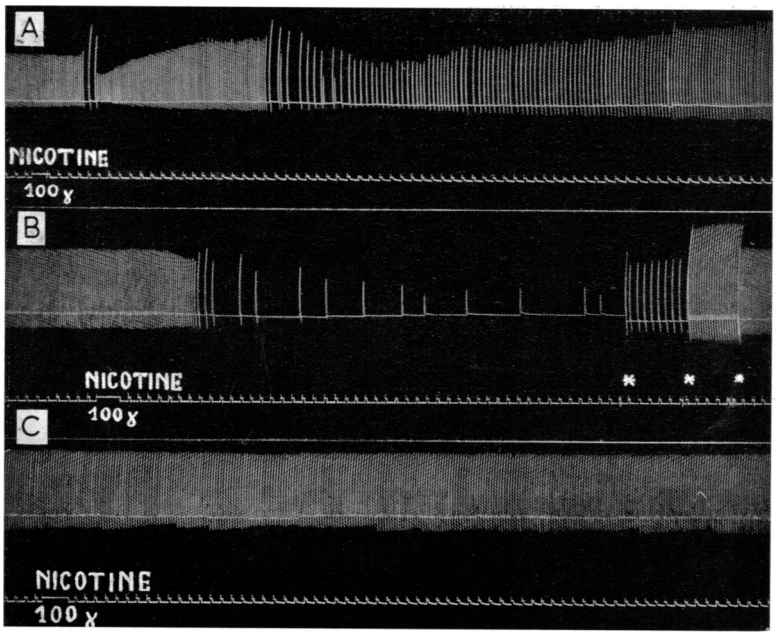

FIG. 7.—The action of nicotine on isolated guinea-pig auricles (A) before and (B) after treatment with adrenaline and (C) after treatment with adrenaline plus ephedrine. 5 μg. adrenaline given between A and B; between B and C 2.5 μg. ephedrine HCl and 5 μg. adrenaline. Adrenaline potentiates the inhibitory action of nicotine, but adrenaline plus ephedrine abolishes the action of 100 μg. N. Nicotine in C was added to the bath 5 min. after ephedrine and 30 sec. after adrenaline. In B, * denotes recording stopped for 1 min. At the end of every record 3 washings. Time, 2 sec.

dynamic rabbit auricles. After adrenaline, 1×10^{-7}, the response to nicotine is changed to a purely inhibitory one (Fig. 6; see, for a similar observation on rabbit auricles, Holz, 1938). If, however, the auricles are pretreated with ephedrine 1×10^{-5} the action of nicotine is prevented (Fig. 7). Hexamethonium is not so effective in protecting against the inhibitory action of nicotine in the presence of adrenaline as it is in protecting against the stimulant action in the absence of adrenaline (Figs. 8a and 8b): a concentration of 2×10^{-5} hexamethonium is necessary to prevent inhibition almost completely (Fig. 8a, line C), whereas 2×10^{-7} fully antagonizes stimulation (Fig. 8b). Hexamethonium 2×10^{-5}, however, does not prevent the inhibitory action of ACh in auricles which have been treated with adrenaline (Fig. 9). Atropine 2×10^{-7} fully prevents the inhibitory action of nicotine in the presence of adrenaline (Fig. 8a, line D).

DISCUSSION

The complex action of nicotine may be considered as the resultant of inhibition and excitation —in agreement with Dixon, 1920; Gronchi, 1939; Kottegoda, 1953, and others—with the possibility that one or other phase may predominate in different experimental conditions. Simultaneous stimulation of parasympathetic and sympathetic structures may explain this type of action, and also the effects of interaction with atropine, ephedrine, eserine, and hexamethonium. If nicotine stimulates autonomic ganglia without liberating ACh at the ganglionic level (Feldberg and Vartiainen, 1935), it is obvious that the antimuscarinic properties of atropine, and the anticholinesterase activity of eserine, may interfere with the action of the ACh liberated at post-ganglionic parasympathetic nerve endings. The stimulant action of nicotine on the sympathetic system has also been abundantly confirmed since the classical studies of Langley and Dickinson (for review, see Heubner, 1947). Evidence that the stimulant effect of a drug on an isolated heart preparation may be due to liberation of sympathin was provided for ACh (in the presence of atropine) by Hoffmann, Hoffmann, Middleton, and Talesnik (1945), and by McNamara, Krop, and McKay (1948). This view can be supported on anatomical, physiological and biochemical grounds. Thus there is chromaffin tissue in the heart (Trinci, 1907; Busacchi, 1912); the normal beating heart produces small quantities of sympathin (Külz, 1928); minced hearts treated with ACh produce a substance which inhibits atropinized rabbit intestine (McDowall, 1946); and heart extracts contain noradrenaline, adrenaline and hydroxytyramine (Goodall, 1951; Holtz, Kroneberg and Schümann, 1951). The antagonism by ephedrine of the stimulant effect of nicotine on atropinized auricles indicates that sympathin is implicated in the action of nicotine (De Jongh, 1951). The similarity between the effects of adrenaline and nicotine in atropinized auricles is evident in both mechanical and electrophysiological records. The following observations also agree with the concept that nicotine simultaneously stimulates both parts of the autonomic nervous system. Firstly, the biphasic type of action—inhibition followed by stimulation—is similar to that

seen on simultaneous stimulation of the vagus and accelerator nerves (Nelemans, 1951); secondly, the action of nicotine is very similar to the action of a mixture of ACh and adrenaline, and the washing-out effects are the same. Lawrentjew's (1929) and Woollard's (1926) opinion, that all intracardiac ganglia are parasympathetic, complicates the question where nicotine acts in the heart (for the nicotinic action of ACh on amphibian hearts, see Pick, 1920; Barlow, 1928; on isolated mammalian hearts, Hoffmann *et al.*, 1945; McNamara *et al.*, 1948). The problem of the site of nicotine action

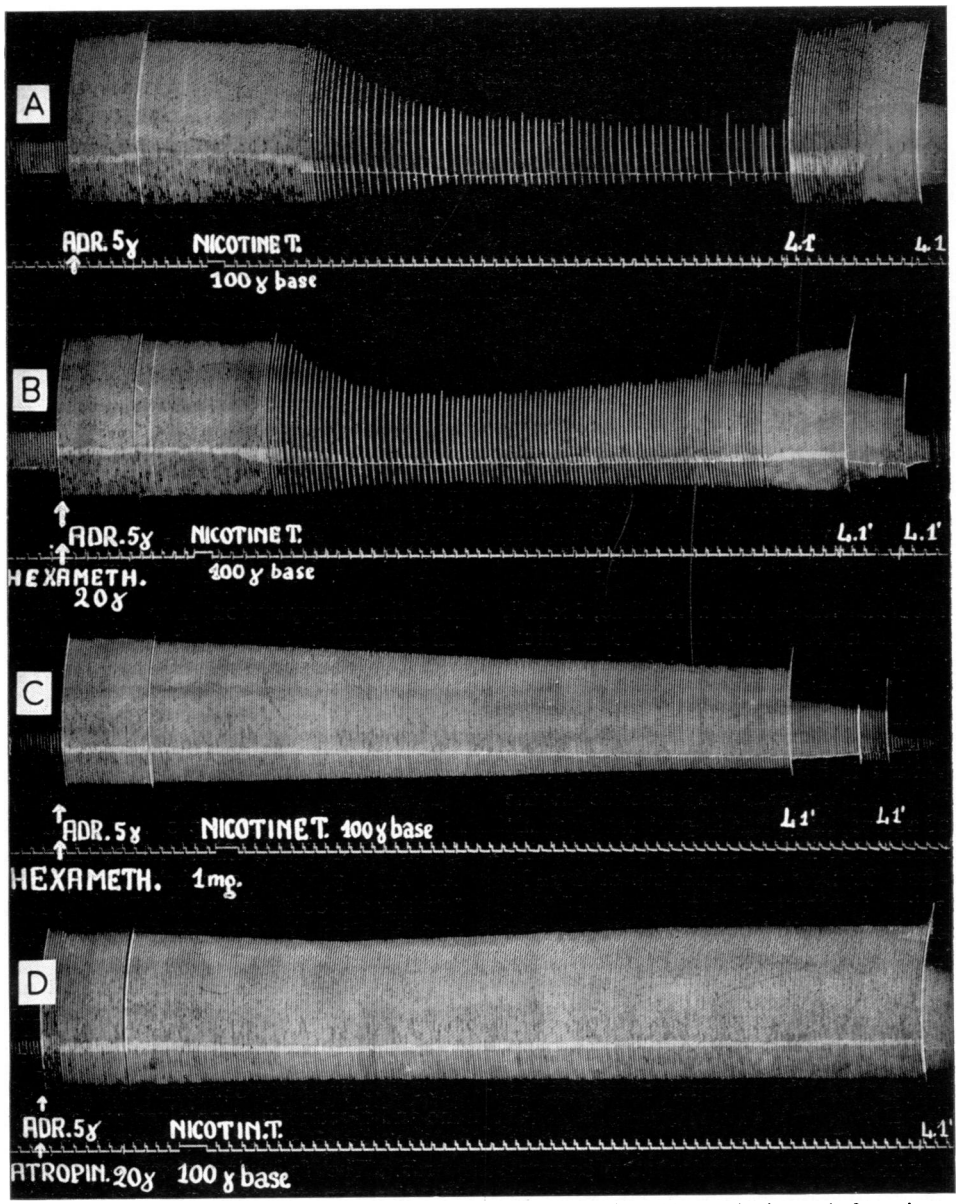

FIG. 8a.—Hypodynamic isolated guinea-pig auricles. To show the antagonism of hexamethonium and of atropine to the inhibitory action of nicotine after adrenaline. Time, 2 sec.; L 1', washings and stop of recording for 1 min.; ADR, adrenaline. Record A shows the effect of 100 μg. nicotine after 5 μg. adrenaline: records B and C show the effect of the same dose of nicotine after adrenaline plus 20 μg. and 1,000 μg. hexamethonium respectively. Record D shows the abolition of the nicotine effect. (Between B and C and C and D the response to 5 μg. adrenaline followed by 100 μg. nicotine remained as in A.)

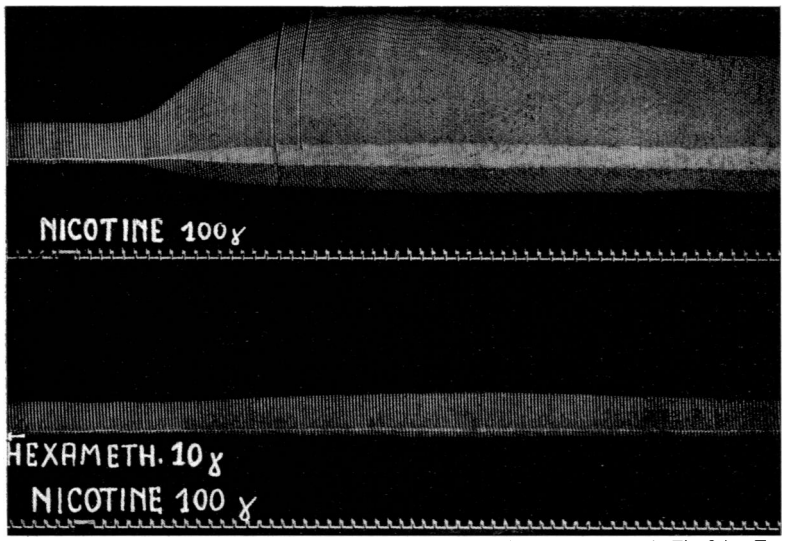

FIG. 8b.—Hypodynamic isolated guinea-pig auricles (continuation of experiment in Fig. 8a). To show the antagonism of hexamethonium towards the stimulating action of nicotine. Time, 2 sec.

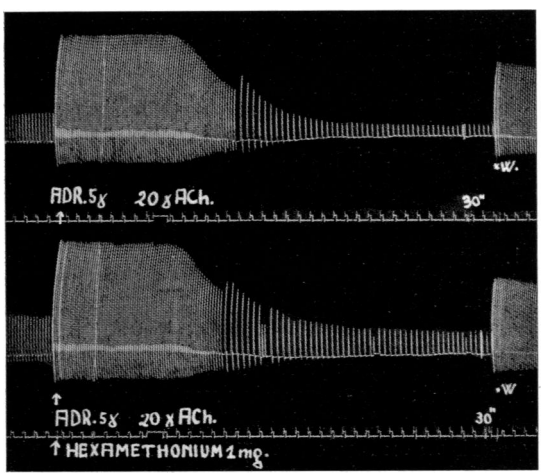

FIG. 9.—Hypodynamic isolated guinea-pig auricles. To show that hexamethonium does not antagonize the inhibitory action of acetylcholine (ACh) after adrenaline. Time, 2 sec.

applies to other tissues. Thus in the isolated ileum nicotine causes parasympathetic (motor) and sympathetic (inhibitor) effects ; inhibition of the ileum is evident when the nicotine is given after atropine or botulinum toxin (Ambache, 1951 ; Ambache and Edwards, 1951). Ambache and Edwards suggest the presence in the intestinal walls of adrenergic ganglion cells despite the lack of direct evidence. Applying the same hypothesis to my results, diagrams like those drawn by Dixon (1920) for the whole heart, and by Ambache and Edwards (1951) for the intestine, will also depict the site of action of nicotine on the auricles: inhibition is prevented by atropine and potentiated by eserine ; stimulation is unmasked by atropine and prevented by ephedrine ; both stimulation and inhibition are prevented by hexamethonium and by large doses of atropine. It is, however, questionable if full antagonism with hexamethonium proves the existence of a synapse in the preparation. If the action of hexamethonium is not restricted to the ganglion " but is fundamentally to prevent such excitation of nervous structures wherever it can be achieved " (Paton and Zaimis, 1952) there is no way to decide where exactly, in the heart, nicotine stimulation takes place (see also Kottegoda, 1953).

If the inhibitory phase of nicotine action in fresh untreated auricles is due, as seems likely, to the stimulation of parasympathetic ganglia, the progressive reduction in this inhibitory response during exhaustion may be explained by a decreased excitability of the parasympathetic ganglia or by a decreased sensitivity of effectors to liberated ACh. A decreased sensitivity to ACh does not seem of prime importance, because the response of auricles to injected ACh is not significantly reduced when they are so exhausted that nicotine stimulates them. Adrenaline may directly or indirectly restore the excitability of parasympathetic ganglia, and consequently the inhibitory response to nicotine, to a level similar to or greater than that of fresh preparations. This hypothesis agrees with the well-known action of small doses of adrenaline on the excitability of the cardiac vagus (Beccari, 1933, 1934a and b), and on synaptic transmission in the autonomic nervous system generally (Bülbring and Burn, 1942). The antagonism of atropine to the inhibition produced by nicotine in the presence of adrenaline accords with the concept that inhibition is mediated through the parasympathetic system, as Amsler (1920) proposed. The effectiveness of hexamethonium suggests that nicotine, in the presence of adrenaline, acts at the ganglionic level. The greater effectiveness of hexamethonium in preventing stimulation by nicotine than in preventing inhibition by nicotine in the presence of adrenaline

is not in disagreement with the fact that even autonomic ganglia are not all equally sensitive to blocking agents (Paton and Zaimis, 1952). The prevention by ephedrine of the inhibitory effect of nicotine in the presence of adrenaline may be explained by assuming that ephedrine–adrenaline competition also takes place at the ganglionic level. We have observed, however, that ephedrine also prevents the inhibition caused by nicotine alone in fresh auricles. This may indicate that: (*a*) in antagonism by ephedrine, competition with adrenaline for common receptors is not, or is not solely, involved (for discussion of what is presumably a direct action of ephedrine on the cardiac vagus see Beccari, 1934a and b); (*b*) adrenaline or sympathin-like substances liberated by nicotine are always somehow implicated in the inhibitory action of nicotine. Small quantities of sympathin are produced even by normal unstimulated hearts (Külz, 1928). Thus sympathin could increase the sensitivity of parasympathetic ganglia to nicotine in fresh auricles as adrenaline does in old preparations. It is possible that the initial inhibitory action which has been described for small doses of adrenaline in isolated hearts, whether treated with ACh or not (Kolm and Pick, 1920; Chiò, 1928; Martini, 1932, in amphibia; Peruzzi, 1950, in mammals; Lands, 1949, in intact animals), may be determined by a similar mechanism acting in presence of a drug, an experimental condition, or a degree of vagal tone, which causes a discharge in parasympathetic ganglia. The prevention of this adrenaline inhibitory action by atropine has indeed been described (Kolm and Pick, 1920; Martini, 1932; Lands, 1949).

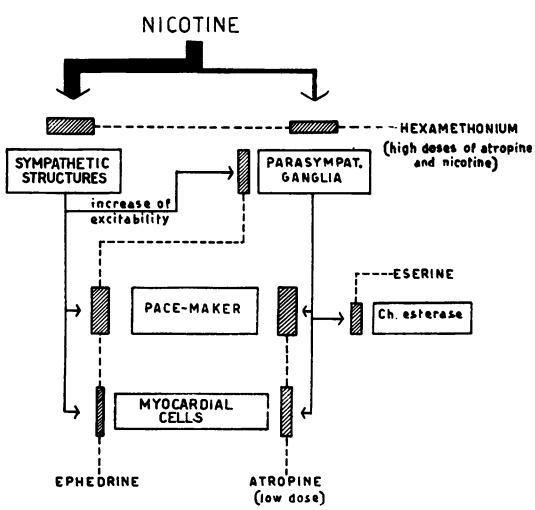

FIG. 10.—Diagram illustrating presumable sites and intensities of action of various drugs on guinea-pig isolated auricles. The action of nicotine varies qualitatively, according to the state of the auricles. A possible interpretation is: *Fresh auricles.*—The high excitability of parasympathetic ganglia, and the high activity of cholinesterase, leads to inhibition in the first phase (owing to the shorter latency of ACh) and to stimulation in the second phase (due, presumably, to sympathins and favoured by the hydrolysis of ACh). *Hypodynamic auricles.*—The low excitability of parasympathetic ganglia, and the increased sensitivity of the pace-maker and myocardial cells to sympathins, leads to predominance of stimulation. *Adrenaline-treated hypodynamic auricles.*—The restored excitability of parasympathetic ganglia (low cholinesterase activity?) results in a predominance of the inhibitory effect on frequency.

Fig. 10 is a diagram in which an attempt has been made to localize the actions of the drugs used in these experiments, and to represent, though only approximately, their different intensities of action on various auricular effector cells. In the legend to the figure the different responses to nicotine of fresh and hypodynamic auricles, and of auricles treated with adrenaline, are explained chiefly as the resultant of a progressive decrease of excitability of parasympathetic ganglia. Bülbring and Burn (1949) have shown that the power of exhausted auricles to synthesize ACh is decreased; it is not improbable that the synthetic power of parasympathetic ganglia is also impaired for, as nervous structures, they are certainly not more resistant to exhaustion than are myocardial cells. There are insufficient data to indicate whether the liberation of sympathin also decreases during exhaustion; but if this happens it would also contribute to the decrease in excitability of the parasympathetic ganglia.

SUMMARY

1. Nicotine (2×10^{-7}–2×10^{-6}) has a complex action on isolated guinea-pig auricles, causing stimulation and inhibition according to the experimental conditions.

2. The inhibitory action on rate and amplitude of contractions is evident in fresh auricles, and is prevented by atropine (2×10^{-7}), and potentiated by eserine (2×10^{-8}); the stimulant action is unmasked by atropine (2×10^{-7}) and prevented by ephedrine (5×10^{-5}); both stimulation and inhibition are prevented by hexamethonium (2×10^{-5}) and atropine (1×10^{-5}).

3. In hypodynamic auricles the nicotine inhibition of rate and amplitude is less intense than in fresh preparations, or is replaced by almost pure stimulation; adrenaline restores inhibition. The inhibitory action of nicotine in the presence of adrenaline is prevented by ephedrine, atropine and hexamethonium.

4. Hexamethonium is approximately 100-fold more effective in preventing the stimulation by nicotine of fresh atropinized auricles or of hypodynamic untreated auricles than it is in preventing

the inhibition by nicotine in the presence of adrenaline. Concentrations of hexamethonium which completely prevent the inhibition from adrenaline + nicotine do not prevent that from adrenaline + acetylcholine.

5. Injected adrenaline is, and sympathin—whether released normally or by nicotine—may be, of fundamental importance in regulating the excitability of parasympathetic ganglia.

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