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EFFECT OF LOW TEMPERATURES ON THE RESPONSES OF GUINEA-PIG ISOLATED ATRIA TO NICOTINE AND TO SYMPATHETIC AND PARASYMPATHETIC STIMULATION

BY

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Nicotine has long been characterized as having two phases of action on the heart (Dixon, 1924), initially a negative and subsequently a positive chrono- and inotropic effect. The first phase is the result of a stimulation of intramural parasympathetic ganglia, but the significance of the second phase is still debatable.

The fact that nicotine fails to cause the positive phase either when the sympathetic receptors have been blocked (Lee & Shideman, 1959; Mannaioni, 1960) or when the catechol amine stores have been depleted by reserpine (Pepeu, Mannaioni & Giotti, 1958; Burn & Rand, 1958; Lee, McCarty, Zodrow & Shideman, 1960) or by guanethedine (Kadzielawa, 1962) suggests the involvement of some adrenergic mechanism. These observations, however, do not indicate whether nicotine liberates catechol amines directly from some peripheral stores or indirectly by ganglionic stimulation.

Kottegoda (1953) postulated the existence of sympathetic ganglia in the heart, and this hypothesis is consistent with the finding that the ganglionic blocking agents prevent the action of nicotine (Kottegoda, 1953; Ginzel & Kottegoda, 1953; Giotti, 1954). However, Middleton, Oberti, Prager & Middleton (1956) and Lee & Shideman (1959) demonstrated a positive chronotropic effect of nicotine on the papillary muscle of the cat, histological examinations of which failed to reveal the presence of ganglionic cells, and on embryonic chick hearts, the sympathetic innervation of which has not been established (Lee *et al.*, 1960). From this, it appears that nicotine might act at extraganglionic sites, liberating catechol amines directly from chromaffin tissue (Ginzel & Kottegoda, 1953; Burn & Rand, 1958) or from particular deposits existing between the sympathetic nerve endings and the adrenergic receptors (Lee *et al.*, 1960) or even from the nerve endings themselves (Lee & Shideman, 1959).

Recently, Leaders & Long (1962) have reported some observations which favour the hypothesis that nicotine liberates catechol amines through a cholinergic mechanism. This hypothesis is based mainly on the fact that hemicholinium, an inhibitor of acetylcholine synthesis (MacIntosh, Birks & Sastry, 1956), completely abolished both the negative and positive effects of nicotine, blocking at the same time the vagal, but not the sympathetic transmission.

In the present paper results obtained by studying the effect of lowered temperature on the response of isolated guinea-pig atria of sympathetic and vagal stimulation are described.

This was done since it has been demonstrated (Eve, 1900; Ambache, 1946; Gillespie & Wishart, 1957; Della Bella, Gandini & Teotino, 1963) that the ganglionic synapses are more sensitive to a fall in temperature than the postganglionic neurones and the peripheral receptors.

METHODS

Male guinea-pigs weighing about 350 g were used. The isolated atria, with vagal and sympathetic innervations, were prepared according to the method of Greef, Kasperat & Oswald (1962).

The right nerve trunks were always stimulated with rectangular, 0.3-msec-duration pulses at frequencies ranging from 5 to 30 shocks/sec. The stimulations were carried out, with supramaximal strength in all instances, at regular intervals for 15 to 30 sec, depending upon the sensitivity of the preparation.

The drugs used were: nicotine hydrogen tartrate, acetylcholine sulphate, adrenaline hydrochloride and hexamethonium bitartrate. Doses given in the text are expressed as the final concentrations in the bath fluid.

RESULTS

By lowering the bath temperature from 30 to 18° C and, in a few experiments, to 16° C, the heart rate progressively decreased while cardiac contractions showed a tendency to increase. Below 25° C arrhythmias occasionally appeared and between 20 and 18° C there was often complete cardiac arrest.

As the temperature was lowered nicotine progressively lost its negative chrono- and inotropic effects, but retained its positive effect. While at 25 to 24° C the negative effect was only slightly reduced in two out of four preparations, at 20 to 21° C there was a marked reduction in two out of seven preparations (Fig. 1) and finally at 18 to 16° C the negative response was completely lost in all preparations (Fig. 2).

In all these preparations nicotine retained its positive chrono- and inotropic effects, although slightly diminished (Figs. 1 and 2), and the response sometimes appeared after a

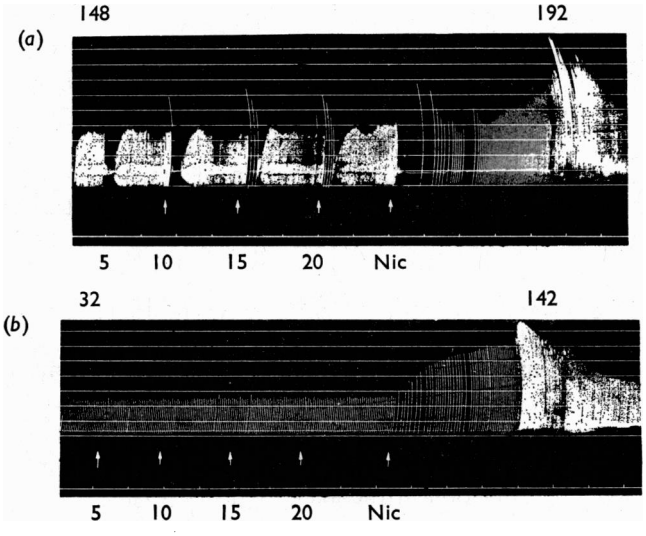

Fig. 1. Guinea-pig isolated atria: effect of lowering of temperature (*a*, 30° C and *b*, 21° C) on vagal stimulation at various frequencies (at arrows, shocks/sec) and on responses to nicotine (at arrows, Nic, 40 μg/ml.). Above the records the atrial rates are shown (beats/min). Time marks, 30 sec.

relatively long latent period compared to that seen at higher temperatures. A similar delayed response was observed by Gillespie & Wishart (1957) who treated the rabbit colon with nicotine at low temperatures.

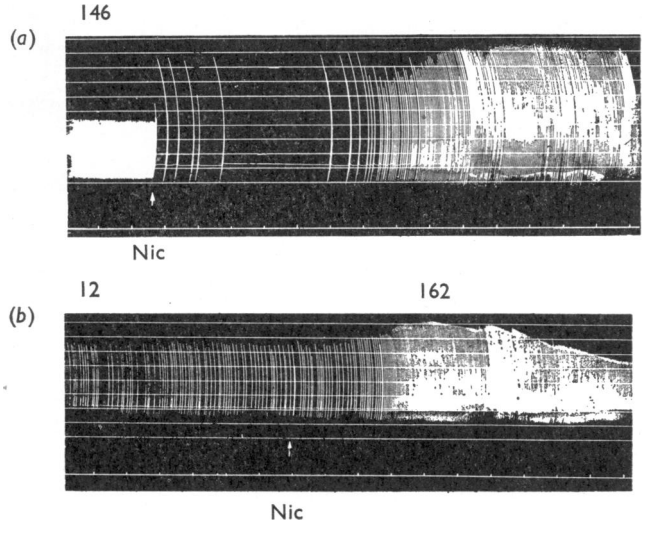

Fig. 2. Guinea-pig isolated atria: action of nicotine (Nic, 40 μg/ml.) at 30° C (*a*) and 16° C (*b*). Above the records the atrial rates are shown. Time marks, 30 sec.

In a second series of experiments, with a progressive fall in temperature from 25 to 20° C the response of the atria to vagal stimulation was greatly reduced or abolished, while the response to sympathetic stimulation continued. In nine out of thirteen preparations the negative chrono- and inotropic effects in response to vagal stimulation were completely absent; in the remaining four they were greatly reduced. In all these preparations simultaneous sympathetic stimulation induced positive chrono- and inotropic effects, the latter being more pronounced (Fig. 3).

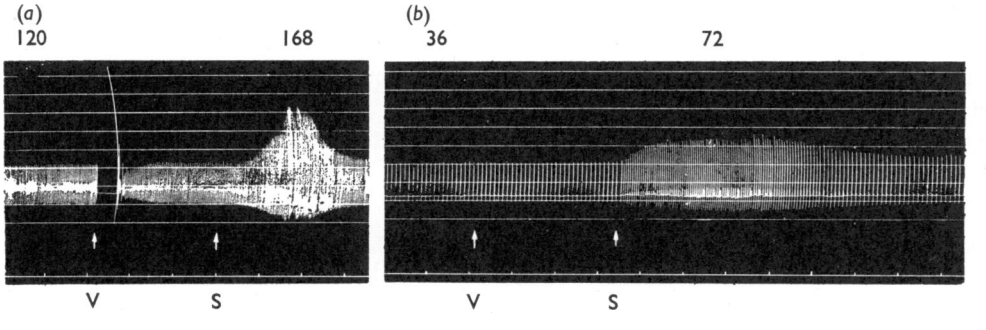

Fig. 3. Guinea-pig isolated atria: influence of lowering of temperature (*a*, 30° C and *b*, 19° C) on the effects induced by vagal (V) and sympathetic (S) stimulation. Above the records the atrial rates are shown. Time marks, 20 sec.

With the progressive lowering of temperature from 25 to 19° C there was a close relationship between the gradual disappearance of the negative chrono- and inotropic phase induced by nicotine and the decrease in the response to vagal stimulation (Fig. 1).

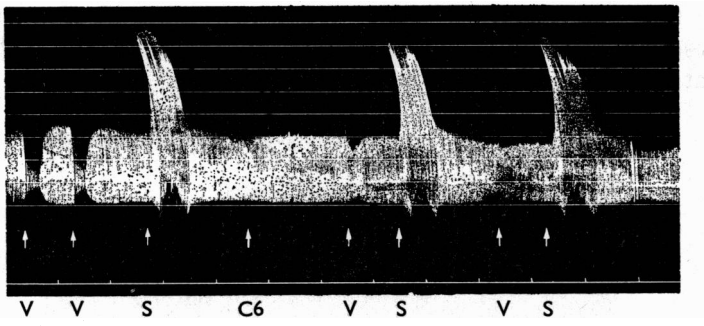

Fig. 4. Guinea-pig isolated atria: action of hexamethonium (C6, 100 μg/ml.) on the effects induced by vagal (V) and sympathetic (S) stimulation. Time marks, 30 sec.

The effects of acetylcholine, adrenaline and noradrenaline remained almost unchanged even at a temperature as low as 18° C.

Hexamethonium (50 to 100 μg/ml.) completely blocked the effect of vagal stimulation, usually within 10 min. Sympathetic stimulation carried out with various frequencies and duration of impulses continued to be effective even in the presence of very large doses (0.5 to 2.5 μg/ml.) of hexamethonium left in the bath for longer periods of time (Fig. 4); 50 μg/ml. of hexamethonium, on the other hand, was sufficient to block completely the typical biphasic action of nicotine.

DISCUSSION

The observations reported in this paper favour the hypothesis that the positive chrono- and inotropic phase induced by nicotine on the guinea-pig atria is not dependent on intramural sympathetic ganglia. This conclusion is based on the fact that both hexamethonium and hypothermia have similar actions, in that they completely block the effects induced by vagal stimulation without modifying those due to sympathetic stimulation. Similar results have been reported by Della Bella, Rognoni & Villani (1959) and by Greef *et al.* (1962).

The results concerning the different effects of low temperature on the vagal and sympathetic systems could be explained by postulating either that sympathetic ganglia are relatively less sensitive than parasympathetic ganglia to hypothermia, or that the sympathetic system is more resistant to cooling because there are no intramural ganglionic synapses along their pathway.

The latter hypothesis appears, however, to be the more convincing of the two as it accords both with the hexamethonium findings and the results which demonstrate that the ganglionic synapses are more sensitive to hypothermia than are the postganglionic neurones and the peripheral receptors (Eve, 1900; Ambache, 1946; Gillespie & Wishart, 1957; Della Bella *et al.*, 1963). Furthermore, there is no evidence that the sensitivity of the sympathetic ganglia to low temperatures is different from that of the ganglia belonging to the parasympathetic division.

With the progressive lowering of temperature there was a good correlation between the decrease of the negative chrono- and inotropic phase of nicotine and the reduction in the response to vagal stimulation. On the other hand, the chrono- and inotropic positive

phase of both nicotine and sympathetic stimulation remains almost unchanged at low temperatures.

All these results agree with the hypothesis that there are no intramural sympathetic ganglia and therefore suggest that nicotine causes the positive chrono- and inotropic effects by direct mobilization of catechol amines from some peripheral stores and not indirectly through an action at ganglionic level.

The fact remains, however, that hexamethonium blocks the positive phase of nicotine. This could be explained by admitting either that hexamethonium has a peripheral antinicotinic activity, or that nicotine acts at the level of parasympathetic ganglia and liberates catechol amines through a cholinergic mechanism.

A peripheral antinicotinic action of hexamethonium, which has been proposed by Paton & Zaimis (1959), is today almost established. Hexamethonium is capable of preventing the activities of nicotine and even of acetylcholine at sites other than the subsynaptic membrane; for instance the excitation of the sensory fibres of the carotid body (Ginzel, Glupp & Werner, 1952; Douglas, 1952), and of the skin (Douglas & Gray, 1953; Diamond, 1959) and the excitation of sympathetic postganglionic C-fibres (Ferry, 1963; Blakeley, Brown & Ferry, 1963).

The hypothesis of a cholinergic mediation in the action of nicotine is mainly supported by the results of Leaders & Long (1962), who demonstrated on the cat and rabbit isolated atria that hemicholinium can abolish both negative and positive chrono- and inotropic effects of nicotine, while stimulation of the sympathetic nerve still produces a positive chronotropic response. They concluded that in both the negative and positive phases of the atrial responses to nicotine only the parasympathetic system is involved and that the mobilization of catechol amines from peripheral stores is probably brought about by the mediation of acetylcholine.

However, our observations, that the chronotropic and inotropic positive effects of nicotine are still present at temperatures at which the parasympathetic system is completely insensitive both to nicotine and to electrical stimulation, seem to exclude an involvement of the cholinergic system as suggested by Leaders & Long (1962).

The different conclusions, drawn by us and by these authors, could be explained on the assumption that hemicholinium, like hexamethonium, has a peripheral antinicotinic action.

This hypothesis can be supported by the recent results of Burn & Gibbons (1964), demonstrating analogous blocking effects of hexamethonium and hemicholinium on the action of acetylcholine and bretylium on sympathetic nerve terminals by preventing their entry into the fibres.

In conclusion, the results reported in this paper can be considered as further evidence in favour of the view that the guinea-pig isolated atria are not provided with intramural sympathetic ganglia, and of the hypothesis that nicotine produces its positive chrono- and inotropic effects by acting at an extraganglionic site and liberating catechol amines directly. This extraganglionic action of nicotine can be abolished by hexamethonium.

SUMMARY

1. The biphasic action of nicotine on the guinea-pig isolated atria, with both vagal and sympathetic nerves intact, has been examined.

2. When the temperature is lowered from 30 to 18° C, the effect due to vagal stimulation tends to disappear gradually, while that due to sympathetic stimulation remains almost unchanged.

3. A similar relationship has been observed for nicotine: the lowering of temperature progressively reduces its negative but not its positive chrono- and inotropic effect.

4. It has been confirmed that hexamethonium does not modify the response to sympathetic stimulation even in relatively large doses.

5. The results can be considered as further evidence in favour of the view that nicotine provokes its positive chrono- and inotropic effects by acting at extraganglionic sites and liberating catechol amines directly. The extraganglionic action of nicotine can be abolished by hexamethonium.

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