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NICOTINE-LIKE ACTIONS IN AURICLES AND BLOOD VESSELS AFTER DENERVATION

BY

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The work to be described in this paper is a continuation of observations made on the action of nicotine and acetylcholine in the isolated auricles of the rabbit heart and in the perfused vessels of the rabbit ear (Kottegoda, 1953a, 1953b). Nicotine and acetylcholine were found to stimulate the auricles in the presence of atropine, and the stimulant action was blocked by hexamethonium. Similarly nicotine was found to constrict the vessels of the rabbit ear; acetylcholine in the presence of atropine or when the perfusion had continued for 24 hours had the same effect. This constrictor action was blocked by hexamethonium; in the presence of benzylimidazoline (Priscol) the constrictor action was reversed to a dilator action, and this dilator action was also blocked by hexamethonium. The observations suggested that the actions of nicotine and of acetylcholine were indirect and were due to the liberation of an adrenaline-like substance. Because of these and various other findings discussed later further experiments were undertaken to test the action of nicotine and acetylcholine on auricles and on vessels which had been denervated some time previously.

For experiments on auricles, cats were used in place of rabbits, as the former were found to stand the operation better. A few auricles from normal cats were tested with nicotine and acetylcholine in the presence of atropine; it was found that they behaved like rabbit auricles towards these substances.

METHODS

Cat Auricles. Both stellate ganglia were removed from cats under ether and pentobarbitone sodium (30 mg./kg. body weight intraperitoneally). After 12 to 14 days the animals were killed, the auricles dissected out, and the response to acetylcholine and nicotine tested according to the method described earlier (Kottegoda, 1953a).

Rabbit Ears. The sympathetic supply to the rabbit's ear consists of post-ganglionic fibres mainly from the superior cervical ganglion, but also of fibres from the stellate ganglion which do not pass through the superior cervical ganglion (Feldberg, 1926). Both ganglia on one side were removed under ether and pentobarbitone sodium anaesthesia. A very great portion of the sensory supply of the rabbit ear is provided by the great auricular (C2 and C3) and great occipital (C2 and partly C3) nerves. For sensory denervation of the ears, one-third to half-an-inch of each of these nerves was removed proximal to the insertion of the ears. The perfused vessels of the isolated ears were tested with nicotine and acetylcholine according to the method described by Burn and Robinson (1951), in which Stephenson's recorder (1948) was used.

RESULTS

Isolated Cat Auricles.—Three auricles were tested with nicotine and acetylcholine in the presence of atropine 12–14 days after denervation. Nicotine was found to increase both the rate and amplitude of the auricular beat (Fig. 1). This stimulant action,

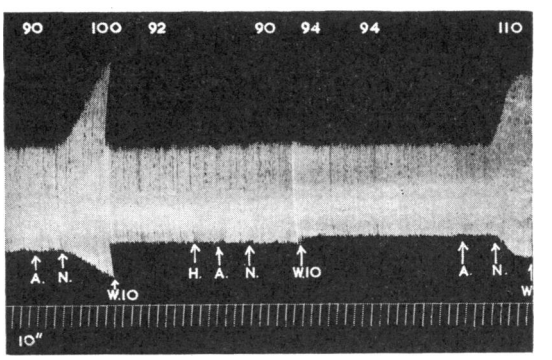

FIG. 1.—Spontaneous contractions of cat auricles which had been sympathetically denervated 2 weeks earlier, showing the stimulant action of nicotine after atropine. Bath volume 40 ml. At A 10 μg. atropine sulphate was added after which 0.4 mg. nicotine hydrogen tartrate was added at N. At W the bath was changed 10 times, and at H 2.5 mg. hexamethonium bromide was added followed by 10 μg. atropine sulphate. Nicotine (N) was then without effect. After washing out the bath at W the stimulant action of nicotine after atropine returned. Figures at top of tracing denote number of auricular beats per minute.

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like that observed in normal auricles, was suppressed by hexamethonium and returned after the hexamethonium was washed out. Similar effects were observed with acetylcholine.

Rabbit Ears after Sympathetic Denervation.—Three isolated ears were examined 1 to 3 weeks after removal of the sympathetic ganglia. In these nicotine caused a marked constriction only, or one which was followed by a small dilatation. Hexamethonium always blocked this constrictor action (Fig. 2), which reappeared when the hexamethonium was washed out. In fresh preparations acetylcholine caused only a dilatation; but if atropine was first injected or if larger doses of acetylcholine were given after the preparation was a few hours old, a constriction was produced which was blocked by hexamethonium. The constrictor action of acetylcholine returned gradually as the hexamethonium was removed. In previous experiments on isolated rabbit ears (Kottegoda, 1953b) the constriction caused by a given dose of nicotine or acetylcholine increased as the perfusion was continued. In the ears in which the sympathetic fibres had degenerated, the initial constrictor response to these substances was always greater than in normal ears, and there was no tendency for the constriction caused by a given dose to increase with time. The responses remained more or less the same or declined as the perfusion was continued Fig. 3.

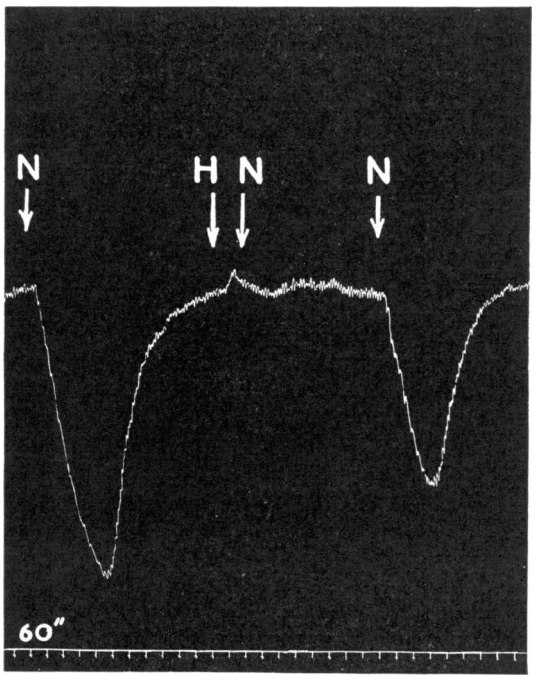

FIG. 2.—Outflow record from perfused rabbit ear, sympathetically denervated 3 weeks earlier. At N injection of 10 μg. nicotine hydrogen tartrate caused a reduction in outflow. At H 100 μg. hexamethonium bromide was injected, followed by the same dose of nicotine at N. In the presence of hexamethonium, nicotine had no effect. After some time, as the hexamethonium was washed away nicotine (N) again caused a reduction in outflow. (A fall in the record in this and subsequent figures indicates a reduction in outflow.)

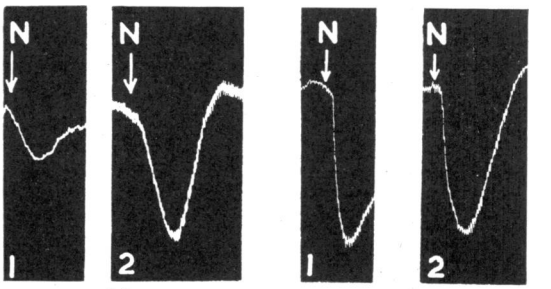

FIG. 3.—Outflow from perfused rabbit ear. (*a*) Normal ear. Effect of 20 μg. nicotine hydrogen tartrate at N on the first day (1) and the same dose of nicotine on the second day (2). Note the bigger reduction in outflow on the second day to the same dose. (*b*) Sympathetically denervated ear. Effect of 10 μg. nicotine hydrogen tartrate (1) on the first day and (2) of the same dose on the second day. The response does not now increase as the perfusion is continued.

Rabbit Ears after Sensory Denervation.—Four ears were examined 1 to 4 weeks after sensory denervation. In these, too, nicotine caused a constriction which was often great. This constriction was sometimes followed by dilatation. Again, hexamethonium suppressed the constriction caused by nicotine. Acetylcholine injected early in the perfusion caused a dilatation only, but after atropine it caused a constriction which, like that caused by large doses of acetylcholine in the older preparations, was blocked by hexamethonium (see Fig. 4). Once the latter was washed away the constrictor action of acetylcholine returned.

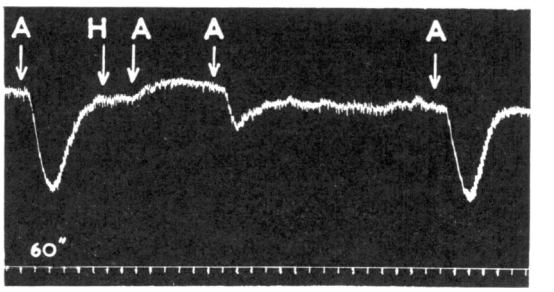

FIG. 4.—Outflow from rabbit ear 10 days after sensory denervation. 2nd day perfusion. At A 40 μg. acetylcholine bromide was injected. At H 100 μg. hexamethonium bromide was injected, followed by the same dose of acetylcholine at A. Now the acetylcholine was without effect. Note the return of the effect of acetylcholine as the hexamethonium is washed out.

Discussion

The well-recognized peripheral effects of nicotine occur at the motor end plate and in ganglia. Several phenomena have now been described by various workers which, in one respect or another, are anomalous. The first of these is the stimulant action of nicotine (Heymans, Bouckaert, and Dautrebande, 1931) and of acetylcholine (v. Euler, 1938) on the carotid body. Moe, Capo, and Peralta (1948) found that tetraethylammonium blocked the action of these substances, but did not modify the response to hypoxia and the effect of cyanide. Ginzel, Klupp, and Werner (1952) and Douglas (1952) showed that hexamethonium behaved like TEA. The second is the pilomotor response which occurs in the skin of man and of the cat after the intracutaneous injection of nicotine or of acetylcholine. This was described in 1940 by Coon and Rothman. The third is the stimulant action of acetylcholine in the heart of the cat or rabbit after treatment with atropine, accompanied by the release of an adrenaline-like substance. This was described by Hoffmann, Hoffmann, Middleton, and Talesnik in 1945. A similar action of acetylcholine and of nicotine has been observed in the isolated rabbit auricles and found to be blocked by hexamethonium (Kottegoda, 1953a). The fourth is the stimulant action of acetylcholine and of nicotine on sensory nerve endings in the skin described by Brown and Gray (1948). Douglas and Gray (1953) have recently shown that this action, like that on the carotid body, is blocked by hexamethonium, but that the receptors in the skin still discharge impulses in response to touch. The fifth is the inhibitory action of nicotine on the isolated intestine in the presence of atropine, which was described by Ambache and Edwards in 1951. The last is the constrictor action of nicotine and acetylcholine in the rabbit ear vessels recently shown by one of us to be abolished by hexamethonium (Kottegoda, 1953b).

The actions of nicotine on the carotid body receptors and on the sensory receptors of the skin are presumably not ganglionic actions, and since they are blocked by hexamethonium, it follows that the action of hexamethonium can exclude other actions of nicotine than those exerted on ganglia. Ambache and Edwards (1951) believed that the inhibitory action of nicotine on the intestine was exerted on ganglion cells of the myenteric plexus which possessed adrenergic neurones. Evans and Schild (1953) found, however, that a transient inhibitory action of nicotine was obtained in ganglion-free specimens of cat jejunum, particularly in preparations stimulated by eserine and acetylcholine.

In the heart, on the other hand, the stimulant action of nicotine seems more likely to be an action on ganglia, or at least an action on tissue able to liberate an adrenaline-like substance, some vagal preganglionic fibres functioning perhaps like the splanchnic terminations in the adrenal medulla. Middleton, Middleton, and Toha (1949) have shown that vagus stimulation liberates an adrenaline-like substance from the isolated heart after treatment with atropine, so that the acceleration occurs after a physiological stimulus as well as when nicotine or large doses of acetylcholine are applied. Our experiments show that the stimulant action of nicotine and acetylcholine is present after degeneration of the sympathetic fibres, and is therefore probably related to this vagus acceleration.

There remains for consideration the pilomotor phenomenon of Coon and Rothman, and the constrictor action in the vessels of the rabbit ear, These two effects must have a different mechanism. since the pilomotor action disappeared after degeneration of the sympathetic fibres. Coon and Rothman came to the conclusion that nicotine stimulated some of the terminations of the post-ganglionic sympathetic fibres and then by an axon reflex caused erection of the hairs. The arguments for this view were that the intracutaneous injection of nicotine or acetylcholine caused the pilomotor response after section of the mixed nerve supply, but not after degeneration of the sympathetic fibres, and not in the presence of concentrations of procaine too low to have an analgesic action, and therefore too low to paralyse the sensory fibres. Their conclusion is of such importance that it would seem desirable that the experiments after sympathectomy should be repeated.

In the rabbit ear vessels we have found that the constrictor action of acetylcholine seen after giving atropine or after prolonged perfusion, and the constrictor action of nicotine, remain unaffected by degeneration of all sympathetic fibres and also by degeneration of the sensory fibres. Hence the constrictor effect cannot be explained by an axon reflex involving either sympathetic or sensory channels. It is an effect which seems to be due to the release of an adrenaline-like substance since in the presence of benzylimidazoline (Priscol) both acetylcholine and nicotine cause dilatation. Moreover, the action, whether constrictor, or after reversal by benzylimidazoline dilator, is abolished by hexamethonium. Evans and Schild say that block by hexamethonium may be due to the action of hexamethonium on the end organ, and that therefore caution is required in drawing a conclusion. However, hexamethonium in very high doses

does not modify the constrictor action of adrenaline, and we have found that when the postganglionic fibres to the rabbit ear vessels are stimulated in the preparation described by Gaddum and Kwiatkowski (1938), the constriction obtained remains unchanged after the injection of hexamethonium. (This result indicates in addition that there are no ganglia on the course of fibres arising from the superior cervical ganglion.)

Hence abolition by hexamethonium suggests that acetylcholine and nicotine cause constriction either by stimulating ganglia with adrenergic neurones or by stimulating tissue resembling chromaffin tissue. At this point histological evidence is required which is at present not available. In the intestine a terminal reticulum has been described by Stöhr (1932, 1934) and by Reiser (1933) and a sympathetic ground plexus by Boeke (1940). These do not degenerate after removal of the ganglion cells. Something of the same kind may be present around the vessels. Nelemans (1948) has described structures resembling ganglia in the nerve plexuses surrounding the smallest blood vessels of the frog's tongue. They persisted after degeneration of the sympathetic fibres and of the somatic fibres. Our observations suggest that the rabbit's ear offers a tissue for study where histological and functional changes may be correlated.

Summary

1. The stimulant action of nicotine and acetylcholine on the atropinized cat auricle has also been found in preparations in which the sympathetic fibres have degenerated. Hexamethonium abolishes this effect in the normal as well as in the denervated auricle.

2. In isolated perfused rabbit ears deprived of their sympathetic and sensory supply 1–4 weeks previously, nicotine and acetylcholine (the latter after atropine or on the second day of perfusion) exert a vasoconstrictor effect as they do in normal ears. Hexamethonium blocks this action in both preparations.

3. The significance of these results in relation to the possible occurrence of peripheral ganglia with adrenergic neurones or tissue resembling chromaffin tissue is discussed.

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