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THE ACTION OF NICOTINE AND ACETYLCHOLINE ON THE VESSELS OF THE RABBIT'S EAR

BY

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The action of nicotine on blood vessels is usually explained as being due to stimulation of sympathetic ganglia, as was shown by Langley (1901). However, Handovsky and Pick (1913) found that nicotine caused vasoconstriction when perfused through the vessels of the frog hindlegs (Läwen-Trendelenburg preparation), from which it appeared that nicotine might exert a more peripheral effect. They used a 1% solution, but in 1937 Loewi made similar observations using a 0.01% solution, and in 1946 Haimovici and Pick obtained vasoconstriction by injecting 5 μg. nicotine; they observed that the effect was blocked by thiamine and thiazole. Later Haimovici (1948) showed that continuous perfusion with nicotine 1 in 50,000 caused initial constriction, but the rate of flow gradually increased and the injection of large doses of nicotine was then without effect. Haimovici found that the action of nicotine was unaffected by removal of the sympathetic chains and of the spinal nerves and that it persisted during perfusion with tetraethylammonium or with an extract of curare. He concluded that the action was peripheral to the postganglionic fibres and perhaps directly on the blood vessels.

The dilator action of small amounts of acetylcholine such as 5 μg. on the perfused vessels of the isolated rabbit ear was described by Dale in 1914, but in 1918 Reid Hunt stated that large amounts caused vasoconstriction both in the rabbit ear and in the vessels of the muscles of cats and of dogs; in the rabbit ear he injected as much as 5 mg.; he concluded that acetylcholine had a constrictor action on blood vessels which was exerted somewhere "beyond the ganglia cells." In discussing these results Feldberg and Minz (1932) state that Bartosch and Nagel found that the constrictor action of acetylcholine was abolished by nicotine, though Hunt had found nicotine ineffective in this way. Further, Hirose (1932) observed that, when acetylcholine was injected into the femoral artery of a cat, the outflow of blood from the leg was diminished.

Feldberg and Minz made intra-arterial injections of acetylcholine into the splanchnic area of cats; they prepared the cats by removing the adrenals, tying the abdominal aorta below the origin of the inferior mesenteric artery, and tying the inferior vena cava below the liver. They found that injection of acetylcholine into the aorta above the origin of the coeliac artery, atropine having first been given, caused a rise of blood pressure by splanchnic vasoconstriction. This effect was diminished but not abolished by removal of the solar plexus. They found the effect was abolished by large doses of nicotine, and thought it possible that acetylcholine exerted its constrictor action by stimulating ganglia placed more peripherally than the solar plexus and inferior mesenteric ganglia.

Burn and Robinson (1951) studied the effect of acetylcholine in the rabbit ear perfused with Locke's solution, and observed that in the first few hours of perfusion it dilated the vessels. They noted that, as the perfusion was continued, the dilatation caused by a given dose of acetylcholine declined and disappeared, and that at this stage a larger dose of acetylcholine caused a constriction. They found that, once a constrictor response to acetylcholine had set in, there was no reversal to a dilator action with any dose. They supposed that both the dilator and the constrictor effects were due to a direct action on the vessels. Burn and Dutta (1948) had previously observed that the constrictor effect of acetylcholine was reversed to a dilator effect when Priscol (2-benzyl-2-imidazoline hydrochloride) was added to the perfusion fluid.

The present work was begun in order to examine the effect of nicotine on the blood vessels, and in particular to observe whether it was modified by hexamethonium.

METHOD

Rabbit ears removed from the freshly killed animal were perfused with oxygenated Locke's solution according to the method described by Burn and Robin-

son (1951), using Stephenson's recorder (1948) to measure the outflow. When the ears were to be tested on the second day, the perfusion was continued overnight without interruption. In some experiments the skin was removed with a sharp scalpel, beginning with a flap on the convex side of the ear near the proximal end. Although it was often found that it was possible to remove the skin completely on the convex surface only, as much of the skin on the rest of the ear was removed as was possible, without damage to the rest of the ear.

All substances for injection were made as concentrated acidified solutions, which were of the following strengths; acetylcholine bromide 4 mg./ml., nicotine hydrogen tartrate 2 mg./ml., atropine sulphate 1 mg./ml., hexamethonium bromide 4 mg./ml. The final dilution of each substance was made in Locke's solution, just before each injection, and the *p*H was tested with Universal indicator. The doses of the injections are expressed in terms of the salts. When the response to any substance was a dilatation, a control injection of the same volume of Locke's solution was given to eliminate any dilator effect due to the volume of the injected fluid. All injections were contained in a volume of 0.05 ml. Locke's solution.

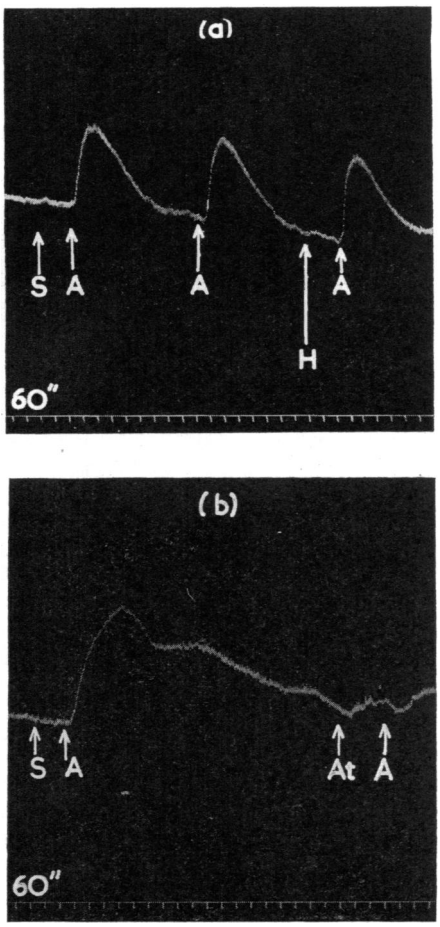

FIG 1.—(*a*) Outflow record from perfused rabbit ear; rise of the record indicates increase of outflow. S=Injection of 0.05 ml. Locke's solution. A=Injection of 2 μg. ACh in 0.05 ml. Locke's solution. H=Injection of 0.1 mg. hexamethonium. (*b*) S as in (*a*). A=Injection of 5 μg. ACh. At=Injection of 4 μg. atropine sulphate

RESULTS

Dilatation Caused by Acetylcholine.—As found by earlier workers the response to acetylcholine was initially a dilatation. This dilatation was not affected by hexamethonium, but was abolished by atropine (Fig. 1), which indicated that the dilator action of acetylcholine was muscarine-like. This observation was made in 8 trials in 4 preparations.

Constriction Caused by Acetylcholine and Nicotine.—The effect of a larger dose of acetylcholine on the ear after the perfusion had continued for some hours was different, for it caused a constriction which was blocked by hexamethonium as shown in Fig. 2*a*. The constrictor action of

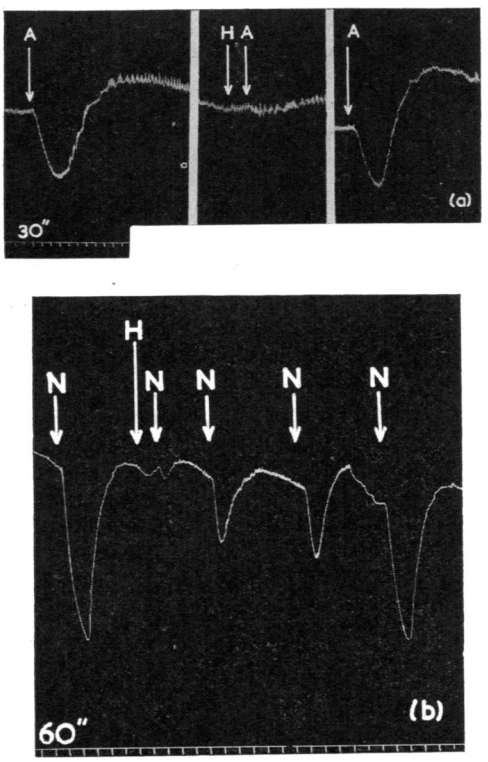

FIG. 2.—Record in this and subsequent figures as in Fig. 1. (*a*) At A, 0.1 mg. ACh was injected; at H, 0.1 mg. hexamethonium was injected. (*b*) At N, 25 μg. nicotine acid tartrate was injected; at H, 0.1 mg. hexamethonium was injected.

acetylcholine reappeared when the hexamethonium was washed out. This was observed in 7 trials in 4 preparations. Nicotine also caused a constriction (Fig. 2*b*), and this constriction was also blocked by hexamethonium. The constrictor action gradually returned as the hexamethonium was removed. The block of nicotine action was complete in 11 out of 14 trials and partial in 3 out of 14 trials ; 9 preparations were used. Nicotine, unlike acetylcholine, usually caused a constriction in the early stages of perfusion, but the constriction increased as the perfusion was continued.

These results suggested that the constrictor action of acetylcholine was a nicotine-like action.

Conversion of a Dilator Action into a Constriction.—If acetylcholine possessed both muscarine-like and nicotine-like actions in this preparation, the injection of atropine should convert the dilator action into a constrictor action under suitable conditions. This is shown in Fig. 3, in which a

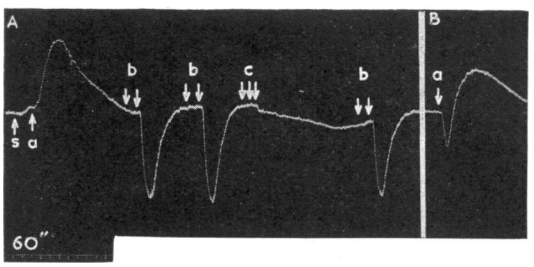

FIG. 3.—In A, at s, 0.05 ml. Locke's solution was injected; at "a," 20 μg. ACh was injected; at "b" 8 μg. atropine followed by 20 μg. ACh was injected; at "c," 0.1 mg. hexamethonium followed by 8 μg. atropine and then by 20 μg. ACh was injected. In B, at "a," 20 μg. ACh was injected.

moderately high dose of acetylcholine (20 μg.) caused a dilatation (Fig. 3a). When this dose of acetylcholine was repeated after injecting 8 μg. atropine it now caused a constriction (Fig. 3b). After the injection of 0.1 mg. hexamethonium and 8 μg. atropine, acetylcholine had no effect (Fig. 3c). The constrictor action of acetylcholine after atropine returned when the hexamethonium was washed out. In part B of Fig. 3 the effect of the same dose of acetylcholine alone is shown which was observed 1½ hr. later. At this point acetylcholine caused an initial constriction followed by a dilatation. The double response showed both nicotine-like and muscarine-like effects, the latter outlasting the former.

Mixed Effects of Acetylcholine and Nicotine.—When acetylcholine caused both constriction and dilatation, hexamethonium blocked only the constrictor action, and had no effect on the dilatation. Nicotine sometimes caused a dilatation after an initial constriction. As with acetylcholine, only the constriction was abolished by hexamethonium, while the dilatation was unaffected (Fig. 4).

The action of tetraethylammonium (TEA) on the constriction caused by nicotine and acetylcholine was similar to that of hexamethonium, though TEA often had an initial constrictor action of its own.

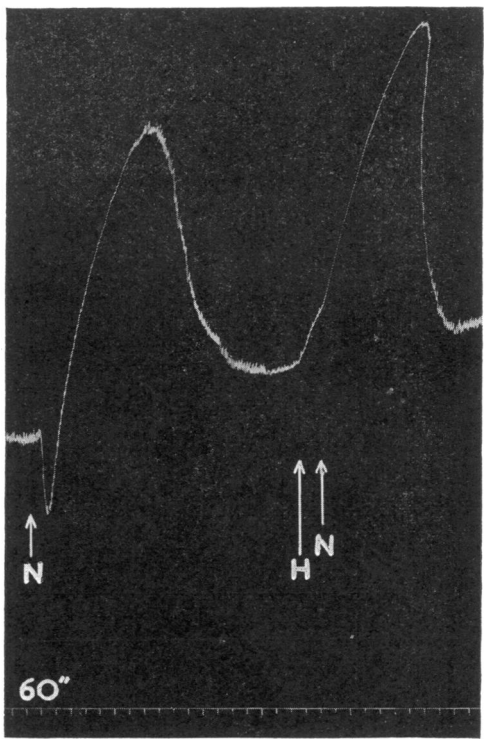

FIG. 4.—This preparation was unusual, because when it was fresh nicotine caused dilatation in 10 μg. and 20 μg. The above record, obtained on the second day, shows the effect of injecting 20 μg. nicotine at N; when 0.1 mg. hexamethonium was given first at H, the constrictor phase of the nicotine action was abolished.

Effect of a Reversing Agent.—Since Burn and Dutta (1948) had shown that the constrictor action of acetylcholine was reversed by Priscol, it seemed possible that acetylcholine and nicotine caused constriction by releasing adrenaline or a similar substance. The constriction caused by nicotine was also found to be reversed by Priscol (Fig. 5*a*). If the dilatation which nicotine caused in the presence of Priscol was due to the release of an adrenaline-like substance, then hexamethonium should abolish this response. Fig. 5*b* illustrates the finding that hexamethonium blocked the dila-

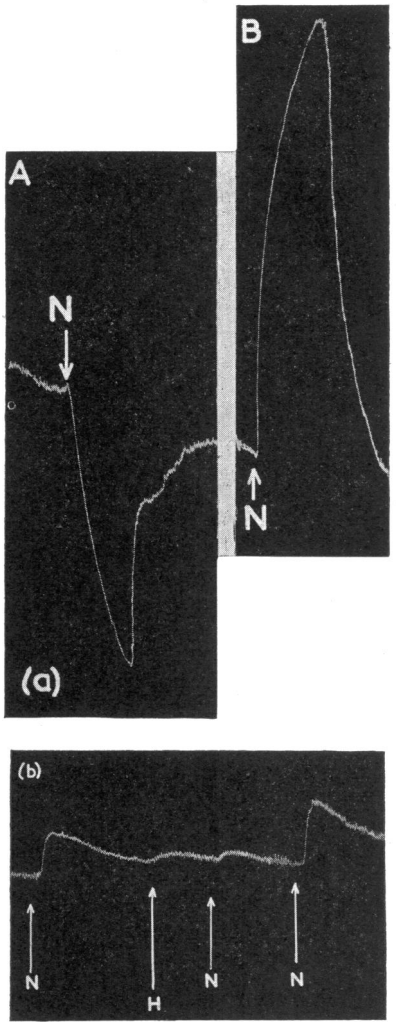

FIG. 5.—(*a*) In A the constrictor effect of injecting 5 μg. nicotine is shown. In B the ear was perfused with Locke's solution containing Priscol (20 mg. per l.). The injection of 1 μg. nicotine caused dilatation. (*b*) During perfusion of the ear with Priscol (10 mg. per l.) 10 μg. nicotine was injected at N. At H, 0.1 mg. hexamethonium was injected.

tation which nicotine caused in the presence of Priscol, and that as the hexamethonium was washed out the dilator action of nicotine returned.

Removal of the Skin.—From the results presented it appeared very likely that the site of the constrictor action of nicotine and acetylcholine was different from that of the dilator action of these two substances. I therefore decided to test the reaction of the vessels to nicotine and acetylcholine before and after the removal of most of the skin in the hope of separating these two sites anatomically. By this method it was possible to separate the dilator and constrictor actions of these two substances. Whereas in the ear with the skin intact the constriction caused by a given dose of nicotine or acetylcholine increased with the duration of the perfusion, after removal of the skin the constrictor action of both these substances was greatly reduced or absent and often a dilator response was seen. Records obtained before and after removal of the skin are shown in Fig. 6 (*a* and *b*). A dose of nicotine which caused constriction in the ear with the skin intact caused a dilatation in the same ear after removal of the skin. This dilatation which nicotine caused in the skinned ear, like the one which sometimes follows the constriction in the unskinned ear (Fig. 4), was not blocked by hexamethonium, which indicated that this was due to a direct action of nicotine. A similar result was obtained with acetylcholine (Fig. 6*b*). In one experiment the vessels of the skinned

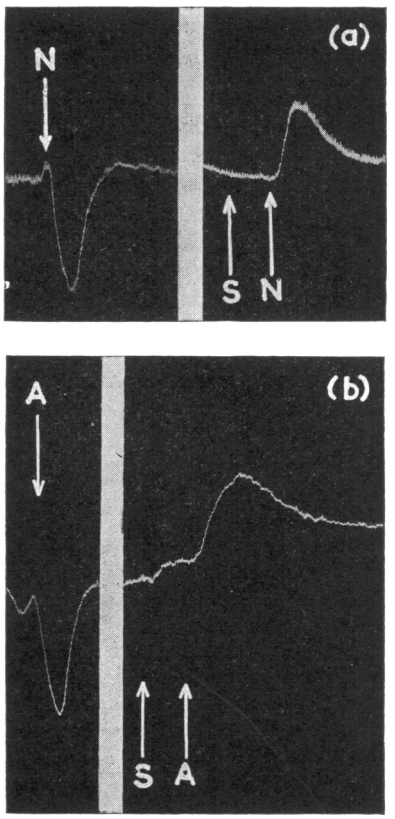

FIG. 6.—(*a*) On left is shown the constrictor action of 40 μg. nicotine. The skin was removed, and the next morning, as shown on the right, the injection of 40 μg. nicotine caused dilatation. S is control injection of perfusion fluid. (*b*) On the left is shown the constrictor action of 5 μg. ACh, at A. The skin was then removed. The next morning, as shown on the right, 5 μg. ACh caused dilatation.

ear responded with a dilatation to a dose of 0.0025 μg. (in 0.05 ml.) of acetylcholine, while the injection of the same volume of Locke's solution had no appreciable effect.

Discussion

The experimental evidence shows that both acetylcholine and nicotine can affect the vessels of the rabbit ear in two ways. Acetylcholine has a direct vasodilator action which is abolished by atropine; it is seen in almost all perfused rabbit ears when the perfusion begins. In a small proportion of preparations nicotine also has a direct vasodilator action, though this is not abolished by atropine. These dilator effects are not affected by hexamethonium. When the perfusion is continued for some hours, the vasodilator effect of acetylcholine disappears or is masked because a constrictor effect appears. This constrictor action of acetylcholine is blocked by hexamethonium. With nicotine there is a similar change from a vasodilator to a vasoconstrictor effect in the few preparations in which the former is seen, and in the majority the initial vasoconstrictor effect increases as the perfusion continues. This vasoconstrictor action is blocked by hexamethonium.

The increase in the constrictor action of nicotine as the perfusion proceeds calls to mind a similar change which was observed in the rabbit auricles when isolated and beating in Locke's solution. When freshly prepared, nicotine had no effect on the rhythm except in large amounts; when the auricles were tested 24 hr. later, after being kept overnight in Locke's solution at 4° C., they were sensitive to smaller amounts (Kottegoda, 1953).

There is a close parallelism between the action of acetylcholine on the isolated rabbit auricles and on the perfused rabbit ear vessels. In both preparations there are two effects. There is an inhibitory or vasodilator action which is abolished by atropine, and there is a stimulant or constrictor action which is blocked by hexamethonium. The stimulant action on the auricles is probably due to a release of an adrenaline-like substance, since Hoffman, Hoffman, Middleton, and Talesnik (1945) have shown that acetylcholine releases an adrenaline-like substance from the isolated and atropinized mammalian heart. There is reason to suppose that the constrictor action on the rabbit ear is also due to the release of an adrenaline-like substance, since this action is reversed to a dilator action by the presence of Priscol in the perfusion fluid.

The parallelism is not quite so complete for nicotine, since its inhibitory action on the auricles is believed to be mediated through cholinergic ganglion cells and its rarely observed dilator action on the ear vessels is not abolished by atropine. However, the stimulant action of nicotine on the auricles appears to be similar to its constrictor action on the vessels.

When the ear has been perfused until all traces of dilator response to acetylcholine is lost and constriction is regularly obtained, the removal of the skin lays bare a network of vessels covering the cartilage which react to acetylcholine and also to nicotine by vasodilatation. After removing the skin it is usually necessary to leave the ear for some hours before this response is obtained. The sensitivity to acetylcholine then is sometimes extreme, vasodilatation being observed with so little as 2.5 mμg. acetylcholine.

The removal of the skin may remove the vessels which are responsible for the constrictor response, leaving behind those which are responsible for the initial dilator response which may have been put out of action by steadily increasing oedema, or the removal of the skin may remove ganglion cells or chromaffin tissue on which acetylcholine or nicotine may act to release an adrenaline-like substance, which is responsible for the constriction. It is difficult to choose between such alternatives. Experiments are now in progress to determine the effect of nerve degeneration on this action, and when the results are available the position should be clearer.

It is interesting to note in conclusion that as long ago as 1890 Langley and Dickinson found that nicotine caused constriction followed by dilatation in the rabbit ear when observations were made in the whole animal. They also found that after large doses of nicotine subsequent doses caused only dilatation.

Summary

1. Nicotine causes vasoconstriction in the perfused vessels of the isolated rabbit ear. This constrictor action is blocked by hexamethonium and tetraethylammonium.

2. When Priscol is added to the perfusion fluid, nicotine causes dilatation. This dilatation is blocked by hexamethonium.

3. It seems probable that both these effects of nicotine are due to the release of an adrenaline-like substance.

4. Acetylcholine causes dilatation in the freshly perfused ear, but when the perfusion is continued on the second day it causes constriction. The

dilator effect is blocked by atropine, but not by hexamethonium, while the constrictor action is blocked by hexamethonium (and tetraethylammonium) but not by atropine. It is already known that the constrictor action of acetylcholine is reversed to a dilator action by Priscol.

5. It seems probable that the constrictor action of acetylcholine, like that of nicotine, is due to the release of an adrenaline-like substance.

6. When a preparation is taken in which both nicotine and acetylcholine exert a constrictor action, the removal of the skin leaves a network of vessels on the cartilage in which both substances cause dilatation.

7. There is a close similarity in the two actions which acetylcholine exerts in the rabbit ear vessels and the two actions it has on the isolated rabbit auricles.

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