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THE EFFECTS OF NICOTINE ON THE BLOOD VESSELS OF SKELETAL MUSCLE IN THE CAT. AN INVESTIGATION OF VASOMOTOR AXON REFLEXES

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From experiments recently performed on the post-contraction hyperaemia in skeletal muscle, it was concluded that the vasodilatation is brought about by an axon reflex in dilator fibres of the sympathetic outflow, probably cholinergic in nature (Hilton, 1953).

Axon reflexes in post-ganglionic sympathetic fibres have already been produced in the mammalian skin in response to intradermal injections of acetylcholine and nicotine. Coon & Rothman (1940) described, as the result of such an axon reflex, a widespread pilomotor response in the cat and man. Wada, Arai, Takagaki & Nakagawa (1952) showed that, in man, the sweating response seen around the site of injection of these drugs is similarly produced. They showed, in addition, that higher concentrations ($1:10^3$) of nicotine abolished the reflex response previously obtained with lower concentrations ($1:10^5$).

These findings suggested the possibility that nicotine could be a useful agent for eliciting axon reflexes in the vasomotor nerve fibres in skeletal muscle and, in addition, for paralysing the axon reflex responsible for the post-contraction hyperaemia. An account of some of the experimental results has already been given in a preliminary report (Hilton, 1952).

METHODS

All the experiments were performed on cats under chloralose anaesthesia (75–80 mg/kg). The venous outflow from the gastrocnemius muscle was recorded, using a drop-chamber and the Gaddum drop timer. The operative procedures used to obtain the venous outflow, to make arterial injections and to stimulate the tibial nerve, and all the apparatus used have been previously described (Hilton, 1953). Heparin (1000 units/kg) was given intravenously before any vessels were opened.

In some experiments the sciatic nerve was sectioned 8–15 days before the actual experiment. In others, unilateral lumbar sympathectomy, consisting of removal of the five lower lumbar ganglia

and the intervening portions of the chain, was performed 14–27 days before the final experiment. All these operations were carried out aseptically under pentobarbitone anaesthesia.

In order to study the effects of drugs on muscles poisoned with botulinum toxin, 25,000 mouse LD50's of type A toxin were injected aseptically into the gastrocnemius under ether anaesthesia 1–3 days before the actual experiment, as previously described (Hilton, 1953).

Except where otherwise stated, arterial injections of vasomotor drugs were made in 0·1 ml. saline. The doses of nicotine given refer to the tartrate and not to the base.

RESULTS

The response to arterial injections of nicotine

It was found that nicotine, when injected arterially into the gastrocnemius muscle, produces vasoconstrictor and vasodilator effects. Fig. 1*a* shows typical changes in the venous outflow from the muscle in response to such

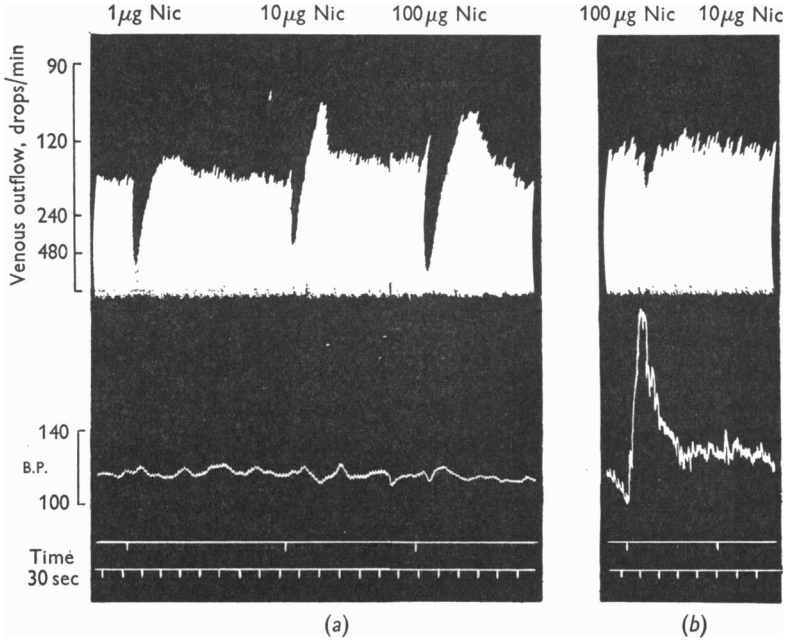

Fig. 1. Records of venous outflow (Gaddum drop timer) from gastrocnemius muscle, and of arterial blood pressure. (*a*) Cat, 3·0 kg, arterial injections of 1, 10 and 100 μg nicotine. (*b*) Cat, 2·5 kg, intravenous injections of 100 and 10 μg nicotine.

injections. These changes occur without any alteration of the arterial blood pressure and are therefore due solely to an action of the nicotine on the vasomotor tone in the muscle. Small doses (1 μg) give rise to a vasodilatation, which is usually followed by constriction when larger doses (10–100 μg) are injected. The complex response to larger doses varies somewhat from one animal to another: in general, the higher the blood pressure the larger is the

constrictor component. Occasionally, 100 μg nicotine produces a pure constriction (Fig. 2) and, in a few animals, a pure dilatation.

These vasomotor effects are certainly not due to the fact that small amounts of nicotine will have entered the general circulation, for when 10 μg nicotine are given intravenously, no change is seen in blood pressure or muscle blood flow, and further, although the intravenous injection of 100 μg nicotine causes

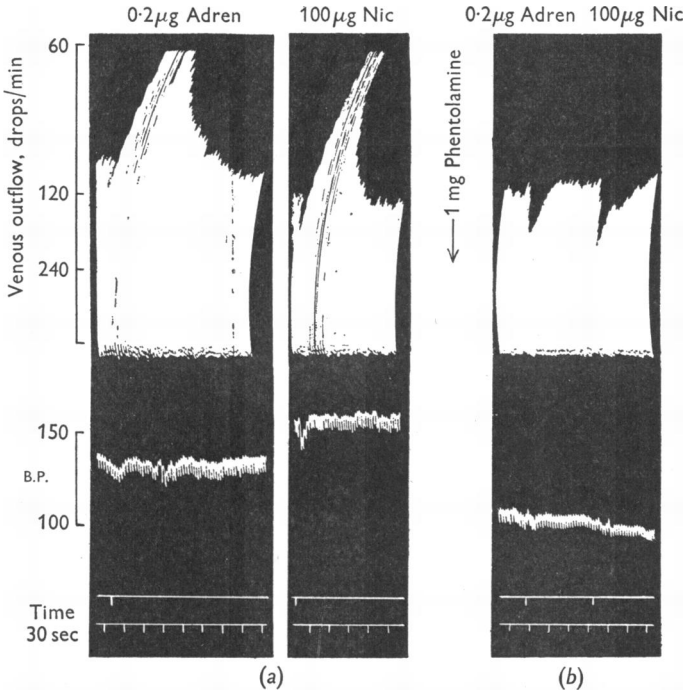

Fig. 2. Cat, 3·0 kg. Records of venous outflow from gastrocnemius muscle, and of arterial blood pressure. Effects of arterial injection of 0·2 μg adrenaline and 100 μg nicotine (*a*) before, and (*b*) after an arterial injection of 1 mg phentolamine in 1 ml. saline.

a large rise in blood pressure, it either has no effect on the venous outflow or causes but a slight increase in flow which is almost certainly passively produced by the increase in blood pressure (Fig. 1*b*). It follows that the arterially injected nicotine produces its effects by acting within the muscle, a conclusion which is in accord with the finding that these effects are not altered by cutting the sciatic nerve. They could only result, therefore, either from an axon reflex or from a direct action of nicotine on the blood vessels. These modes of action do not exclude one another, and the effects may be produced in both ways.

Analysis of the vasoconstrictor effect

Although the vasoconstrictor effect of nicotine injected arterially into the muscle is not altered by acute nerve section, it disappears on nerve degenera-

tion. Eight days after section of the sciatic nerve such vasoconstriction is no longer seen. The nerve fibres responsible belong to the sympathetic system because the vasoconstrictor effect is also lost 14 days after removal of the ipsilateral lumbar sympathetic chain and ganglia. Hence, it appears that the constrictor component of the nicotine response is produced by stimulation of sympathetic constrictor nerve endings: it should therefore be abolished by adrenolytic drugs. That this is so is shown by the experiments illustrated in Figs. 2 and 3.

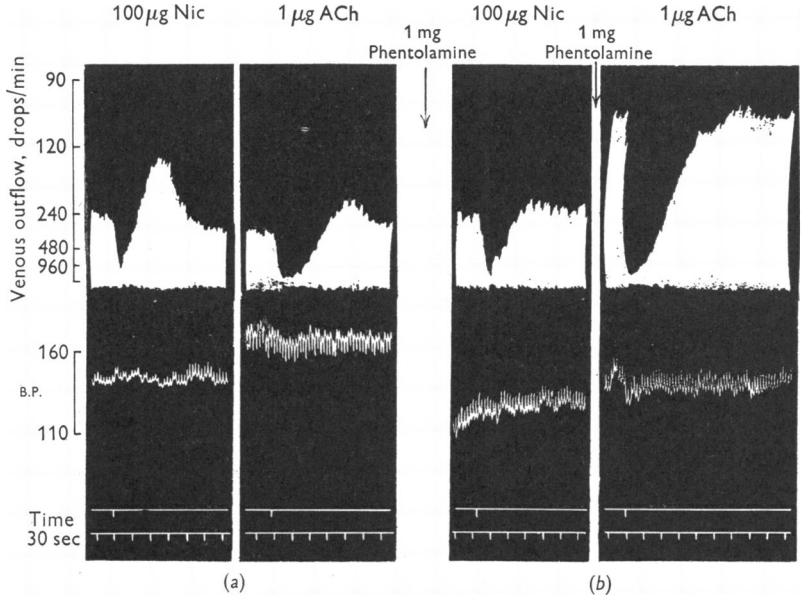

Fig. 3. Cat, 5·0 kg. Records of venous outflow from gastrocnemius muscle, and of arterial blood pressure. Effects of arterial injection of 100 μg nicotine and 1 μg acetylcholine (*a*) before, and (*b*) after arterial injection of phentolamine. Botulinum toxin had been injected into the muscle 3 days previously.

In Fig. 2*a*, the response of an innervated muscle to 0·2 μg adrenaline hydrochloride (Parke Davis) is compared with that to 100 μg nicotine: the constrictor effect of nicotine is particularly well marked. From Fig. 2*b*, it is seen that an arterial injection of 1 mg phentolamine (Regitine, Ciba) reverses the response to both adrenaline and nicotine. It might appear from this experiment as though phentolamine not only abolishes the constrictor response of nicotine but converts it, like that of adrenaline, into a vasodilator effect. In fact, it has only unmasked the normal dilator effect of nicotine. This is evident from experiments such as that of Fig. 3, in which nicotine produces the usual complex response: i.e. vasodilatation followed by constriction. In such experiments the effect of phentolamine is to abolish the vasoconstrictor response

while the vasodilator effect is but slightly accentuated, which would be expected from the removal of the vasoconstrictor component.

Results similar to those with nicotine are occasionally obtained with acetylcholine. In a few animals, arterial injection of 1 μg acetylcholine produces, in addition to the usual vasodilator response, a vasoconstriction (Fig. 3*a*) which again is abolished by phentolamine (Fig. 3*b*), and which is therefore probably also produced through constrictor nerve fibres.

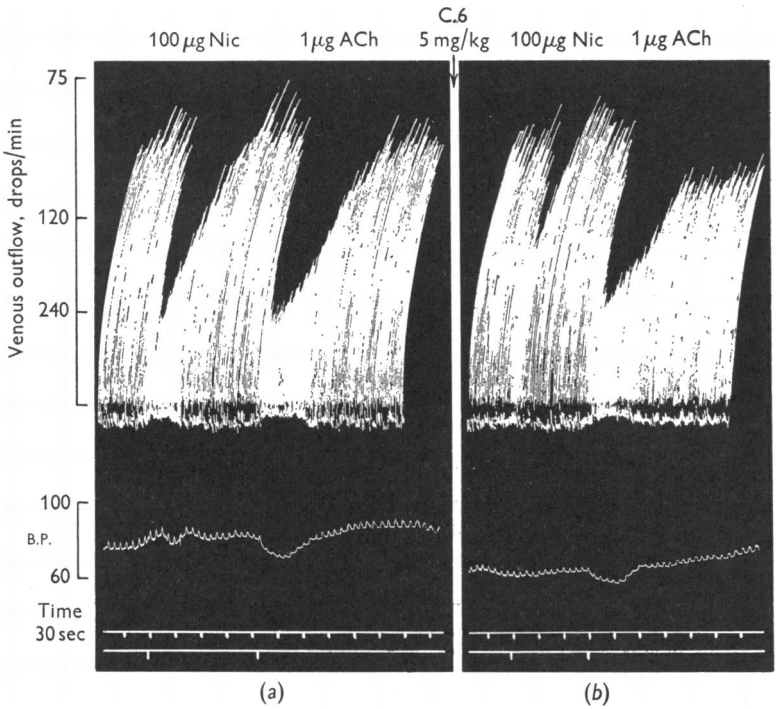

Fig. 4. Cat, 2·3 kg. Records of venous outflow from gastrocnemius muscle, and of arterial blood pressure. Effects of arterial injection of 100 μg nicotine and 1 μg acetylcholine (*a*) before, and (*b*) after arterial injection of 5 mg/kg hexamethonium bromide in 1 ml. saline.

Analysis of the vasodilator effect

The vasodilator response to an arterial injection of 100 μg nicotine is usually slightly smaller than that of 1 μg acetylcholine similarly injected. This is seen in the experiment of Fig. 4*a*, in which a previous arterial injection of 1 mg phentolamine had been given to abolish the constrictor component of the nicotine response.

This vasodilator action of nicotine is greatly reduced by ganglionic blocking agents. Both tetraethylammonium bromide and hexamethonium bromide, which are known to block the stimulating action of nicotine and acetylcholine

not only on autonomic ganglia but also on sensory and autonomic nerve endings, were used for this purpose. After either of these compounds has been injected arterially into the muscle, the vasodilator response to a subsequent injection of nicotine is found to be greatly reduced, while the acetylcholine dilatation is scarcely affected. This is shown for hexamethonium bromide in Fig. 4*b*. In order to be effective, the hexamethonium has to be left in the muscle for 2–3 min: this is achieved by temporarily excluding the venous outflow immediately after the injection. The tetraethylammonium was injected in a dose of 15 mg/kg, and did not have to be left in the muscle in order to be effective.

These findings suggest that the dilator effect of nicotine is largely the result of stimulation of vasodilator nerve endings, whereas the dilator effect of acetylcholine (at least that obtained with the relatively large doses used in these experiments) is independent of such vasodilator nerve fibres.

The small vasodilator response to nicotine which persists after the application of ganglionic blocking agents must be due to a direct action of the drug on the muscle vessels themselves. Further evidence for such a non-nervous component of the vasodilator response is provided by the finding that nicotine does not completely lose its vasodilator effect after degeneration of the sciatic nerve or after inactivation of the cholinergic nerve endings by injection of botulinum toxin. The experiments shown in Fig. 3, in which nicotine produced a good dilator effect, were performed in an animal which had had botulinum toxin injected into its gastrocnemius 3 days previously. The vasodilator action of acetylcholine was enormously enhanced, probably as a result of the functional denervation produced by the toxin. In chronically totally denervated or chronically sympathectomized muscles and in those poisoned with botulinum toxin, the dilator effect of a small dose of nicotine (1 μg) is usually greater than that obtained in normal muscles; but on increasing the dose, the vasodilator effect does not increase, so that the response to 100 μg is often smaller than that obtained with this dose in the normal muscle (Figs. 5*a* and 6*a*). As a result of the reduction in the dilator response to larger doses of nicotine and the enhanced effectiveness of acetylcholine, 100 μg nicotine produce in these muscles a response which is always somewhat smaller than that to even 0·1 μg acetylcholine, and which may be no greater than that to 0·01 μg acetylcholine. In a few experiments with muscles that had been injected with botulinum toxin, and in one chronically denervated muscle, the vasodilator response to 100 μg nicotine was found to be as resistant to hexamethonium bromide as was that to 1 μg acetylcholine, both being only slightly reduced. This is illustrated in Fig. 6. Phentolamine was given first as in the experiments with hexamethonium on normal muscles, so as to provide similar experimental conditions for the comparison. As seen in Fig. 6*b*, there is a small reduction of both responses, which could be accounted for by the fall in arterial blood pressure (following

the injection of hexamethonium bromide), but the nicotine response is no more affected than the acetylcholine response. The relative vasodilator effects of nicotine and acetylcholine in the muscle treated with botulinum toxin are very similar to those obtained in the normal muscle after hexamethonium bromide, as seen from a comparison of Fig. 6*a* with Fig. 4*b*.

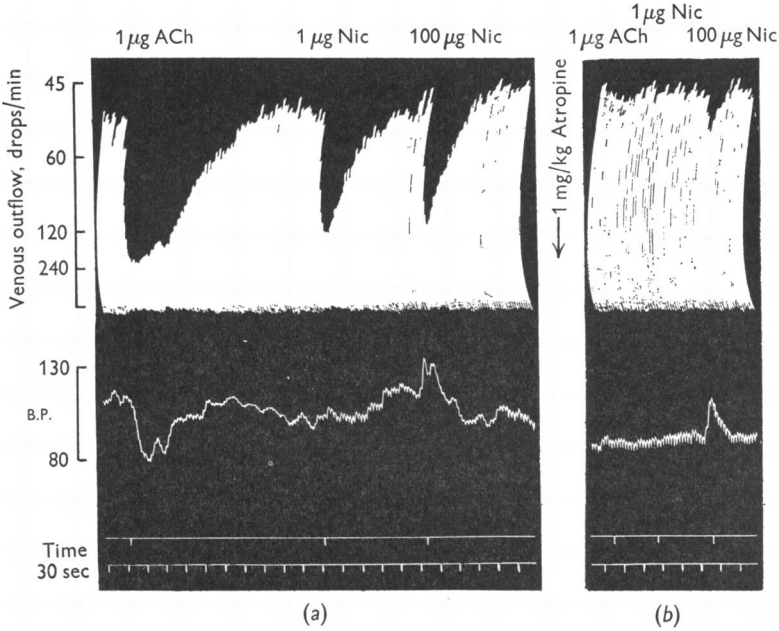

Fig. 5. Cat, 2·8 kg. Records of venous outflow from gastrocnemius muscle, and of arterial blood pressure. Botulinum toxin had been injected into gastrocnemius muscle 24 hr previously. Effects of arterial injection of 1 μg acetylcholine, 1 and 100 μg nicotine (*a*) before, and (*b*) after 1 mg/kg atropine, injected intravenously.

This hexamethonium-resistant nicotine vasodilatation is abolished by atropine in the same doses as those required to abolish the vasodilator effect of acetylcholine (Fig. 5*b*).

The effect of large 'paralysing' doses of nicotine

The conclusion that the vasodilatation produced by nicotine in the muscle of the normal cat is partly nervous in origin, partly a direct effect, is supported by the results obtained after arterial injection of a large, 'paralysing' dose of nicotine (1 mg). Such a dose of nicotine does not affect either the dilator response to acetylcholine or the constrictor response to adrenaline, but it abolishes the vasoconstrictor effect of nicotine itself (Fig. 7). The vasodilator action of a small dose (1 μg) of nicotine is also abolished, probably because it is nervous in origin; a larger dose (100 μg) of nicotine retains some vasodilator effect which is probably a direct action of this drug on the vessels.

To obtain these results the paralysing dose of nicotine has to be left in the muscle for 2–3 min by occluding the venous outflow. Immediately after releasing the clamp and restoring the circulation through the muscle, all drug responses are sluggish: the results shown in Fig. 7*b* are obtained after an interval of 10 min.

Large, paralysing doses of nicotine also greatly reduce the hyperaemic after-effect of a short tetanic contraction of the muscle. In Fig. 8*a* are seen the response to 1 μg acetylcholine and the hyperaemia following a 10 sec tetanus of the gastrocnemius muscle, obtained by stimulation of the tibial nerve.

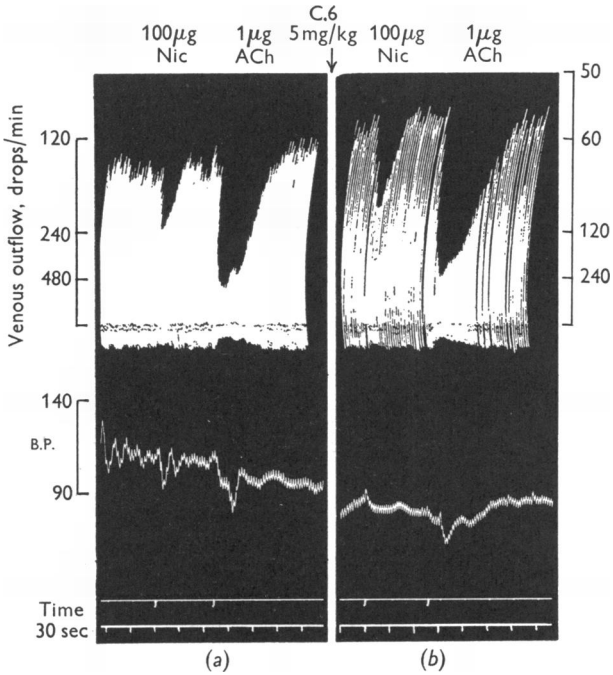

Fig. 6. Cat, 1·8 kg. Records of venous outflow from gastrocnemius muscle, and of arterial blood pressure. Botulinum toxin had been injected into gastrocnemius muscle 3 days previously. Effects of arterial injection of 100 μg nicotine and 1 μg acetylcholine (*a*) before, and (*b*) after arterial injection of 5 mg/kg hexamethonium bromide in 1 ml. saline.

Between *a* and *b*, 1 mg nicotine tartrate was injected. Ten minutes later an injection of 200 μg nicotine still produced some vasoconstriction (Fig. 8*b*), probably because the axon reflex paralysis was not complete. At this stage, a 10 sec tetanic contraction of the muscle was maintained at about 90% of normal tension, but the post-contraction hyperaemia was practically abolished. 1 μg acetylcholine still produced its normal, or only a slightly reduced vasodilatation. When the effect of the paralysing dose of nicotine has worn off (after about 45 min), the post-contraction hyperaemia returns.

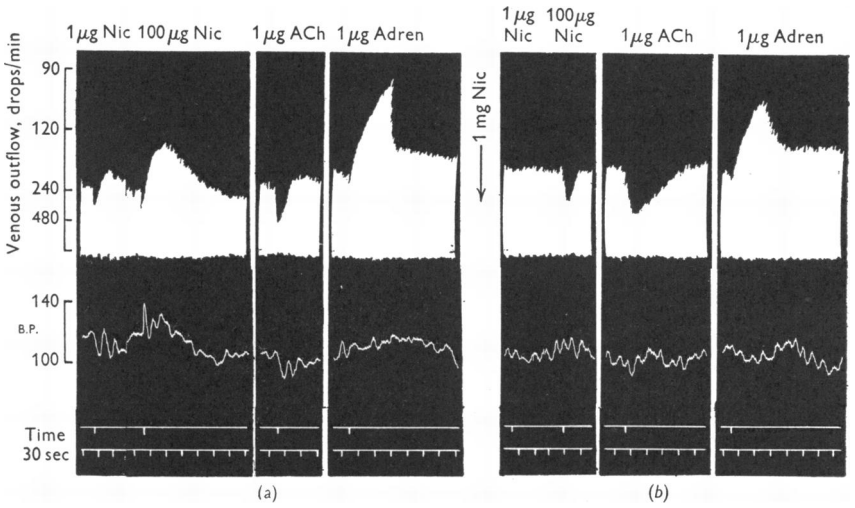

Fig. 7. Cat, 3·0 kg. Records of venous outflow from gastrocnemius muscle, and of arterial blood pressure. Effects of arterial injection of 1 and 100 μg nicotine, 1 μg acetylcholine and 1 μg adrenaline (*a*) before, and (*b*) 10 min after arterial injection of 1 mg nicotine in 1 ml. saline.

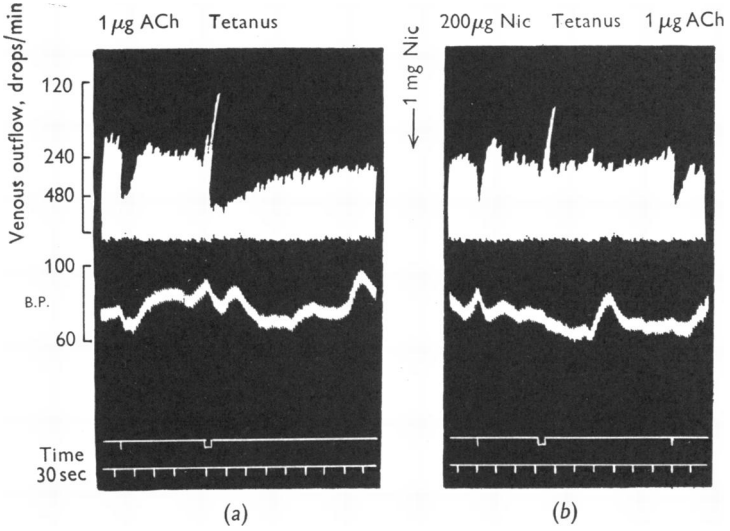

Fig. 8. Cat, 2·8 kg. Records of venous outflow from gastrocnemius muscle, and of arterial blood pressure. Effects of arterial injection of 1 μg acetylcholine and of 10 sec tetanus of gastrocnemius (*a*) before, and (*b*) 10 min after arterial injection of 1 mg nicotine in 1 ml. saline. Effect of 200 μg nicotine also shown (*b*).

DISCUSSION

The finding that nicotine elicits axon reflexes in sympathetic nerve fibres in skeletal muscle is in line with the previous observations of similar axon reflexes in the mammalian skin. Since nicotine also stimulates sensory nerve endings in the cat's skin (Brown & Gray, 1948; Douglas & Gray, 1953) and carotid body (Douglas, 1952) it is clear that, far from acting in a specific manner at autonomic ganglia, this drug is quite indiscriminate in its effects on nervous structures.

In skeletal muscle, nicotine produces, by means of an axon reflex in sympathetic fibres, both vasoconstriction and vasodilatation. The constrictor effect of nicotine, on arterial injection into the muscle, is entirely the result of such an axon reflex, while the vasodilatation is mainly, though not wholly, accounted for in this way. A small part of the vasodilator effect appears to result from a direct action of nicotine on the vessel wall, even in the normally innervated muscle.

It seems, therefore, that nicotine can stimulate sympathetic nerve endings in the same doses as those required to stimulate ganglion cells, and, further, that in both cases it acts as readily on adrenergic as on cholinergic fibres. Certainly the vasoconstriction seen in these experiments must be effected via adrenergic fibres, and the results obtained in the muscles injected with botulinum toxin suggest that that part of the vasodilator effect which is nervous in origin is mediated by cholinergic fibres.

The fact that nicotine can produce effects through axon reflexes must be kept in mind when interpreting the actions of nicotine on isolated organs. For instance, Ambache (1951) and Ambache & Edwards (1951) obtained with nicotine an adrenaline-like effect on the isolated intestine of the rabbit and the kitten's stomach, which they considered to be evidence for the presence of local ganglion cells giving rise to adrenergic fibres. Accepting that this effect of nicotine is nervous in origin, there is no need to postulate the existence of such ganglion cells in the wall of the intestine; for the effect could as well be explained as the result of an axon reflex in adrenergic nerve fibres supplying the intestine. Furthermore, the inhibitory effect of nicotine occasionally observed by Evans & Schild (1953) on preparations of the circular muscle layer of the cat's jejunum containing no ganglion cells might also be due to the action of nicotine on adrenergic nerve fibres—a possibility which was considered by these authors.

It was previously shown that the intramuscular injection of local anaesthetics and botulinum toxin practically abolished the hyperaemic response to muscular contraction, while leaving the vessels with their normal sensitivity to substances acting directly on the vessel wall, such as acetylcholine (Hilton, 1953). In the muscle poisoned by botulinum toxin, the vasodilator response

to acetylcholine was even increased, a finding which is attributed to the functional denervation produced by the toxin. It has now been shown that botulinum toxin affects the axon reflex vasodilatation produced by nicotine in the same way as it affects the post-contraction hyperaemia, suggesting that the nerve fibres activated are the same in both vascular reactions. This suggestion is strengthened further by the results obtained with large, paralysing doses of nicotine. These abolish the vasoconstrictor response to smaller doses of nicotine and reduce the vasodilator response, both effects being probably due to the abolition of the axon reflexes in vasomotor nerve fibres. The fact that these paralysing doses of nicotine also abolish the post-contraction hyperaemia lends further support to the concept of an axon reflex as the main mechanism producing the post-contraction hyperaemia, and leads to the conclusion that the nerve fibres activated in this reaction are the same as those which are stimulated by nicotine and responsible in the main for its vasodilator effect also.

SUMMARY

1. Nicotine, injected arterially into the gastrocnemius muscle of the cat, produces vasoconstrictor and vasodilator responses which have been analysed.

2. The vasoconstrictor response is attributed to an axon reflex in adrenergic sympathetic fibres whose endings are stimulated by the nicotine. This conclusion is based on the following findings: (*a*) the vasoconstriction is no longer obtained if the sciatic nerve is sectioned, or the lumbar sympathetic chain and ganglia are removed, and time is allowed for nerve degeneration; (*b*) acute section of the sciatic nerve does not affect the vasoconstrictor response; and (*c*) phentolamine, in doses which reverse the constrictor action of adrenaline, abolishes the constrictor response to nicotine.

3. The vasodilator response is attributed partly to an axon reflex in cholinergic vasodilator fibres, and partly to a direct action of nicotine on the muscle blood vessels. The evidence for this conclusion is as follows: (*a*) in normal muscles, hexamethonium and tetraethylammonium bromide considerably reduce, but do not abolish, the vasodilator response: (*b*) in chronically denervated muscles and in muscles poisoned with botulinum toxin, the vasodilator response to nicotine (100 μg) is usually smaller than normal. This response is scarcely affected by hexamethonium bromide but is abolished by atropine.

4. In large doses, nicotine paralyses these axon reflex vascular reactions, while leaving the vessels normally responsive to the vasodilator action of acetylcholine and the vasoconstrictor action of adrenaline.

5. Large, 'paralysing' doses of nicotine also abolish the post-contraction hyperaemia. This result lends support to the concept of an axon reflex as the main mechanism producing the post-contraction hyperaemia in skeletal

muscle, and leads to the conclusion that the nerve fibres activated in this reaction are the same as those which are stimulated by nicotine and responsible also, in the main, for its vasodilator effect.

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