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BLOCKING ACTION OF DECAMETHONIUM AT DIFFERENT SITES IN THE AUTONOMIC NERVOUS SYSTEM OF THE CAT

BY

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Doses of decamethonium sufficient to paralyse skeletal and respiratory muscles in the cat for 20 to 30 min can reversibly block transmission at several sites in the autonomic nervous system. The sympathetic vasodilator outflow to skeletal muscle was blocked at the post-ganglionic nerve endings, probably by preventing the release of acetylcholine. The effects of vagal stimulation on heart-rate and intestinal contraction were blocked in most experiments, possibly by an action on pre-ganglionic as well as post-ganglionic nerve endings. However, decamethonium did not block all cholinergic nerve endings—for example, it did not diminish either the effects of stimulation of the chorda tympani on the submandibular salivary gland or those of pelvic nerve stimulation on the bladder.

Decamethonium iodide is extensively used as a neuromuscular blocking agent in animal experiments. It was being used for this purpose, during experiments on the activation of cholinergic vasodilator nerves to skeletal muscle in decerebrate cats (Abrahams, Hilton & Zbrozyna, 1960), when it was found that the dose used to paralyse skeletal and respiratory muscles for 20 to 30 min also abolished the action of the vasodilator nerve fibres. Further experiments have shown that these doses of decamethonium affect transmission at a number of sites in the autonomic nervous system of the cat.

METHODS

The experiments were performed on cats anaesthetized with chloralose (70 mg/kg) given intravenously after the induction of anaesthesia with ethyl chloride and ether.

Regional blood flows were registered by the venous outflow technique using a transistorized drop recorder. The preparations for muscle, skin and submandibular salivary gland blood flow were those previously described (Hilton & Lewis, 1955; Abrahams, Hilton & Zbrozyna, 1960). Salivary secretion was recorded from the cannulated duct of one submandibular salivary gland: the gland was stimulated via a fluid electrode attached to the cut chordo-lingual nerve. The heart rate was recorded by means of a Statham strain gauge transducer connected to a cannula in one femoral artery, records being made with a high-speed pen recorder.

Records of intestinal activity were made *in situ* by introducing a small rubber bag into a loop of small intestine through a small slit. About 1 ml. of normal saline was introduced into the rubber bag, which was then connected by polythene tubing to a Statham strain gauge. One vagus nerve was dissected free in the neck, divided, and the peripheral stump placed on platinum stimulating electrodes and immersed in liquid paraffin. Stimuli were supramaximal square waves, 10/sec, with a pulse width of 1 msec.

To record bladder pressures, the urethra was exposed and cannulated with wide bore polythene tubing. A few ml. of normal saline were introduced into the bladder, and the cannula was connected to a Statham strain gauge. The pelvic nerve was dissected free on one side, and the cut distal end introduced into a fluid electrode for electrical stimulation.

Localized regions of the hypothalamus were stimulated through monopolar steel electrodes placed stereotactically, as previously described (Abrahams, Hilton & Zbrozyna, 1960).

In some cats the spinal roots L6, L7 and S1 were sectioned aseptically under pentobarbitone anaesthesia. In the final experiment carried out 10 to 14 days later, the sciatic nerve was stimulated via platinum electrodes mounted in a perspex cuff. The stimuli were supramaximal square waves at 32/sec, pulse width being 10 msec.

Decamethonium was given as the iodide, and gallamine as the triethiodide (Flaxedil). All doses are given as the salt.

RESULTS

In 5 cats the cholinergic vasodilator nerves to skeletal muscle were activated by localized electrical stimulation of brain stem regions concerned with the defence

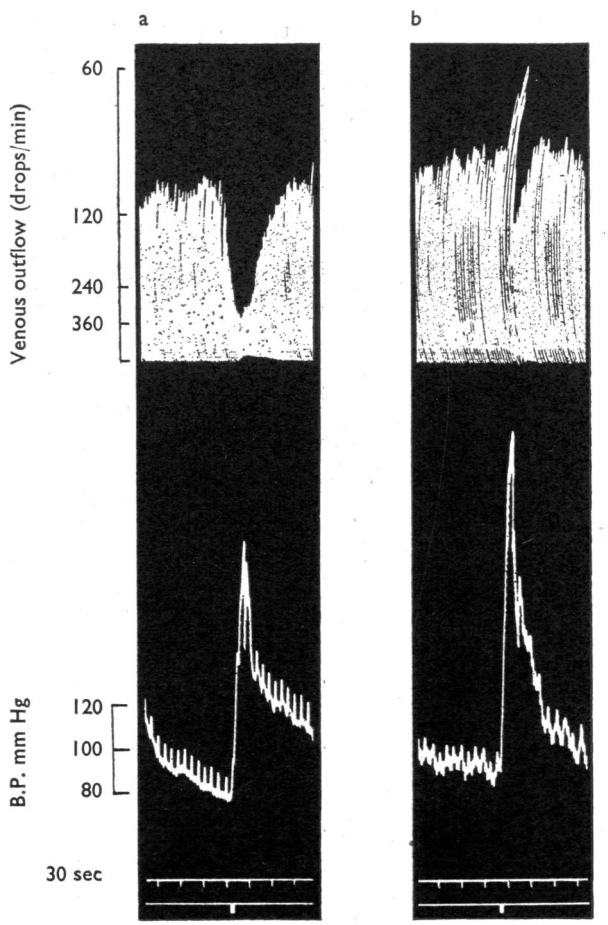

Fig. 1. Cat, 3 kg: records of venous outflow from skinned hind-limb (Gaddum drop-timer) and arterial blood pressure. Effect of electrical stimulation at a point in tegmentum ventral to superior colliculus before (*a*) and after (*b*) decamethonium (100 μg/kg) injected intravenously.

reaction (Abrahams, Hilton & Zbrozyna, 1960). The muscle vasodilatation was abolished in 3 cats by the intravenous injection of 100 μg/kg of decamethonium, as shown in Fig. 1. In the 2 other cats this dose reduced the vasodilatation, which, however, was abolished by administering further doses.

The initial injection of decamethonium itself caused a small dilatation of the muscle blood vessels. In the 2 cats in which a single dose of decamethonium did not abolish the vasodilatation produced by brain stem stimulation, it was found that subsequent injections of decamethonium still produced an increase in muscle blood flow. When a dose level had been reached at which further injection of decamethonium no longer had a vasodilator effect, brain stem stimulation also no longer produced a vasodilatation. In one cat this required a dose of 500 μg/kg.

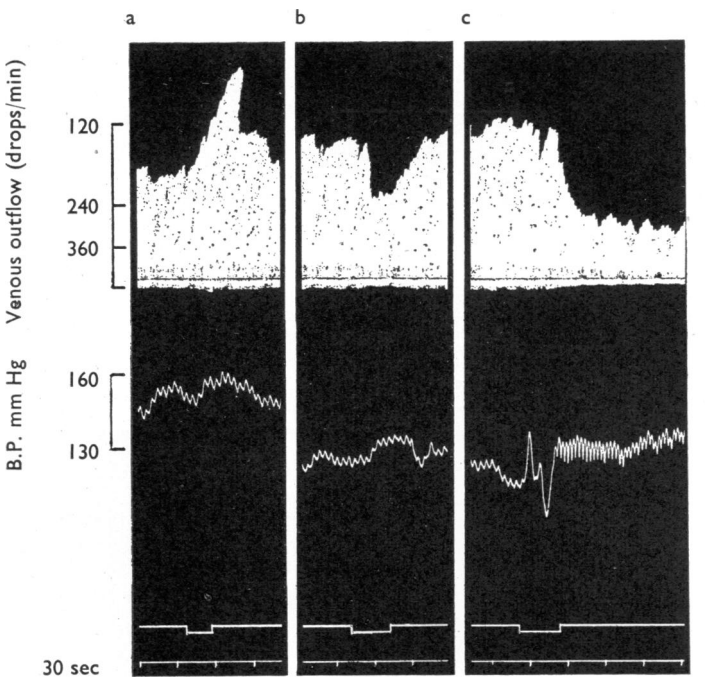

Fig. 2. Cat, 3.6 kg: records of venous outflow from gastrocnemius muscle, and of arterial blood pressure, 14 days after section of spinal roots L6–S1. Effect of sciatic nerve stimulation before (*a*) and after (*b*) 1(N-ethyl-N-2-bromoethylaminomethyl)naphthalene, SY/28 (100 μg), injected arterially. At (*c*) effect of decamethonium (100 μg/kg), injected intravenously.

Electrical stimulation of the regions of the brain stem which produce active muscle vasodilatation also elicits a number of other autonomic effects. These include vasoconstriction in skin, a rise in arterial blood pressure, pupil dilatation, retraction of the nictitating membrane, and pilo-erection. None of these responses was affected by the doses of decamethonium used. It therefore seemed likely that decamethonium was exerting its effect not on the muscle vasodilatation centrally, nor at the autonomic ganglia, but rather at the vasodilator nerve endings. This was confirmed by experiments in which the post-ganglionic vasodilator nerve fibres were stimulated

directly, along their course through the sciatic nerve. The spinal roots L6 to S1 had been sectioned 10 to 14 days previously, so that sciatic nerve stimulation only excited sympathetic nerve fibres to the muscles of the lower leg. The action of vasoconstrictor fibres as seen in Fig. 2*a* was blocked by two different drugs, in 5 experiments by guanethidine and in 2 experiments by 1(N-ethyl-N-2-bromo-ethylaminomethyl)naphthalene (SY-28). The latter was given by intra-arterial injection after which sciatic nerve stimulation then produced a vasodilatation as seen in Fig. 2*b*. Guanethidine was given either intravenously in a dose of 3 mg/kg, or by local intra-arterial injection in a dose of 30 μg/kg ; in either case, paralysis of the vasoconstrictor endings was complete after about 20 min.

When decamethonium was injected after one of these two drugs, it caused a prolonged vasodilatation (Fig. 2*c*). This vasodilatation gradually disappeared over a period of about 20 min. It was then possible to test again the vasodilator effect of sciatic nerve stimulation. This was usually blocked for a further period of 20 min (Fig. 3*b*).

There are similarities between some of the effects of decamethonium on the vasodilator fibres, and its effects on the neuromuscular junction. On intravenous

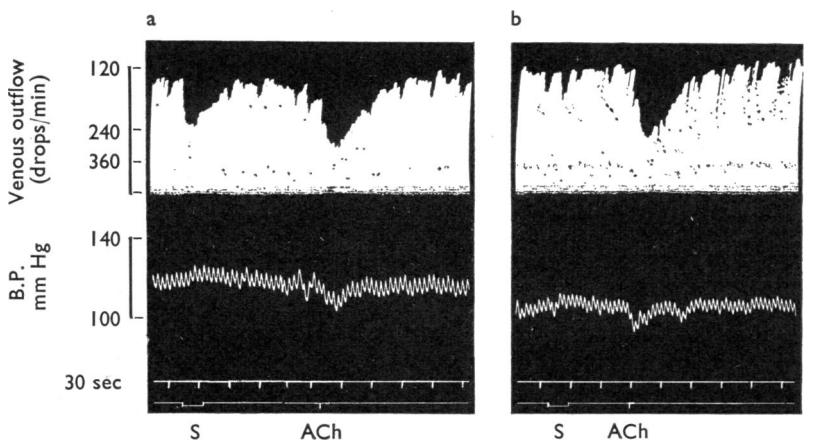

Fig. 3. Cat, 2.25 kg: records of venous outflow from gastrocnemius muscle and of arterial blood pressure, 14 days after section of spinal roots L6–S1. Guanethidine (100 μg) administered intra-arterially. Effects of arterial injection of 0.1 μg acetylcholine (ACh) and of sciatic nerve stimulation (S) before (*a*) and after (*b*) decamethonium 100 μg/kg injected intravenously.

injection, decamethonium causes muscular fasciculation, an excitatory action, before it produces neuromuscular block (Paton & Zaimis, 1949). This is paralleled by the vasodilatation that it produces on injection. Another similarity is found in the antagonism of other blocking agents towards decamethonium. Paton & Zaimis (1959) showed that both curare and gallamine could antagonize the neuromuscular blocking action of decamethonium. We have found that gallamine itself has no action on the vasodilator fibres, but that it can antagonize the action of decamethonium. If an injection of decamethonium is preceded by a paralytic dose of gallamine (2 mg/kg), then the decamethonium is without effect on muscle

vasodilatation (Fig. 4*c*). If time is allowed for the muscular paralysis following these injections to wear off, and a further dose of decamethonium is injected, the vasodilatation is markedly reduced (Fig. 4*d*).

Notwithstanding these findings, the mode of action of decamethonium at the vasodilator nerve endings is not identical with that at the neuromuscular junction,

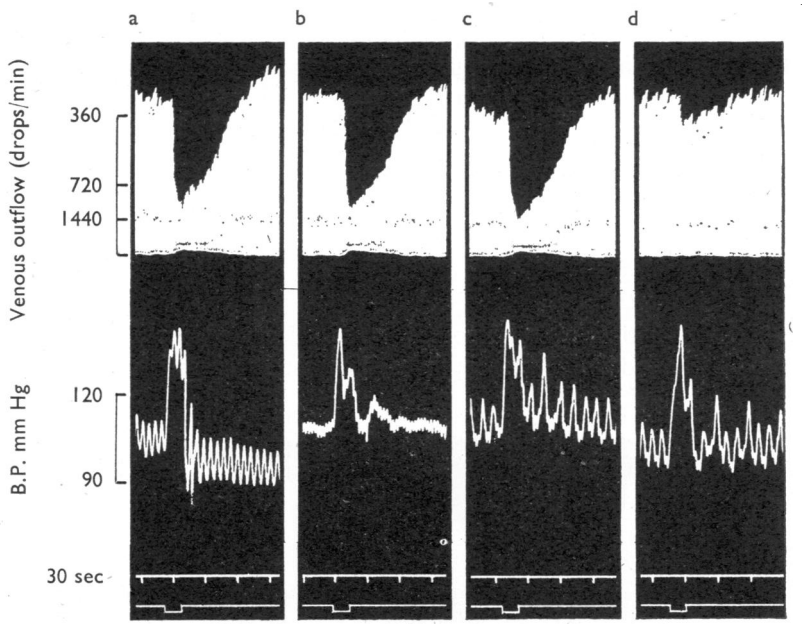

Fig. 4. Cat, 2.8 kg: records of venous outflow from skinned hind limb and arterial blood pressure. Effect of electrical stimulation at a point in hypothalamus. Gallamine (2 mg/kg) injected intravenously between (*a*) and (*b*); followed by decamethonium (100 μg/kg) a few min later between (*b*) and (*c*), and again after all drug effects had worn off, between (*c*) and (*d*).

for in 2 experiments in which the response to nerve stimulation was blocked by decamethonium, an intra-arterial injection of acetylcholine still produced a muscle vasodilatation (Fig. 3*b*).

Effects on the vagus innervation of the heart

Curare and gallamine, as well as blocking neuromuscular transmission, abolish the action of the vagus on the heart (Mautner & Luisada, 1941 ; Bovet, Depierre, Courvoisier & de Lestrange, 1947 ; Jacob & Depierre, 1950 ; Riker & Wescoe, 1951). We found decamethonium to have similar actions, although they were not regularly obtained. In 4 out of 9 cats a single dose of decamethonium of 100 μg/kg abolished the slowing of the heart normally produced by vagal stimulation (Fig. 5). Of the remaining cats, vagal slowing was much reduced in 3, but in 2 cats increasing the dose of decamethonium to as much as 500 μg/kg was without any effect.

Both curare and gallamine are thought to exert their effects on the heart in a manner similar to atropine, since, at the time the response to vagal stimulation is abolished, the slowing due to injected acetylcholine is also abolished.

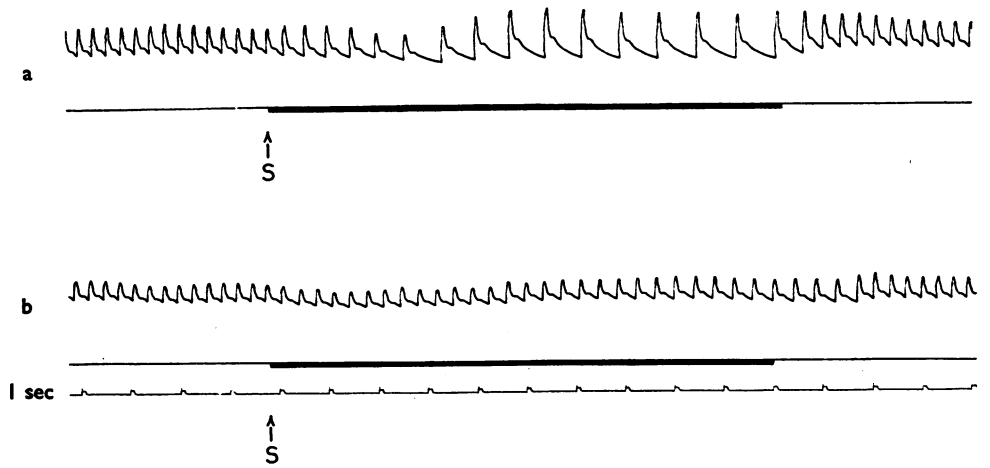

Fig. 5. Cat, 2.5 kg: records of arterial pulse pressure. Effect of vagal stimulation (bar marked S) before (*a*) and after (*b*) decamethonium (100 μg/kg) injected intravenously.

Decamethonium, however, has a variable effect on the cardiac slowing produced by injections of acetylcholine. This effect was examined in 6 cats. In 2 of the cats in which decamethonium had abolished the effect of vagal stimulation, acetylcholine was still effective in slowing the heart. In one cat where decamethonium had reduced the response to vagal stimulation, the effect of injected acetylcholine appeared to be enhanced. In the 3 remaining cats the effect of acetylcholine was much reduced by decamethonium, even though in one of these animals decamethonium had no effect on the response to vagal stimulation.

Effects on contractions of the small intestine in situ

Decamethonium had effects on intestinal activity in 5 out of 7 cats. An injection of 100 μg/kg caused a slowing and then cessation of the spontaneous activity of

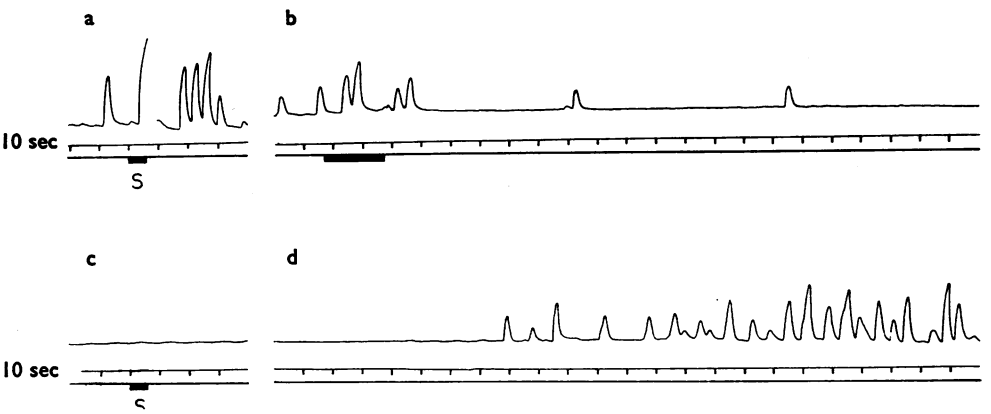

Fig. 6. Cat, 2.5 kg: records of contractions of small intestine *in situ*. Effect of decamethonium (100 μg/kg) injected intravenously at signal in (*b*) on spontaneous contractions and those evoked by vagal stimulation at signal marked S in *a* and *c*. An interval of 6 min elapsed between panel *b* and *c* and of 8 min between *c* and *d*.

the intestine (Fig. 6); and after a few minutes vagal stimulation became ineffective, although acetylcholine still produced contractions. These effects of decamethonium wore off in 4 cats several minutes after spontaneous respiration had reappeared. In the fifth cat spontaneous activity did not return during the remaining 2 hr of the experiment, neither was it possible to elicit contractions by vagal stimulation.

Effects at other sites of cholinergic transmission

The effects of decamethonium so far described are those at sites where transmission is thought to be mediated by acetylcholine. However, this drug does not exert a

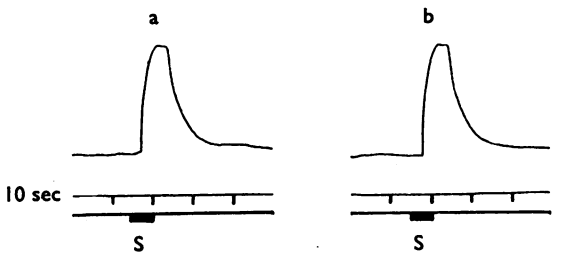

Fig. 7. Cat, 2.4 kg: records of bladder pressure. Pelvic nerve stimulated (S) before (*a*) and after (*b*); decamethonium (100 μg/kg) injected intravenously.

blocking action at all such sites. In 3 experiments we could find no effect of decamethonium on submandibular salivary gland secretion and the increase in blood flow elicited by chorda tympani stimulation. Also, in 4 experiments we were unable to demonstrate any effect of decamethonium on bladder contractions elicited by pelvic nerve stimulation (Fig. 7). In 2 of these experiments, spontaneous increases

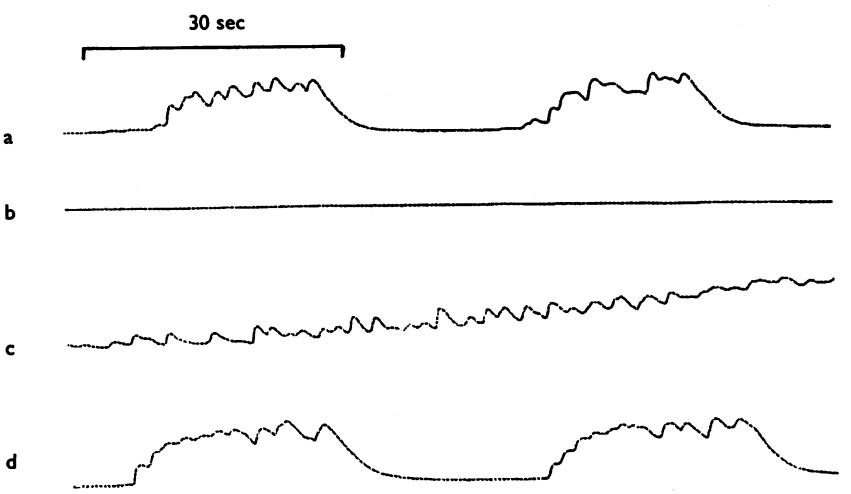

Fig. 8. Cat, 2.8 kg: records of bladder pressure. Effect of decamethonium (100 μg/kg), injected intravenously, on spontaneous contractions. Before (*a*) and immediately following injection (*b*); 17 min after injection (*c*) and 19 min after injection (*d*).

in bladder tone were observed. These were abolished in 1 or 2 min by decamethonium, reappearing 15 to 20 min after the return of spontaneous respiration (Fig. 8).

DISCUSSION

Since the extensive study of Paton & Zaimis (1949 ; 1951) on the polymethylene bistrimethylammonium salts, it has generally been assumed that, whereas those with a carbon chain length of 5 or 6 act predominantly at autonomic ganglia, the blocking action of decamethonium is exerted at the neuromuscular junction. In the cat this was the only action described in response to small doses, and in particular an atropine-like effect was denied (Paton & Zaimis, 1949). The present experiments show that the dose of decamethonium ordinarily used to paralyse skeletal and respiratory muscles of the cat for 20 to 30 min (100 μg/kg) commonly blocks at several sites in the autonomic nervous system.

The block of the sympathetic vasodilator outflow to skeletal muscle is exerted at the endings of the post-ganglionic nerve fibres ; for when these fibres are stimulated directly, their vasodilator action is regularly abolished by decamethonium. While we could not exclude, on the basis of our experiments, a concomitant block of the pre-ganglionic nerve endings, this seemed to be unlikely, for when the muscle vasodilatation was elicited by hypothalamic stimulation as part of a whole pattern of autonomic response, this single vasodilator component was abolished by decamethonium while the others were unimpaired.

The nature of the block of the post-ganglionic endings seems a curious one. The drug " stimulated " before paralysing, in that its initial effect was a vasodilatation. Its blocking action was antagonized by gallamine triethiodide. Thus far, the facts run parallel with those originally described at the neuromuscular junction (Paton & Zaimis, 1949) which led to the hypothesis that the block was due to a persistent depolarization of the muscle end-plates (Zaimis, 1951 ; Burns & Paton, 1951) ; but there are significant differences. We found gallamine triethiodide itself to have no blocking action on the vasodilator nerve endings ; and, secondly, during a decamethonium block, and when vascular tone had returned almost to its previous resting level, acetylcholine injected arterially exerted its usual vasodilator action. The latter finding also rules out competitive block by decamethonium, as was more recently proposed to explain later findings at the neuromuscular junction (Zaimis, 1953 ; Thesleff, 1955a & b). Since virtually nothing is known of the physiology of neuro-effector transmission at the sites of the autonomic system with which we have been dealing, it is particularly hazardous to guess at the implications of our results. The easiest explanation is that decamethonium had blocked the vasodilator nerve-endings by preventing release of acetylcholine.

When we turn to the action of decamethonium on the effect of vagal stimulation on heart-rate, the situation is still more complicated, for the electrical stimuli were being applied to pre-ganglionic fibres. Thus, the finding in some experiments of a block of the effect of nerve stimulation with a persistence of the effect of injected acetylcholine could be explained by an action interfering with transmission from pre- to post-ganglionic fibres. But since, in other experiments, the effect of injected acetylcholine was considerably reduced, some action on neuro-effector transmission

is definitely indicated. This could well be produced in more than one way, that is, by prevention of the release of acetylcholine and by prevention of the action of acetylcholine on the S-A node, one or the other of these two actions being more conspicuous in a particular experiment.

In the small intestine, not only the effect of vagal stimulation, but also the spontaneous activity of the gut was prevented, which may indicate an action on the myenteric plexus. The spontaneous activity of the bladder was also blocked, but without any action on transmission from the pelvic nerve.

Decamethonium can thus act on many different types of neurone: it has even been reported recently to interfere with transmission in the spinal cord of the cat, in a dose of 70 μg/kg, which is smaller than that we used (Fujimori & Eldred, 1961). The present findings show that decamethonium is not the drug of choice to prevent muscular movements in the cat, when studying centrally regulated adjustments of the cardiovascular system, and this consideration may also apply to other animal species. On the other hand, some use might be made of findings which otherwise seem to present unwanted complications; for we now have a means of selectively blocking the vasodilator fibres to skeletal muscle in the cat, leaving the other vasomotor fibres still functional. It would be useful if a similarly selective block could also be obtained in the human subject, in view of the recent interest in a similar nerve supply to muscle blood vessels in man (Blair, Glover, Greenfield and Roddie, 1959; Barcroft, Brod, Hejl, Hirsjarvi & Kitchin, 1960).

All in all, we are far from the simple statement of the mode of action of decamethonium that once seemed possible. The results of these experiments serve to show once more that it cannot be assumed that the effects of a drug are limited to its best-known and most commonly described action.

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