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THE BLOCKING ACTION OF CHOLINE 2:6-XYLYL ETHER BROMIDE ON ADRENERGIC NERVES

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Choline 2:6-xylyl ether bromide (TM 10), given systemically to cats in doses of 5 to 15 mg./kg., abolishes the effects of adrenergic nerve stimulation whilst leaving the reactions of the effector organs to adrenaline unimpaired. The effects of a single dose may take up to one hour to become fully established and last for more than twenty-four hours. Apart from transitory ganglionic blockade, cholinergic autonomic nerves are unaffected even by large doses of TM 10. Doses of TM 10 which produce effective blockade do not impair conduction along adrenergic nerve trunks; the drug must, therefore, act at, or close to, the nerve terminals. TM 10 prevents the output of noradrenaline from the spleen on stimulating the splenic nerves; but, in acute experiments, it does not influence the liberation of pressor amines from the stimulated suprarenals. Examination of some ethers related to TM 10 revealed no correlation between TM 10-like adrenergic blocking activity and local anaesthetic activity. The action of TM 10 on adrenergic nerves does not, therefore, seem to be accounted for by axonal block.

In 1954 Hey and Willey described the synthesis of choline 2:6-xylyl ether bromide (TM 10) and discovered that it abolished, for many hours, the effects of stimulating the postganglionic sympathetic supply to the nictitating membrane in cats, without affecting—except initially and transiently—the reactions to injected adrenaline.

Assuming that this paralysis might result from axonal block, they examined TM 10 for local anaesthetic activity when given intracutaneously to guinea-pigs, and found it to be a powerful and long-lasting local anaesthetic. The hypothesis of axonal block thus fitted the known facts. But whether conduction in postganglionic fibres was in fact abolished by doses of TM 10 which annulled the effects of stimulation was not directly tested.

The experiments described in this paper extend the observations of Hey and Willey to other nerves and effectors, and show that TM 10 does not abolish the effects of adrenergic nerve stimulation by preventing the passage of impulses along the nerve trunk, but by exerting an action at, or close to, the nerve terminals so that the impulses arriving there are rendered ineffective. A preliminary account of this work has already appeared (Exley, 1956).

METHODS

Experiments were done on the nictitating membrane, heart, pilomotor muscles, uterus, salivary glands, sweat glands, spleen, and suprarenals, in anaesthetized cats. Anaesthesia was induced with ether and maintained by chloralose (80 mg./kg., i.v.). Drugs were administered through a cannula in the femoral vein. Arterial blood pressure was recorded from a carotid artery by a mercury manometer. Some unanaesthetized cats and guinea-pigs were given TM 10 subcutaneously and their general reactions observed.

Electrical Stimulation.—Stimulation of nerve trunks was by rectangular pulses (0.5 msec. duration) applied through shielded electrodes. Contractions of the nictitating membrane were recorded in the usual way.

Nerve Action Potentials.—These were picked up through platinum electrodes and led into condenser-coupled amplifiers (time constant, 1 sec.). The output from these was displayed on oscilloscopes, one of which was fitted with a Cossor camera for recording purposes. The inferior cardiac nerve was prepared for stimulation close to the stellate ganglion in cats receiving artificial respiration and with the upper six ribs of the same side removed. All connexions to the stellate ganglion other than the cardiac branches were cut; action potentials were picked up from the distal part of the nerve, within 1 cm. of the heart.

The right cervical sympathetic trunk, with its superior cervical ganglion and postganglionic nerve, was exposed. The adjacent vagus nerve and nodose ganglion were excised. The preganglionic trunk was stimulated with either single or repetitive shocks, and biphasic action potentials in the postganglionic trunk were picked up by electrodes placed as far as possible from the ganglion. The ability of the nictitating mem-

brane of the same side to contract during a short period of tetanic stimulation at 10 shocks/sec. was checked from time to time. The tissues were covered with warm paraffin to prevent drying.

Heart Rate.—This was recorded on smoked paper by a Thorp electro-magnetic impulse counter operated by a relay and mercury switch. The latter was connected to the right ventricle by a hook and thread. The impulse counter was tripped to zero once every 10 sec. The thorax was opened and all nerve connexions to the right stellate ganglion were severed, except the cardiac branches which were laid on stimulating electrodes. The left vagus in the neck was cut and the distal trunk prepared for stimulation.

Uterine Movements.—The cervical end of one uterine horn of a non-pregnant cat was fixed *in situ*; the other end, after being detached from the ovary, was connected by a thread to a recording lever. The hypogastric nerves were ligated proximally and stimulated under warm paraffin.

Salivary Secretion.—The submaxillary duct was cannulated and the saliva allowed to displace saline, from a reservoir, into tubing connected to a Palmer Silver Drop Tube; a small amount of detergent was added to the saline to reduce the size of the drops, each of which operated an electro-magnetic marker. The chorda tympani nerve and the preganglionic cervical sympathetic trunk were prepared for stimulation.

Splenic Venous Blood.—This was obtained by the technique of Peart (1949), who showed that the transmitter of splenic nerve impulses was almost exclusively noradrenaline, a fact later confirmed by Mann and West (1950). Cats were pre-treated with atropine and had both adrenals ligated. Heparin (2,000 U./kg.) was administered after completing the dissection. Three-minute samples of blood, under the various control and experimental conditions, were collected into ice-cooled tubes containing heparin. The blood was immediately centrifuged; the plasma was drawn off, measured, and stored on ice until assayed. Between periods of collection, the venous blood was returned to the circulation through a polythene tube leading into the right femoral vein. The amine content of the plasma was assayed on the blood pressure of a cat which had been treated with atropine and ergotamine as described by Euler (1950). In later experiments, mepyramine maleate (5 mg., i.m.) also was given. The standard solution was of (−)-noradrenaline bitartrate, in terms of which the amine content of the plasma was estimated.

Results

Action of TM 10 on Cervical Sympathetic Nerves

Transmission to Nictitating Membrane.—The blocking action of TM 10 on the sympathetic supply to the nictitating membrane of the cat, discovered by Hey and Willey (1954), was readily confirmed. Fig. 1 shows a record of the contractions of the membrane during periodic postganglionic stimulation (15 sec. in each min., rate 50 shocks/sec.). Direct responses to adrenaline (10 μg., i.v.) were obtained before, and at various times after, the injection of 5 mg./kg. TM 10, the electrical stimulus being temporarily withheld during the adrenaline observations. The record shows the gradual development of the characteristic postganglionic block, accompanied, in the earliest stages, by a weak antisympathomimetic effect. The paralysis was persistent and never wore off during acute experiments such as this. Evidence will be presented later to show that the action of TM 10, though taking up to 1 hr. to become fully established, persists for 24 hr. or longer.

TM 10 also produced a failure of the nictitating membrane to respond to cervical sympathetic stimulation in rabbits anaesthetized with pentobarbitone.

Transmission to Iris.—In a cat not given atropine, a larger dose of TM 10 (10 mg./kg.) prevented dilatation of the iris on cervical sympathetic stimulation, though dilatation was readily obtained with intravenous injection of adrenaline.

Comparison of the Action of TM 10 on Adrenergic and Cholinergic Nerves

Though TM 10 is highly active in paralysing adrenergic nerves, the observation was made that the drug appears to exert no such effect upon postganglionic cholinergic nerves. This is illustrated by the following three types of experiment on animals not given atropine.

Actions on Parasympathetic and Sympathetic Nerves to the Heart.—The upper part of Fig. 2 shows the effects of 15 sec. periods of stimulation (50 shocks/sec.) to the cardio-accelerator nerve and to the left vagus, together with the effect of an intravenous dose of adrenaline. TM 10 was then administered; this caused an initial increase in heart rate, followed by a decrease which persisted throughout the experiment. (The drug invariably produced bradycardia in anaesthetized cats, whether atropine had been given or not.) The lower part of Fig. 2 shows the effects of nerve stimulation, and of adrenaline, some 20 min. after giving TM 10. The response to sympathetic stimulation was abolished whereas that to parasympathetic stimulation was unimpaired; the chronotropic effect of adrenaline appeared to be slightly augmented. Thus the effects of TM 10 were clearly confined to the sympathetic nerves to the heart: indeed, in similar experiments doses of TM 10 as high as 50 mg./kg. failed to produce sustained block of the vagus, though there was a

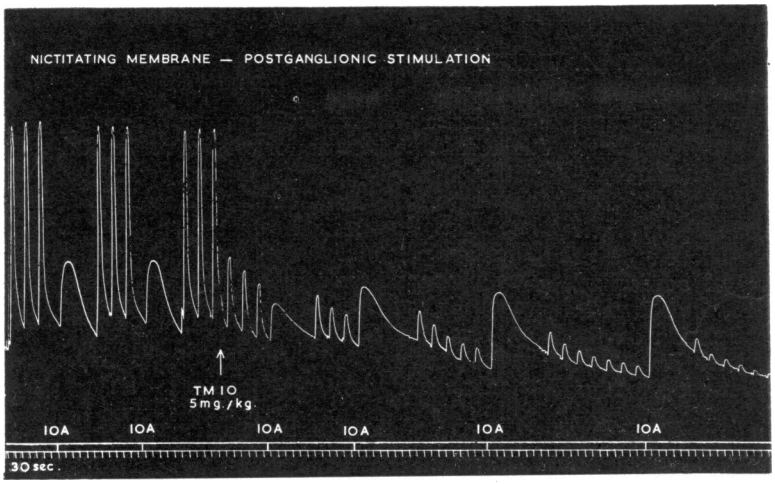

FIG. 1.—Cat, 2·1 kg., atropine, chloralose. Contractions of the nictitating membrane in response to postganglionic stimulation (15 sec. periods, 50 shocks/sec.) and to adrenaline (10 μg., i.v., at 10A) before and after TM 10 (5 mg./kg., i.v.). The onset of characteristic block to postganglionic nerve stimulation is seen after TM 10 with the transient anti-adrenaline action of the drug.

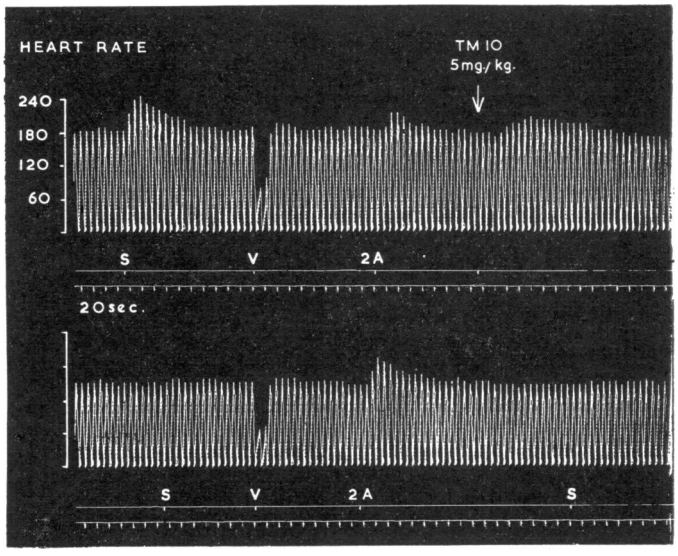

FIG. 2.—Cat, 3·7 kg., chloralose. Record of heart-rate response to stimulation (15 sec. periods, 50 shocks/sec.) of sympathetic (right cardio-accelerator, S) and parasympathetic (left vagus, V) nerves, and to adrenaline (2 μg., i.v., at 2A), before and after TM 10 (5 mg./kg., i.v.). Lower record started 20 min. after giving TM 10. The effect of sympathetic nerve stimulation is abolished by TM 10 while that of vagal stimulation remains. The response to adrenaline is not diminished.

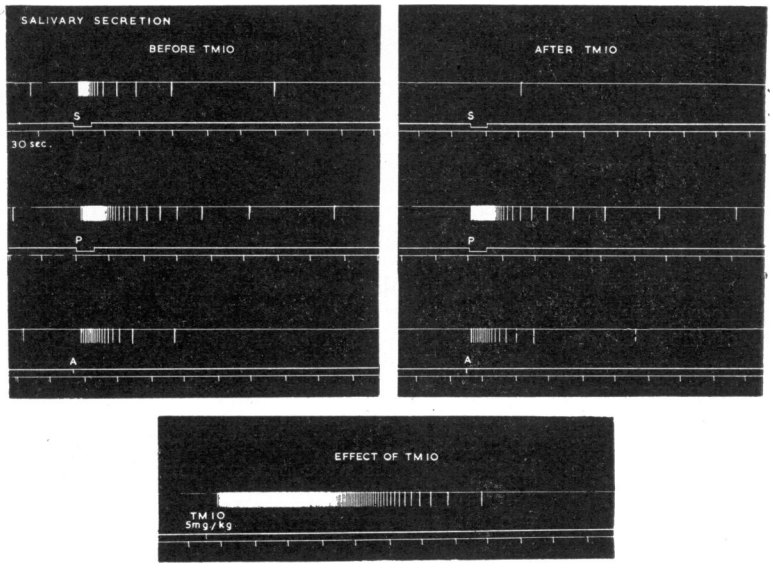

FIG. 3.—Cat, 2·1 kg., chloralose. Salivary secretion in response to stimulation (15 sec. periods, 50 shocks/sec.) of cervical sympathetic (S), or of chorda tympani (P), or to injection of adrenaline (10 μg., i.v., at A), before (upper left-hand records) and after (upper right-hand records) TM 10 (5 mg./kg., i.v.). The immediate effect of TM 10 is shown on the bottom record. The upper right-hand tracings began 20 min. after giving the TM 10. Each signal mark represents one drop of saliva. It is seen that only the response to sympathetic nerve stimulation is affected by TM 10.

paralysis for a few minutes after the injection, probably attributable in part to ganglionic blockade and in part to the weak antiparasympathomimetic action of the drug (Willey, 1957).

Actions on Parasympathetic and Sympathetic Nerves to Submaxillary Gland.—The effects of stimulation of these nerves, and of adrenaline, before and after 5 mg./kg. TM 10, are shown in Fig. 3. The normal responses are shown on the left, and those obtained 20 min. after TM 10 on the right. Again there was a clear-cut abolition of the sympathetic component without impairment of the effects of parasympathetic stimulation or of injected adrenaline. The injection of TM 10 itself evoked a brisk flow of saliva (lower block, Fig. 3). Further experiments showed that this secretion could be prevented by atropine, but not by hexamethonium. It is thus a further example of the muscarine-like action of TM 10, first noted by Willey (1957) on isolated guinea-pig ileum.

Actions on Sympathetic Nerves to Sweat Glands and Pilomotor Muscles.—It is well known that sweating is abolished by atropine, and Dale and Feldberg (1934) established the cholinergic nature of the sympathetic nerves supplying certain sweat glands in cats.

Following their technique, the caudal end of the cut left lumbar sympathetic chain was stimulated for periods of 30 sec. (50 shocks/sec.) at suitable intervals. Two responses were observed—the appearance of beads of sweat on the foot pads, and erection of the fur on the tail. Skin resistance in one foot pad was measured, and this showed an abrupt fall when sweating began. Twenty minutes after the injection of TM 10 (10 mg./kg.) the lumbar sympathetic was again stimulated; the pilomotor response was now absent, though sweating, with diminution in skin resistance, occurred exactly as before. Sweating was readily abolished, however, by a subsequent dose of atropine. Once more, therefore, it was evident that TM 10 was blocking adrenergic nerves, while leaving cholinergic nerves unaffected.

Effect of TM 10 on Action Potentials in Adrenergic Nerves

The fact that cholinergic postganglionic nerves were unaffected by TM 10 made it doubtful whether the characteristic action of the drug on adrenergic nerves could be ascribed to axonal block as such. In order to settle this point, therefore, the effect of TM 10 upon the action potentials picked up from three different adrenergic nerve trunks in the cat, the inferior cardiac, the splenic, and the postganglionic cervical sympathetic nerves, was investigated.

A series of three oscillograms of action potentials from the left inferior cardiac nerve, at a point within 1 cm. of its termination in the heart, are shown on the left of Fig. 4. The nerve was stimulated close to its exit from the stellate ganglion. The first potential was recorded before the drug was given, and the second 15 min. after the intravenous injection of 10 mg./kg. TM 10. The third was recorded 40 min. after giving the drug and was immediately preceded by a 30 sec. period of tetanic stimulation at a frequency of 50 shocks/sec. It is clear from these oscillograms that TM 10 did not depress the action potentials.

Exactly comparable results were obtained with the splenic nerves.

Oscillograms reproduced on the right of Fig. 4 were obtained from the postganglionic trunk of the

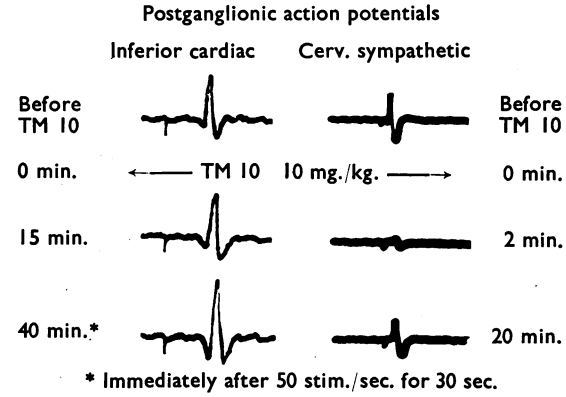

FIG. 4.—Cat, atropine, chloralose. Oscillograms of postganglionic action potentials from the inferior cardiac nerve (left) and from postganglionic fibres of the cervical sympathetic (right) following stimulation of the preganglionic trunk. TM 10 does not reduce the action potentials recorded from the inferior cardiac nerve, but it does produce a transient reduction in action potentials from the cervical sympathetic—probably because of ganglionic blockade. For further details, see text.

cervical sympathetic, and were evoked by preganglionic shocks. The upper oscillogram represents a normal biphasic action potential. Immediately after the intravenous injection of TM 10 (10 mg./kg.) there was an almost total suppression of the postganglionic action potential, as illustrated by the middle oscillogram taken 2 min. after giving the drug. This reduction was transitory and was probably due to a fleeting ganglionic blockade—as might be expected with a quaternary ammonium compound of this nature. Twenty minutes later the potential had returned almost to normal. But, at this stage, when the postganglionic potentials were passing normally along the nerve, preganglionic tetanic stimulation failed to evoke a response from the nictitating membrane.

It thus seems clear that the blocking action of TM 10 on the effects of adrenergic nerve stimulation cannot be attributed to a block of axonal conduction in adrenergic nerve trunks.

Action of TM 10 on Output of Pressor Amines

It is evident, since TM 10 does not impair the response of effector cells to adrenaline or noradrenaline, except immediately and transitorily, that it must reduce, in some way, the amount of mediator liberated by stimulated adrenergic nerves. It was of interest, therefore, to see whether this could be confirmed on a typical adrenergic nerve; and, if so, to determine if there was an analogous interference with the liberation of pressor amines from the stimulated suprarenal medulla.

Output of Noradrenaline from Spleen.—Samples of splenic venous blood, collected under various experimental conditions, were assayed for their pressor-amine content in terms of (−)-noradrenaline bitartrate. For the "post-stimulus" samples, the splenic nerves were stimulated (6 V., 50 shocks/sec.) during the first min. of a 3 min. collection period. Table I shows the results of one such experiment. The first two plasma samples were collected before the TM 10 was given. Stimulation of the nerves increased the total noradrenaline output/3 min., more than eightfold, and evoked a visible contraction of the splenic capsule. TM 10 (10 mg./kg. i.v.) was then given. A further resting sample (No. 3) was collected some 20 min. later. Subsequent stimulation of the splenic nerves now caused no detectable increase in amine output (Sample No. 4) over the resting level, nor did it evoke visible contraction of the splenic capsule. It was thus clear that TM 10 had prevented the liberation of noradrenaline in response to splenic nerve stimulation.

TABLE I

RESTING AND POST-STIMULATION NORADRENALINE CONTENTS OF SPLENIC VENOUS BLOOD PLASMA BEFORE AND AFTER TM 10

Sample No. and Description	Vol. of 3 min. Sample (ml. Plasma)	Noradrenaline Equivalent (μg./ml. Plasma)	Total Noradrenaline Output (μg./3 min.)
1. Resting	1·4	0·05	0·07
2. Post-stimulation	1·7	0·35	0·60
3. Resting, 20 min. after TM 10 (10 mg./kg.)	1·6	0·05	0·08
4. Post-stimulation, 25 min. after TM 10	1·6	0·05	0·08

Output of Amines from Suprarenals.—Experiments were done on the suprarenals, stimulated either through the splanchnic nerves or by chemical means.

For the splanchnic nerve experiments, cats were given atropine, anaesthetized with chloralose, eviscerated, and the kidneys tied off from the circulation. The distal portion of the cut left splanchnic nerve was stimulated for periods of 30 sec. (50 shocks/sec.). The resulting output of pressor amines was estimated on the arterial blood pressure and nictitating membrane of the same animal, by equating with suitable mixtures of (−)-adrenaline and (−)-noradrenaline given intravenously. The response of the nictitating membrane to stimulation of the preganglionic trunk of the cervical sympathetic was also tested occasionally.

Records of the arterial blood pressure, and of the contractions of the nictitating membrane, in such an experiment, are shown in Fig. 5. Before giving TM 10 the membrane responded fully to sympathetic nerve stimulation. The effects of splanchnic nerve stimulation were fairly accurately matched by a mixture of 2 μg. adrenaline and 3 μg. noradrenaline. One hour after TM 10 (5 mg./kg.) the response to cervical sympathetic stimulation was abolished; on the other hand, there appeared to be no change in the response to splanchnic stimulation. Larger doses of the drug (10 mg./kg.) similarly failed to modify the output of pressor amines on splanchnic stimulation.

In other experiments the suprarenals were stimulated by injections of tetramethylammonium bromide (TMA). Fig. 6 shows the response of the nictitating membrane of an atropine-treated cat to intravenous doses of adrenaline and of TMA, and to a lengthy period of postganglionic stimulation of the cervical sympathetic of the same side (10 shocks/sec.). The TMA caused a spiky contraction of the membrane, typical of ganglionic stimulation, followed by the familiar slow contraction owing to release of pressor amines from the suprarenals. TM 10 (5 mg./kg.) was given during the period of tetanic stimulation; the onset of nerve block is clearly demonstrated. Some time later the same doses of adrenaline and of TMA were repeated; the response to the former was not altered (lower half of Fig. 6), but that to TMA lacked the spiky ganglionic component. The slower component of the TMA response was still present, suggesting that the suprarenals were still discharging pressor amines. The suprarenals were then ligated, after which operation the response to TMA was abolished. A final electrical stimulus to the cervical sympathetic confirmed the persistence of a complete block.

The results of these experiments lead to the conclusion that TM 10 does not interfere, either qualitatively or quantitatively, with the release of

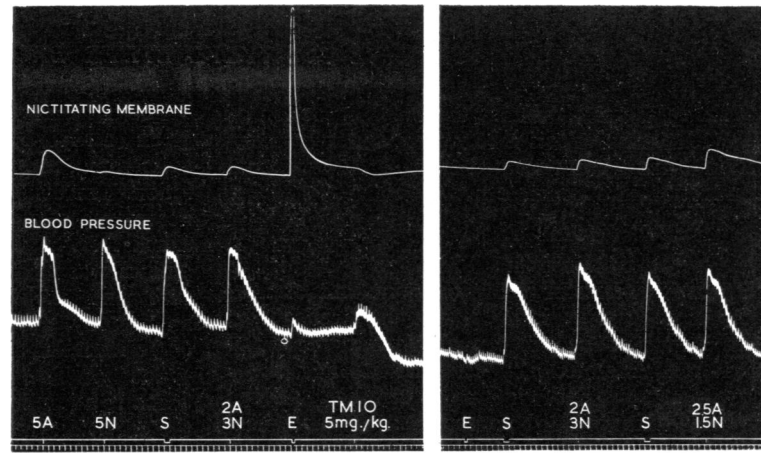

FIG. 5.—Cat, 2·8 kg., atropine, chloralose, eviscerated. Upper record, contractions of nictitating membrane; lower record, arterial blood pressure. Periods of left splanchnic nerve stimulation (30 sec., 50 shocks/sec.) at S and of cervical sympathetic stimulation (15 sec.) at E. Doses of adrenaline (A), noradrenaline (N), or mixtures containing the two amines were injected intravenously. The dose of each amine in μg. is indicated by the numerals preceding A or N. The record on right began 1 hr. after giving TM 10. Time, 30 sec. There is an abolition of effect of cervical sympathetic stimulation (E) after giving TM 10, while effect of splanchnic stimulation (S) persists.

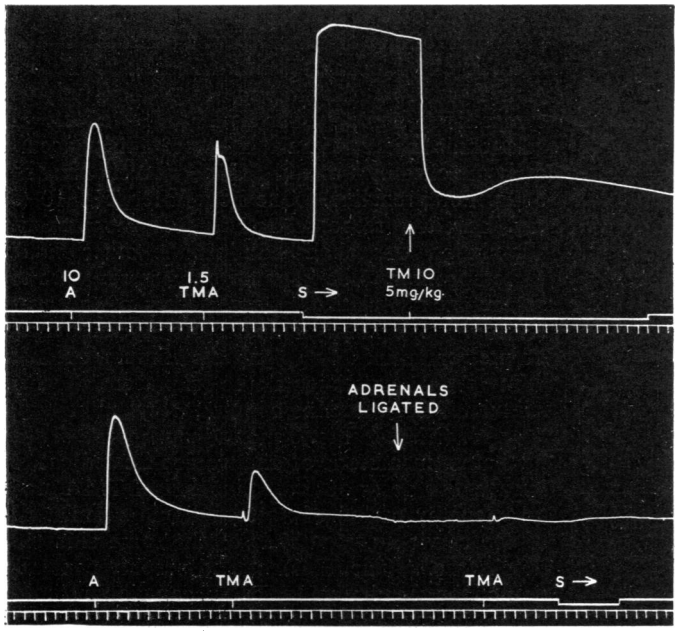

FIG. 6.—Cat, 2·9 kg., atropine, chloralose. Record of contractions of nictitating membrane evoked by 10 μg. adrenaline i.v. (10A), 1·5 mg. tetramethylammonium bromide i.v. (1·5 TMA), or by postganglionic stimulation of the cervical sympathetic at 10 shocks/sec. (S). Time, 30 sec. Lower record started 30 min. after TM 10 was given. For explanation, see text.

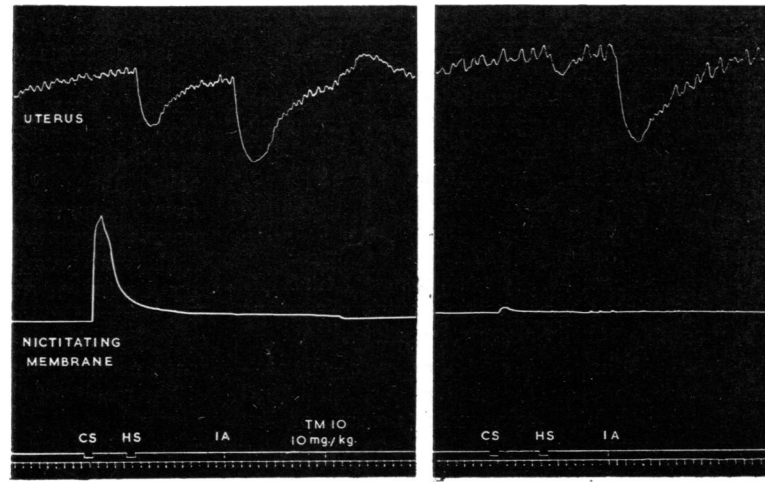

FIG. 7.—Cat, non-pregnant female, atropine, chloralose. Upper record, uterine tone; lower record, contractions of nictitating membrane. At 1A, adrenaline 1 μg. i.v. Stimulation (50 shocks/sec., for 30 sec.) of cervical sympathetic at CS, and of hypogastric nerves at HS. Time, 30 sec. Record on right started 1 hr. after TM 10. TM 10 greatly reduces the response of the uterus to hypogastric nerve stimulation while it appears to increase slightly the response to injected adrenaline.

pressor amines from the suprarenals stimulated under the conditions of these acute experiments. But experiments of this kind do not, of course, afford information about possible long-term disturbances within the gland. Such effects have been investigated, and will be reported separately.

Effect of TM 10 on Sympathetic Nerves to the Uterus

Dale (1906) first drew attention to the fact that the sympathetic nerves to the non-pregnant uterus exert an almost purely inhibitory action. As this inhibitory action, produced either by adrenaline or by nerve stimulation, is not readily blocked by antisympathomimetic drugs, it was of interest to see whether it would be affected by TM 10.

Such an experiment is illustrated in Fig. 7. Contractions of the nictitating membrane evoked by cervical sympathetic stimulation were recorded together with the relaxations of the uterus evoked by hypogastric nerve stimulation and by intravenous injections of adrenaline. One hour after giving TM 10 (10 mg./kg.) the responses to cervical sympathetic and to hypogastric nerve stimulation were greatly reduced, the former somewhat more than the latter. The sensitivity of the uterus to adrenaline was increased. In general, it has been found that hypogastric nerves are less readily blocked by TM 10 than are the other adrenergic nerves so far tested in cats, and as much as 15 mg./kg. of the drug may be required. On the other hand, the inhibitory effects of sympathetic nerve stimulation to the rabbit small intestine are readily abolished by TM 10 (Bain and Fielden, 1956).

Effects of TM 10 on Blood Pressure and Vasomotor Nerves

Doses of TM 10 sufficient to paralyse adrenergic nerve responses in cats tend to produce complex effects upon the blood pressure. The commonest pattern consists of a preliminary hypertension, developing slowly during the 5 min. following the injection, and lasting up to 30 min.; thereafter the blood pressure usually falls slowly to below its initial level. The nature of this preliminary hypertension has not been fully elucidated, but it is seen less frequently in animals whose adrenals have been ligated beforehand; the hypertension may occur in spinal cats and in animals treated with hexamethonium. It may be cut short by a dose of piperoxan (933F) or may be prevented from developing at all by a previous dose of this antisympathomimetic drug.

The vasomotor nerves appear to be paralysed by TM 10. As will be described later, the ears of unanaesthetized rabbits show a marked flushing after a systemic dose of the drug. Furthermore, the pressor responses, evoked in anaesthetized cats by stimulating the cut peripheral ends of the mesenteric nerves accompanying the superior mesenteric artery, are abolished by the usual doses of TM 10. In contradistinction, the pressor effect of splanchnic nerve stimulation may not be abolished by TM 10 even after the suprarenals have been ligated; the explanation for this may well be found in the large amount of extra-adrenal chromaffin tissue which cats possess. Such tissue is believed to be innervated by the splanchnic nerves, and would probably behave, after TM 10, in the same way as do the suprarenals themselves.

Effects of TM 10 on Unanaesthetized Animals

Hey and Willey (1954) investigated the toxicity of TM 10 when given intraperitoneally to mice. In cats, however, the drug appears to be poorly absorbed by this route. For the observations reported below, therefore, it was always given subcutaneously. Four guinea-pigs, given 20 mg./kg. TM 10, showed no outward changes in behaviour, or any immediate or delayed signs of prostration; there was, however, increased lachrymation for a short period after the drug was given. Much larger doses (60 mg./kg.) caused obvious muscular weakness.

Two cats were given 10 mg./kg. of TM 10 and observed during the ensuing 24 hr. Within an hour of the injection both animals had relaxed nictitating membranes and shivered; there were no signs of muscular weakness. Photographs of the eyes of one of these animals (*a*) before, and

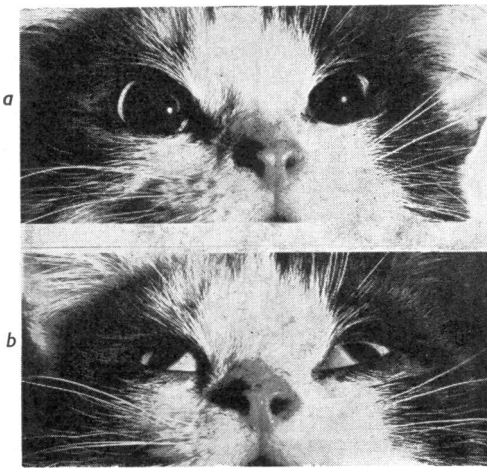

FIG. 8.—Eyes of unanaesthetized cat (*a*) before and (*b*) 24 hr. after a single dose of TM 10 (10 mg./kg., s.c.). The relaxation of the nictitating membranes is still present 24 hr. after the administration of TM 10.

(*b*) 24 hr. after the injection, when the effect on the nictitating membranes was still evident, are shown in Fig. 8. Forty-eight hours after giving the drug, partial recovery had occurred. One of the cats was then anaesthetized with chloralose; the response of the nictitating membrane to nerve stimulation was weak. On the other hand, the responses of the blood pressure and nictitating membrane to splanchnic nerve stimulation, after the animal had been eviscerated, were good, suggesting that the suprarenals were not affected.

The shivering produced by the drug in cats may result from increased heat loss, occasioned by cutaneous vasodilatation. Rectal temperature measurements in a rabbit confirmed that there was a fall in body temperature after TM 10, together with marked flushing of the ears.

TM 10-like Blocking Activity in Other Compounds

Hey and Willey (1954) made no mention of sympathetic-nerve blocking activity save in TM 10, though they found local anaesthetic properties in several related compounds. Some of the other ethers which they prepared were accordingly tested for TM 10-like activity.

The results, from experiments on the cervical sympathetic-nictitating membrane, can be summarized as follows: The *o*-tolyl homologue (choline 2-tolyl ether bromide, TM 18), seemed as active as TM 10 in producing the adrenergic-nerve block; but it has powerful nicotine-like stimulant properties and so the action could only be shown in animals given hexamethonium bromide (3 mg./kg.) and artificial respiration. The introduction of a third methyl group in the *para* position (choline 2:4:6-mesityl ether bromide, TM 17) markedly diminished activity.

With the cationic head of TM 10 altered to triethylammonium (β-(2:6-xylyloxy)ethyltriethylammonium bromide, TE 10), the specific blocking activity was lost, though, as reported by Hey and Willey (1954), this change did not destroy or impair the local anaesthetic activity. The tertiary analogues of TM 10 and TE 10 both had antisympathomimetic activity. But their action on the cervical sympathetic-nictitating membrane was brief, and could be fully accounted for by this antisympathomimetic action. There is no evidence that they interfere, as does TM 10, with the liberation of the transmitter. In their antisympathomimetic effects the tertiary amines (TTE 10 and TTM 10) are thus reminiscent of their parent phenyl homologues, whose properties have been described by Bovet and Maderni (1933) and by Justin-Besançon, Kohler, and Lévy (1937).

It can be concluded, therefore, that there is no direct relation between local anaesthetic activity in these compounds and TM 10-like adrenergic-nerve blocking activity.

Discussion

Hey and Willey (1954), who first made and investigated choline 2:6-xylyl ether bromide (TM 10), thought that its action upon postganglionic adrenergic nerves was sufficiently accounted for by the powerful and long-lasting local anaesthetic effect that they showed the drug to possess. The evidence presented in this paper, however, casts doubt upon the validity of this interpretation and raises the possibility that the drug may act upon adrenergic nerves in some other way. In particular, it has been demonstrated that conduction of impulses along three different adrenergic nerve trunks is not impaired by systemic doses of TM 10 sufficient to block the effect of adrenergic-nerve stimulation.

Furthermore, in parallel tests of the functional activity of both cholinergic and adrenergic nerves, such as those to the heart and salivary glands, cholinergic nerves are unaffected by doses of TM 10 that completely paralyse adrenergic nerves. Indeed, doses of TM 10 as high as 50 mg./kg. fail to block the effects of stimulation of the cardiac vagus nerve once the initial effects of interference with ganglionic transmission have worn off. If TM 10 were acting in virtue of its local anaesthetic properties, then postganglionic cholinergic fibres would be expected to show at least some vulnerability to the drug, especially fibres which run a long anatomical course, such as those to the sweat glands.

It might, of course, be argued that the local anaesthetic effect is not exercised on the main part of the sympathetic axon, but upon the delicate terminal fibrils which, being almost or completely devoid of myelin, might be expected to be specially vulnerable to circulating local anaesthetics, whether these be tertiary amines or quaternary ammonium compounds. But this would still fail to explain the immunity of pre- and postganglionic cholinergic nerve terminals to such an action—there being no reason to suppose that these are less susceptible to local anaesthetics than are the corresponding elements of adrenergic nerves.

Finally, quaternary compounds closely related to TM 10 may exhibit local anaesthetic properties equal to TM 10 itself (Hey and Willey, 1954), but possess, on systemic administration, little or no action upon adrenergic nerves. Thus the triethylammonium analogue (TE 10) is completely devoid

of the peculiar action of TM 10 on adrenergic nerves.

If, then, the adrenergic blockade produced by TM 10 is not connected with its local anaesthetic action it can result only from interference in some other way with the release or synthesis, or both, of the adrenergic mediators. Its action on adrenergic nerves may thus be analogous to that of botulinum toxin on cholinergic nerves.

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