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SOME PHARMACOLOGICAL ACTIONS OF CHOLINE 2:6-XYLYL ETHER BROMIDE

BY

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The following pharmacological properties of choline 2:6-xylyl ether bromide are described: (1) nicotine-like stimulant action on the arterial blood pressure, on the nictitating membrane, and on the soleus and gastrocnemius muscles of cats; (2) muscarine-like action on the arterial blood pressure of cats, and on the isolated guinea-pig ileum; (3) antihistaminic and antimuscarinic actions on the isolated guinea-pig ileum; (4) antisympathomimetic action on the arterial blood pressure of cats; and (5) neuromuscular blocking action on skeletal muscle of cats.

Hey and Willey (1953, 1954) described the long-lasting local anaesthetic action of choline 2:6-xylyl ether bromide (TM 10). They also described its nicotine-like stimulant action on the arterial blood pressure, and on the nictitating membrane, when given intravenously to atropine-treated cats (an action not obtained with subsequent doses); its transient blocking action of the pressor effect of adrenaline; and its action in abolishing the responses of the nictitating membrane to stimulation of its postganglionic nerve trunk. Brown and Hey (1956) have shown that this and related compounds inhibit the enzymic destruction of adrenaline by guinea-pig liver.

In view of the renewed interest in choline 2:6-xylyl ether (Exley, 1956; Bain and Fielden, 1956; Edge, Mason, and Wyllie, personal communication), it seemed worth while to put on record some observations that were not mentioned in the previous papers. These relate to the nicotine-like stimulant, neuromuscular blocking, muscarine-like, antisympathomimetic, antihistaminic, and antimuscarinic actions of the compound.

METHODS

Nicotine-like stimulant activity was tested by standard methods on the blood pressure of spinal cats and on the nictitating membrane and skeletal muscle of cats anaesthetized with chloralose (100 mg./kg., i.v.). Contractions of the soleus and gastrocnemius muscles were recorded with a Brown–Schuster mammalian-muscle myograph. Drugs were injected into the cannulated stump of the cut anterior tibial artery during occlusion of the popliteal artery.

Muscarine-like action was tested on the blood pressure of atropine-free cats anaesthetized with chloralose, and on the isolated guinea-pig ileum suspended in a 10 ml. bath of Tyrode solution at 35° C.

Antisympathomimetic activity was estimated on the blood pressure of spinal cats. The responses to doses of adrenaline and noradrenaline were determined before and after intravenous doses of TM 10.

Antihistaminic and antimuscarinic actions were studied on the isolated guinea-pig ileum.

Neuromuscular blocking activity was studied on the gastrocnemius muscle of anaesthetized cats. The sciatic nerve was stimulated by periodic condenser discharges (4/min.). Drugs were injected into the femoral vein.

RESULTS

Nicotine-like Stimulant Activity

Blood Pressure. — Intravenous injection of TM 10 to atropine-treated cats produces a brief rise in the arterial blood pressure, a characteristic effect of these nuclear-substituted choline phenyl ethers. But this effect of TM 10 is seen only on the first injection of the compound to an animal (Hey and Willey, 1954). Consequently, in assaying the activity of TM 10 against choline phenyl ether, only one estimation can be made on any one animal.

TM 10 is a very weak nicotine-like stimulant compound. It has, on a molar basis, approximately one-hundredth the activity of choline phenyl ether. This corresponds to one-half the activity of acetylcholine.

Despite the long-lasting abolition of the pressor responses to TM 10 that follows the first injection of this compound, the pressor responses to choline

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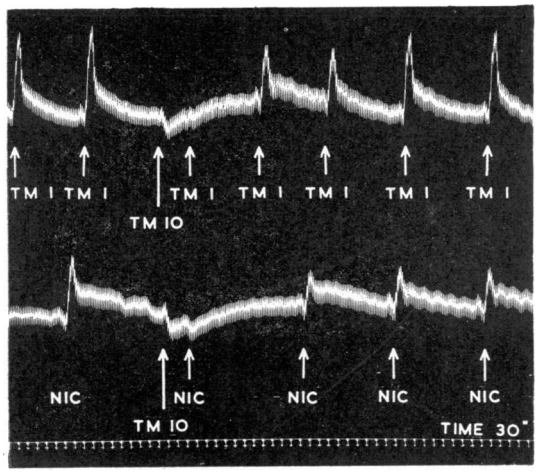

FIG. 1.—Cat, spinal, atropine. Record of arterial blood pressure showing transient blocking effect of TM 10 (10 mg., i.v.) on the pressor responses to i.v. injection of 50 μg. choline phenyl ether bromide (TM 1) and 0.5 mg. nicotine acid tartrate (Nic). Note the absence of pressor response to TM 10 itself, owing to the previous injection (not shown on the record) of 10 mg. of the compound.

phenyl ether and to nicotine are only abolished when injected immediately after TM 10. This is illustrated in Fig. 1. The first injection of TM 10 (not shown in the record) had caused the usual marked pressor response. Subsequent doses of TM 10 no longer raise the blood pressure. The pressor responses to choline phenyl ether and to nicotine, injected immediately after the TM 10, are, however, completely abolished. Recovery then occurs fairly rapidly, the pressor response to choline phenyl ether returning to its original level, whilst a reduction in the response to nicotine is still apparent. (Since the first injection of TM 10 —the one that caused a pressor response—is not shown in Fig. 1, it must be pointed out that there was no lasting change in the responses to choline phenyl ether and to nicotine before and after that injection.) Thus, though TM 10 blocks its own pressor response for a long time, it causes only a transient block of the responses to choline phenyl ether and nicotine. This would suggest that TM 10 differs from the other two drugs in the way in which it produces its initial pressor effect. Nevertheless, it is known that the stimulant action of TM 10 is exercised, at least in part, at autonomic ganglia (see below).

Nictitating Membrane.—Hey and Willey (1954) showed that TM 10 causes a contraction of the nictitating membrane—an effect that is abolished by the removal of the superior cervical ganglion. The contraction evoked by TM 10, however, becomes progressively smaller with subsequent injections, until finally no contraction is produced.

The effect of TM 10 on the contractions of the nictitating membrane caused by electrical stimulation of the cervical sympathetic nerve, by nicotine, and by adrenaline, is shown in Fig. 2. Injection of 5 mg. TM 10 during electrical stimulation first reduces and finally abolishes the response. The

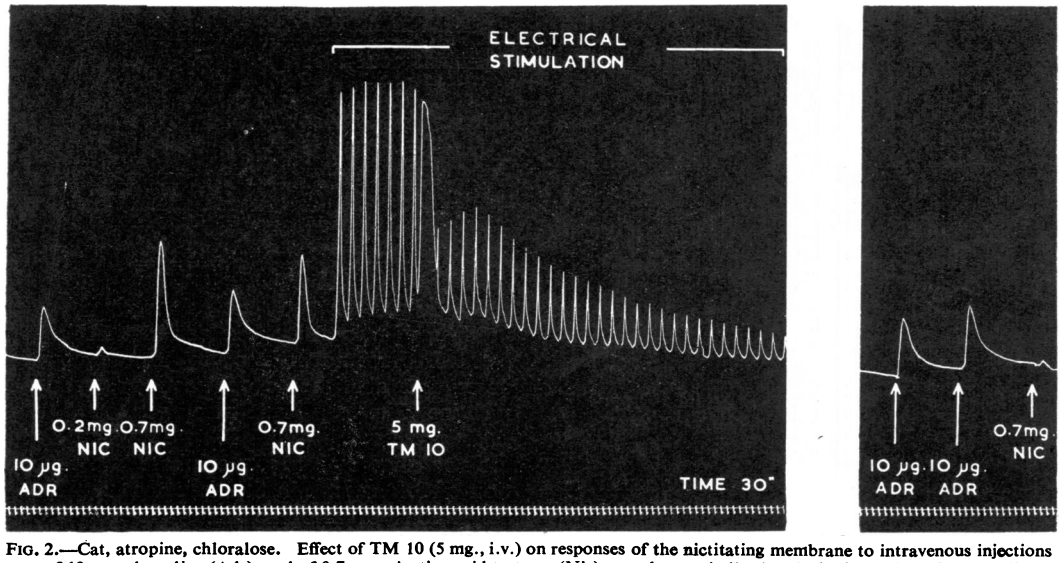

FIG. 2.—Cat, atropine, chloralose. Effect of TM 10 (5 mg., i.v.) on responses of the nictitating membrane to intravenous injections of 10 μg. adrenaline (Adr), and of 0.7 mg. nicotine acid tartrate (Nic); and to periodic electrical stimulation of preganglionic nerve trunk (70/sec. for 2 sec. in each min.). Time interval of about 30 min. between the two parts of the record.

stimulant effect of nicotine is also virtually annulled. The contraction produced by adrenaline is, however, unaffected.

In addition to the block of the response to preganglionic stimulation noted above, Hey and Willey (1954) showed that the response to postganglionic stimulation is also abolished by TM 10. It has already been mentioned that the contraction of the nictitating membrane evoked by TM 10 is abolished by previous administration of the drug; this is an effect analogous to that obtained on the arterial blood pressure. However, the effect of nicotine on the blood pressure is abolished for a much shorter time than is the corresponding effect on the nictitating membrane.

These curious differences between choline 2:6-xylyl ether on the one hand, and choline phenyl ether and nicotine on the other, are clearly worthy of systematic investigation.

Skeletal Muscle.—When injected into the cannulated stump of the cut anterior tibial artery

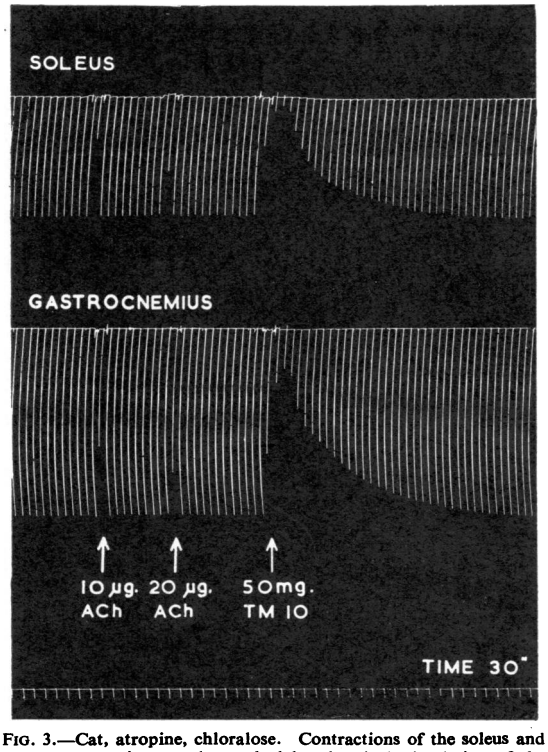

FIG. 3.—Cat, atropine, chloralose. Contractions of the soleus and gastrocnemius muscles evoked by electrical stimulation of the sciatic nerve (periodic condenser discharges, 4/min.), and by injection of acetylcholine and of TM 10. Injections made into the anterior tibial artery during cessation of the electrical stimulation. Note the transient neuromuscular blockade following the stimulant action of TM 10.

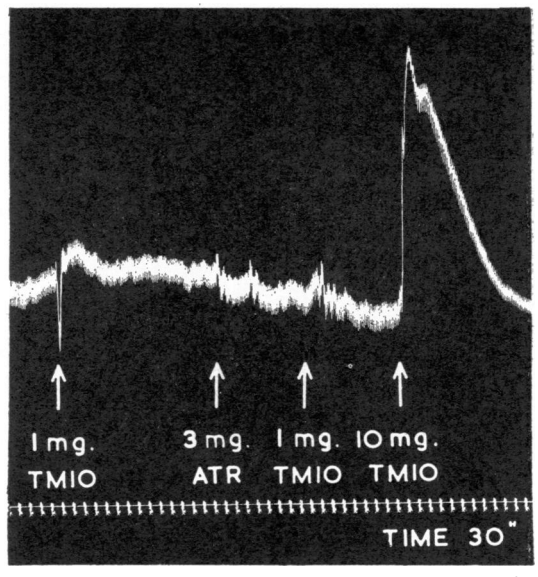

FIG. 4.—Cat, chloralose. Record of arterial blood pressure to show the abolition of the depressor response to TM 10 by atropine, and the pressor effect produced by a subsequent larger dose of TM 10.

TM 10 causes contractions of the soleus and gastrocnemius muscles. Fig. 3 shows the contraction produced by 50 mg. TM 10 compared with those produced by two different doses of acetylcholine. Applying this (2 + 1) assay technique, TM 10 was found to have approximately one five-thousandth the activity of acetylcholine as a stimulant of skeletal muscle. Fig. 3 also shows the transient neuromuscular blockade that follows the stimulant action of TM 10; this will be referred to again later.

Muscarine-like Activity

Intravenous injection of 1 mg. TM 10 causes a marked fall in the blood pressure of atropine-free cats under chloralose anaesthesia. This depressor response is abolished by the intravenous injection of 1 mg./kg. of atropine sulphate. This is illustrated in Fig. 4.

TM 10 also contracts the guinea-pig ileum in the absence of atropine. The contractions produced by TM 10 are not reduced by hexamethonium, in doses that abolish the contractions produced by nicotine (Fig. 5). Fig. 5 also shows that contractions produced by TM 10 and by acetylcholine are abolished by atropine sulphate in a concentration of 0.1 µg./ml. in the tissue bath. With concentrations of atropine that reduce, but do not abolish, the responses to acetylcholine and to TM 10 (20 ng./ml.), the reduction in the effect is the same for both drugs.

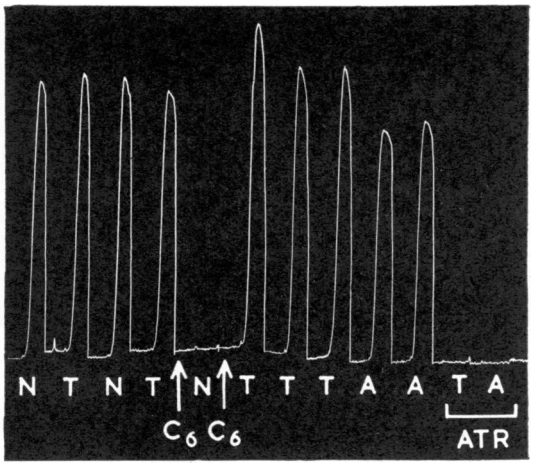

FIG. 5.—Guinea-pig ileum in 10 ml. bath of oxygenated Tyrode solution at 35° C. 4 min. cycle. N, 22 μg. nicotine; T, 40 μg. TM 10; A, 0.4 μg. acetylcholine chloride. In the presence of 4 mg. hexamethonium (C_6) the response to nicotine is abolished whereas the response to TM 10 is increased. Atropine sulphate, 0.1 μg./ml., during signal, abolishes the responses to acetylcholine and to TM 10.

The muscarine-like activity of TM 10 is approximately one-hundredth that of acetylcholine. But comparisons of TM 10 with acetylcholine are complicated. Thus the response to acetylcholine given immediately after TM 10 is frequently reduced—presumably because of an anti-acetylcholine action of TM 10 (Fig. 6). The effect of a subsequent dose of acetylcholine may, however, be increased; this may be accounted for by the weak anticholinesterase action of TM 10 (Willey, unpublished).

The doses of TM 10 generally used to produce this muscarine-like contraction in the isolated guinea-pig ileum ranged from 10 to 40 μg. in the bath. But in one experiment a large contraction was produced by 0.5 μg. TM 10. Repeated administration of the same dose led to progressively smaller responses. This phenomenon has been described by Ambache and Robertson (1953) for the 3-bromo- and 3:5-dibromo-phenyl ethers of choline, and was attributed by these authors to a nicotine-like paralysis.

Antihistaminic and Antimuscarinic Activities

Fig. 6 shows contractions of the isolated guinea-pig ileum to standard doses of histamine, acetylcholine, and barium chloride. Addition of 5 μg./ml. of TM 10 to the Tyrode solution caused a transitory contraction. In the presence of this concentration of TM 10, the response to the "musculotropic" spasmogen barium was unaffected, whereas the responses to acetylcholine and histamine were reduced, the latter to a more marked degree. Similar experiments, carried out with a higher concentration of TM 10 (20 μg./ml.), resulted in the complete abolition of the responses to histamine, whereas the responses to acetylcholine and barium were merely reduced.

It would appear, therefore, that TM 10, though possessing some general spasmolytic action, shows particular antimuscarinic and antihistaminic actions, more especially the latter. Estimations of the antihistaminic activity by Schild's method (Schild, 1947) gave mean pA_2 values of 5.03 (2 min. contact) and 4.98 (14 min. contact).

Antisympathomimetic Activity

In addition to the transient antagonistic action of TM 10 on the blood-pressure responses to adrenaline (Hey and Willey, 1954), the compound also blocks the pressor effect of noradrenaline. Fig. 7 shows the complete abolition of the blood-pressure responses to adrenaline and to noradrenaline after the intravenous injection of TM 10 to a spinal cat treated with atropine. Abolition of the responses, though complete for the doses used, is only transient; complete recovery occurs in about 30 min. (Fig. 7 also shows a potentiation of the responses to adrenaline and noradrenaline after the injection of TM 10—an

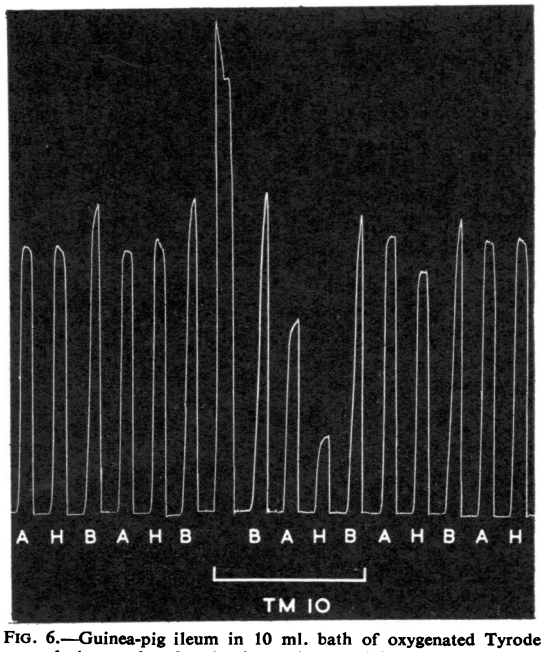

FIG. 6.—Guinea-pig ileum in 10 ml. bath of oxygenated Tyrode solution at 35° C. 4 min. cycle. A, 0.03 μg. acetylcholine chloride; H, 0.06 μg. histamine; B, 0.5 mg. barium chloride. TM 10, 5 μg./ml., during signal, reduces the responses to acetylcholine chloride and to histamine, but does not affect the response to barium chloride.

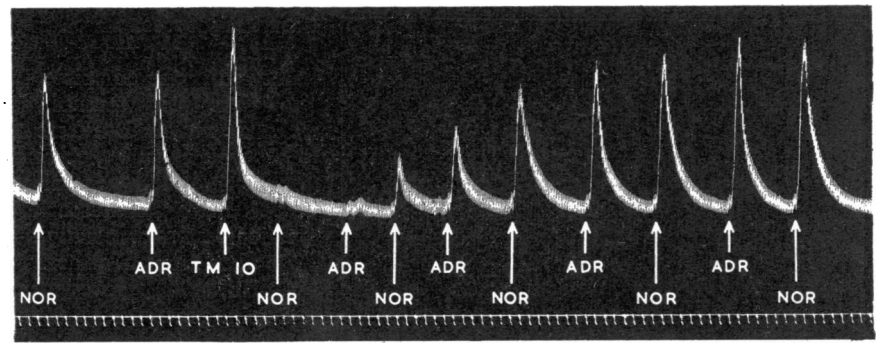

FIG. 7.—Cat, spinal, atropine. Record of arterial blood pressure to show the transient blocking effect of TM 10 (10 mg., i.v.) on the pressor responses to intravenous injections of 3 μg. adrenaline (Adr) and 1 μg. noradrenaline (Nor). Time signal, 30 sec.

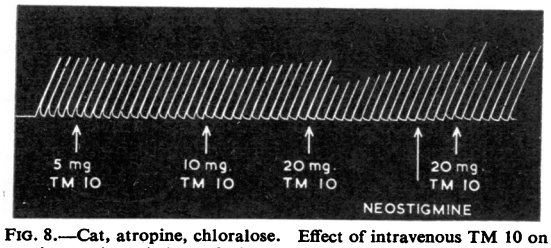

FIG. 8.—Cat, atropine, chloralose. Effect of intravenous TM 10 on isometric twitches of the gastrocnemius muscle evoked by electrical stimulation of the sciatic nerve (periodic condenser discharges, 4/min.). The block is unaffected by an injection of neostigmine methylsulphate (0.75 mg./kg.).

observation described by Brown and Hey (1956) for the related choline *p*-tolyl ether bromide.) The antisympathomimetic action of TM 10 has also been demonstrated on the nictitating membrane of the cat ; but, again, this action is of short duration, and adrenaline rapidly becomes effective again.

Neuromuscular Blocking Action

It has already been noted that TM 10 stimulates skeletal muscle, and then produces a transient neuromuscular blockade (Fig. 3). It has also been shown that the response to a close-arterial injection of acetylcholine is antagonized by TM 10.

In atropine-treated cats under chloralose anaesthesia, intravenous TM 10 causes a transient depression of the twitch tension of the gastrocnemius muscle stimulated through the sciatic nerve. This paralysis is unaffected by a preceding injection of neostigmine methylsulphate (Fig. 8). This last observation, taken together with the fact that TM 10 produces an initial stimulation of skeletal muscle (Fig. 3), suggests that the neuromuscular paralysis produced by TM 10 is of the decamethonium type.

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