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SOME ACTIONS OF SUBSTITUTED CHOLINE PHENYL ETHERS, PARTICULARLY OF CHOLINE 2:6-XYLYL ETHER

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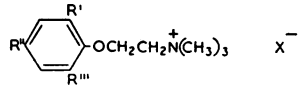
Marked nicotine-like stimulant properties are possessed by choline phenyl ether and choline *o*-tolyl ether, and to a decreasing extent by choline 2:6-xylyl ether and choline 2:4:6-mesityl ether. The compounds all show neuromuscular blocking properties, which are of short duration and pass from mainly decamethonium-like to mainly curare-like as more methyl groups are added to the phenyl nucleus. This series of compounds also possesses muscarinic, weak anti-adrenaline and vasodilator properties, as well as long-lasting local anaesthetic effects in the two compounds tested by intradermal injection.

Hey and Willey (1953, 1954), whilst examining a series of substituted choline phenyl ethers, discovered that some of these, and, in particular, choline 2:6-xylyl ether bromide (TM 10), had local anaesthetic properties, characterized by an unusually long duration of action. Nádor, Herr, Pataky, and Borsy (1953) have also described local anaesthesia of long duration with quaternary ammonium derivatives of well-known local anaesthetics.

Because of the potential clinical usefulness of a compound with this property we have further examined choline 2:6-xylyl ether bromide and compared some of its properties with other closely related quaternary ammonium compounds. As a result of this study and those of Exley (1956), Bain and Fielden (1956), and Willey (1957), other interesting properties in this series have been revealed. Furthermore, choline phenyl ether has a marked and well-known nicotinic effect on the blood pressure (Hunt and Renshaw, 1929; Hey, 1952).

The compounds examined were:



Choline phenyl ether methylsulphate (TM 1) ($R'=R''=R'''=H$; $X=Me_2SO_4$). Choline *o*-tolyl ether bromide (TM 18) ($R'=CH_3$; $R''=R'''=H$; $X=Br$). Choline 2:6-xylyl ether bromide (TM 10) ($R'=R'''=CH_3$; $R''=H$; $X=Br$). Choline 2:4:6-mesityl ether bromide (TM 17) ($R'=R''=R'''=CH_3$; $X=Br$).

The unsubstituted compound has long been known as choline phenyl ether, and Hey and Willey (1953, 1954) used a similar nomenclature for TM 10. To avoid confusion we have adopted these names and used the same system in naming the other members of the series. The third and fourth compounds listed above were first obtained from Dr. P. Hey. Later supplies, as well as the other two compounds listed, were kindly prepared by our colleague, Dr. K. Gaimster.

METHODS

Acute toxicities were determined in groups of 10 albino mice. Mice of either sex weighing 16 to 24 g. were used. LD50, limits of errors, and the slopes of the regression lines were determined graphically.

Local anaesthesia was tested by three methods. These were the guinea-pig weal and frog lumbar plexus methods of Bülbring and Wajda (1945), and the guinea-pig corneal method. In the first of these, observations on groups of four or six guinea-pigs were performed every 5 min. for 30 min. and subsequently at 10 or 15 min. intervals until anaesthesia became minimal. Activities were determined by comparison of the response/time graphs plotted for each experiment, cocaine and sometimes lignocaine being used as standards for comparison.

Experiments on the blood pressure and neuromuscular transmission were performed in cats under chloralose anaesthesia or in spinal preparations; the compounds were injected intravenously. In experiments on the superior cervical ganglion-nictitating

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membrane preparation intra-arterial injections were employed. The cervical sympathetic nerve trunk was stimulated peripherally at 20/sec. and the sciatic nerve at 6/min. or 50/sec. using supramaximal rectangular pulses of 0.5 msec. duration.

Anti-adrenaline activity was examined using the perfused rabbit ear preparation in the manner described by Fleckenstein (1952). The concentration of drug was found which reduced the sensitivity of the preparation to adrenaline by a factor of 10. The perfusing fluid was that recommended by Page and Green (1948).

Other methods are described with the results. Cocaine, lignocaine, and adrenaline were used as the hydrochlorides, acetylcholine and (+)-tubocurarine as the chlorides, hexamethonium as the bromide, gallamine as the triethiodide, neostigmine as the methyl sulphate, atropine as the sulphate, histamine as the acid phosphate, and nicotine as the hydrogen tartrate. All doses in the text refer to these salts unless otherwise stated.

RESULTS

Toxicity in Mice

The acute intravenous toxicities are shown in Table I. The acute subcutaneous LD50 of choline 2:6-xylyl ether was 240 mg./kg. compared with 8.5 mg./kg. intravenously. The toxic symptoms included muscular weakness and clonic convulsions. In addition, choline phenyl ether produced muscular twitching. After intravenous injection death occurred within a few minutes and followed respiratory arrest.

TABLE I

ACUTE INTRAVENOUS TOXICITY OF SUBSTITUTED CHOLINE PHENYL ETHERS IN MICE

Compound	LD50 (mg./kg.)	% Limits of Error ($P=0.05$)	Slope of Regression Line
Choline phenyl ether methylsulphate	2·20	82·0–121·5	4·8
Choline *o*-tolyl ether bromide	0·87	90·5–110·5	14
Choline 2:6-xylyl ether bromide	8·50	90·5–110·5	12·4
Choline 2:4:6-mesityl ether bromide	7·80	90·5–111·0	12·5

Local Tolerance

Injected intradermally into the shaved backs of guinea-pigs and into the ears of rabbits, 0.2 ml. of a 2.0% w/v solution of choline 2:6-xylyl ether caused a slight erythema, which resolved in two or three days. In one rabbit, a few small petechiae were found after close examination with a lens.

Local Anaesthetic Effects

Guinea-pig Weal.—We have confirmed the finding of Hey and Willey (1954) that choline 2:6-xylyl ether produces long-lasting local anaesthesia. In our experiments, the compound was compared directly with both cocaine and lignocaine. The results obtained with the latter two compounds were practically identical when the same concentrations were used.

Using 0.33% solutions, both cocaine and lignocaine produced virtually complete anaesthesia within 5 min., which persisted for 30 min. and then declined fairly rapidly. Choline 2:6-xylyl ether did not produce complete anaesthesia until 20 to 30 min. after injection and this was followed by a slow return of sensation (Fig. 1*a*). In this

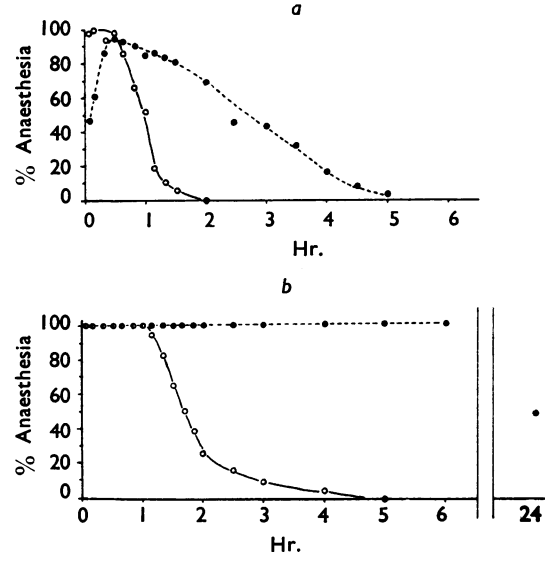


FIG. 1.—Time/response relationship of intradermal local anaesthesia produced in guinea-pigs by cocaine and choline 2:6-xylyl ether. (*a*), Mean results on 12 guinea-pigs (2 experiments) using 0.33% solutions. (*b*), Mean results on 4 guinea-pigs using 3.0% solutions. ○——○, cocaine; ●- - -● choline 2:6-xylyl ether. Ordinates, % anaesthesia. Abscissae, time in hr.

detail, our results differed from those of Hey and Willey (1954), who found that this concentration produced a 75% effect within 5 min. of injection.

When the concentration was increased to 1.0%, we found no appreciable modification of the response to cocaine, but the response to choline 2:6-xylyl ether was much quicker in onset and the duration of anaesthesia was also increased. A concentration of 3.0% produced some increase in the duration of anaesthesia with cocaine, whereas choline 2:6-xylyl ether produced complete anaesthesia for more than 6 hr. and sensation was still reduced after 24 hr. (Fig. 1*b*).

We have also examined choline 2:4:6-mesityl ether by this method. Again it was found that, with a concentration of 0.33%, maximal anaesthesia occurred only after an interval of 30 to 40

min. With this concentration there was complete recovery only after 4½ hr.

Guinea-pig Cornea.—By this method choline 2:6-xylyl ether had no activity when a 2.0% solution was continuously applied to the cornea for 10 min. This agrees with the results of Hey and Willey (1954) in the rabbit.

Frog Lumbar Plexus.—On this preparation, a 0.8% solution of choline 2:6-xylyl ether was effective only after 23 min. contact, whereas a 0.1% solution of cocaine produced anaesthesia in not more than 14 min.

Cardiovascular Effects

Effects on Blood Pressure and Heart Rate.—In the cat under chloralose anaesthesia, the rise in pressure produced by 0.1 mg./kg. of choline phenyl ether was preceded by a brief fall. The rise was abolished by hexamethonium (10 mg./kg.), leaving only a small depressor response which could be inhibited by atropine (3 mg./kg.) (Fig. 3).

Choline *o*-tolyl ether also produced profound effects with doses of 0.05 mg./kg. and greater. These consisted in a brief period of bradycardia followed by tachycardia and a rise of blood pressure. For example, a dose of 0.3 mg./kg. produced a fall in heart rate from 96/min. to 60/min. followed by a rise to 144/min. accompanied by a rise in blood pressure of 90 mm. Hg. This response was regularly obtained (Fig. 2). The pressor response was greatly reduced (to less than 10 mm. Hg) although not abolished by hexamethonium (10 mg./kg.). After hexamethonium, the compound did not cause a fall in blood pressure.

Choline 2:6-xylyl ether had a complex action (Fig. 3). Initially, provided a moderately large dose of 2 mg./kg. or more was given, there was a very brief fall in pressure followed by a marked rise which lasted for 4 to 5 min. The heart rate fell during the latter part of the response; in one experiment an initial heart rate of 200/min. was reduced to 168/min. Subsequent doses caused only a marked fall in blood pressure of 30 to 60 sec. duration. If an initial dose caused only a fall in blood pressure, then a pressor response could not subsequently be obtained. Despite tachyphylaxis of the pressor action seen with this com-

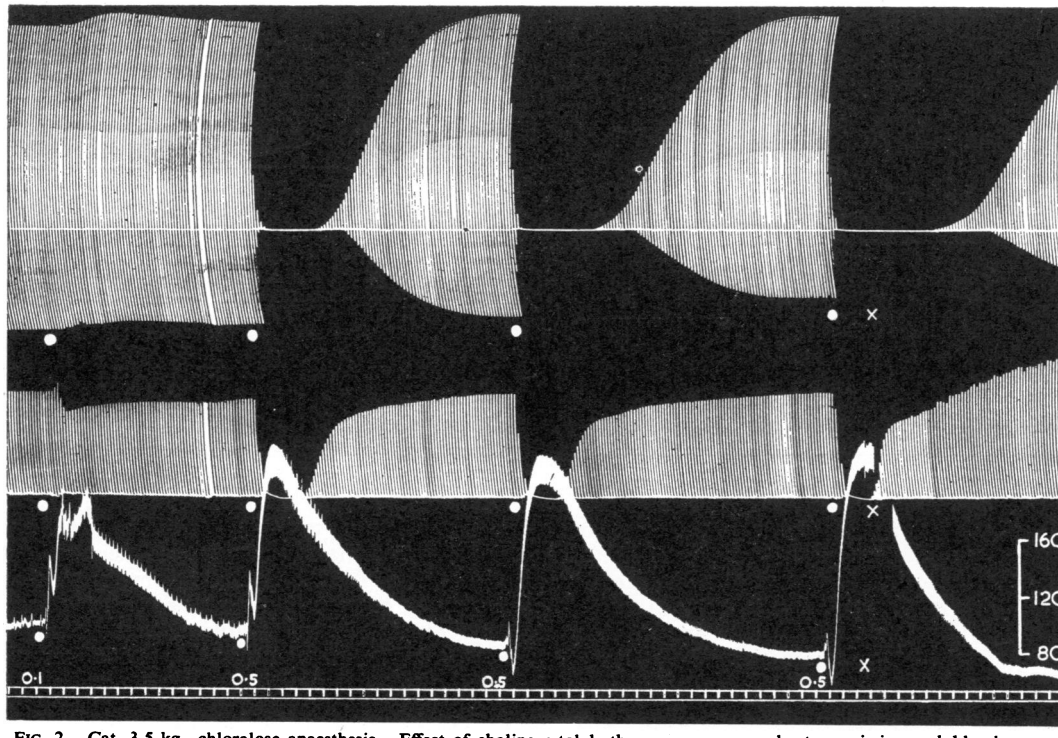


FIG. 2.—Cat, 3.5 kg., chloralose anaesthesia. Effect of choline *o*-tolyl ether on neuromuscular transmission and blood pressure. Tracings from above downwards: contractions of tibialis; of soleus; blood pressure; time marker. At ●, i.v. injections of choline *o*-tolyl ether in doses shown in mg./kg. Each injection was followed by micturition. At X, 0.1 mg./kg. neostigmine. Vertical scale, blood pressure in mm. Hg. Time, 30 sec.

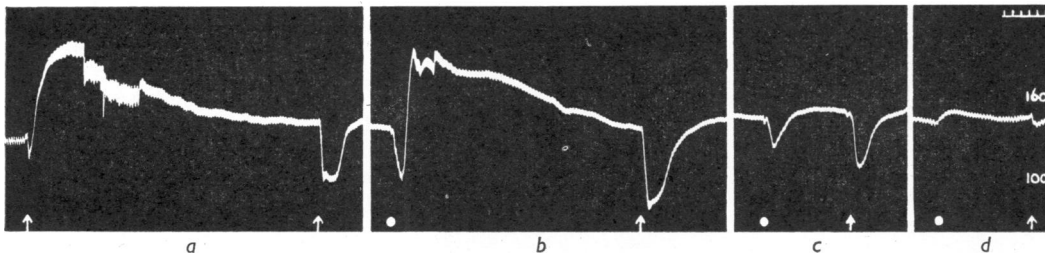


FIG. 3.—Cat, 2.5 kg., chloralose anaesthesia. Blood pressure record. At arrows, 2 mg./kg. choline 2: 6-xylyl ether. At ●, 0.1 mg./kg. choline phenyl ether. (*a*), Note that only the first dose of choline 2 : 6-xylyl ether caused a pressor response. (*b*), A pressor response was still obtained with choline phenyl ether. Between (*b*) and (*c*) 10 mg./kg. of hexamethonium was given. Between (*c*) and (*d*) 3 mg./kg. of atropine was administered. Vertical scale, blood pressure in mm. Hg. Time, 10 sec.

pound, a nicotine-like pressor response could still be obtained with choline phenyl ether (Fig. 3). The depressor response obtained with choline 2:6-xylyl ether could be inhibited by atropine. (This muscarinic property was not reported by Hey and Willey (1954), because they worked on spinal cats which had received atropine). The effect of peripheral stimulation of the vagus nerve on the blood pressure and heart rate was temporarily reduced when doses of about 8 mg./kg. were employed.

Choline 2:4:6-mesityl ether had very little pressor action in the cat under chloralose anaesthesia. Usually only a fall in blood pressure was observed with doses of 1 to 10 mg./kg. This fall was sometimes preceded by a slight rise in pressure. The depressor response could not be abolished by atropine although some reduction of the response occurred. In the spinal cat, doses of 2 to 10 mg./kg. caused only a rise in blood pressure. No tachyphylaxis of this pressor action was observed.

Effects on the Perfused Rabbit Ear.—Choline 2:6-xylyl ether has some anti-adrenaline activity on the cat blood pressure (Hey and Willey, 1954). This property is dealt with in some detail by Exley (1956) and Bain and Fielden (1956). In our experiments on the isolated perfused rabbit ear, the compounds caused vasodilatation and exhibited weak anti-adrenaline activity (Fig. 4). There was little difference between the potency of the four compounds, the concentrations required to reduce the constrictor action of adrenaline to 1/10 ranging from 50 to 150 μg./ml. Choline phenyl ether was the least active and choline *o*-tolyl ether the most active compound.

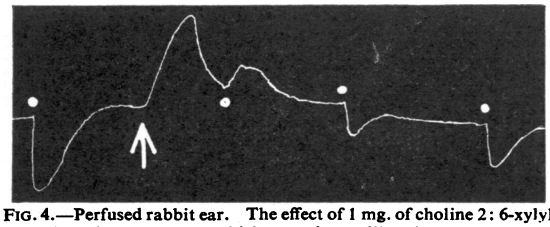


FIG. 4.—Perfused rabbit ear. The effect of 1 mg. of choline 2: 6-xylyl ether, given at arrow, which caused vasodilatation, on responses to 0.05 μg. adrenaline (●).

Effects on Superior Cervical Ganglion

It was observed in the cat that choline 2:6-xylyl ether and choline *o*-tolyl ether caused micturition and a brief but copious flow of saliva, even when the pressor response to the former compound could no longer be obtained. We considered that these effects were probably due to nicotine-like stimulation and decided to examine the compounds on the superior cervical ganglion-nictitating membrane preparation of the cat under chloralose anaesthesia, using intra-arterial injections.

Stimulant effects were repeatedly obtained with choline phenyl ether and choline *o*-tolyl ether in doses as low as 10 μg. Large doses (100 to 400 μg.) of these two compounds caused a biphasic contraction of the nictitating membrane (Fig. 5*c*), the secondary contraction occurring about 20 to 25 sec. after the primary spike contraction. This secondary contraction was probably due to the release of adrenaline from the adrenal medulla, but may have been a muscarinic action directly on the membrane. It could be eliminated if the blood supply to the membrane was interrupted by clamping the external carotid artery during and after the injection.

The results obtained with choline 2:6-xylyl ether and choline 2:4:6-mesityl ether were very variable. Sometimes no stimulation could be obtained with doses ranging from 10 to 400 μg. In preparations where stimulation was obtained, sometimes with doses as small as 20 μg., sensitivity rapidly declined until no effect could be obtained with doses up to 400 μg. Similarly, in preparations where the preganglionic fibres were stimulated electrically, choline 2:6-xylyl ether produced a brief relaxation of the membrane, but

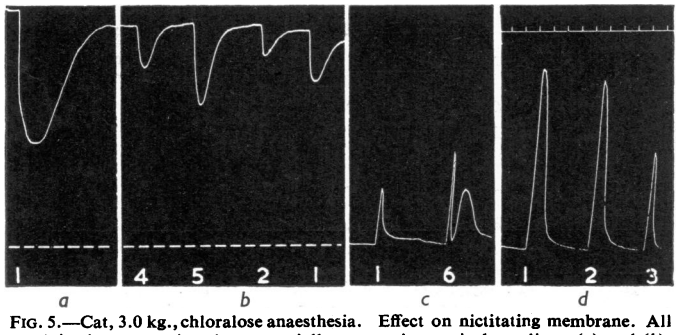


FIG. 5.—Cat, 3.0 kg., chloralose anaesthesia. Effect on nictitating membrane. All injections were given intra-arterially to superior cervical ganglion. (*a*) and (*b*), continuous preganglionic stimulation of cervical sympathetic nerve trunk; (*c*) and (*d*), no electrical stimulation. At (1), 200 μg., at (2), 100 μg., at (3), 50 μg. choline 2:6-xylyl ether. At (4), 4 μg., at (5), 8 μg. hexamethonium. At (6), 200 μg. choline phenyl ether. Note decreased sensitivity to choline 2:6-xylyl ether between (*a*) and (*b*) and potentiation of stimulant effect of this compound by choline phenyl ether between (*c*) and (*d*). Time, 60 sec.

the response to repeated injections rapidly declined. Both effects are shown in Fig. 5, where 200 μg. of choline 2:6-xylyl ether at first produced marked relaxation of the membrane stimulated preganglionically. Sensitivity to the compound rapidly declined, but, when preganglionic stimulation was withdrawn, injection of the same dose caused the membrane to contract. Since both the relaxation and the contraction were rapid in onset, and appeared even though the clamp on the external carotid artery was left in position, they are probably due to a direct effect on the ganglion. Hey and Willey (1954) have shown that this compound has no stimulating action on the nictitating membrane after removal of the superior cervical ganglion, but they were again using animals treated with atropine, so that muscarinic effects would not be seen. In one experiment, the stimulating action of choline 2:6-xylyl ether was potentiated by choline phenyl ether (Fig. 5*c* and *d*).

Effects on Isolated Guinea-pig Ileum

On the isolated guinea-pig ileum suspended in a 5 ml. bath containing oxygenated Tyrode solution, choline phenyl ether and choline *o*-tolyl ether produced submaximal contractions in concentrations of the order of 2 μg./ml. The other two compounds produced comparable contractions in concentrations of the order of 10 to 20 μg./ml.

Contractions following choline phenyl ether and nicotine were blocked by hexamethonium (100 μg./ml.), leaving contractions elicited by acetylcholine and histamine unaffected.

In other experiments, the effect of choline *o*-tolyl ether was reduced or abolished by hexamethonium. When only reduction of the response was obtained, the effect of nicotine was reduced to a greater extent. Atropine (1.0 μg./ml.) greatly reduced the effects of approximately equipotent doses of choline *o*-tolyl ether and nicotine.

It was not possible to block the effects of choline 2:6-xylyl ether or choline 2:4:6-mesityl ether with hexamethonium, although the effects of both these compounds and of nicotine were reduced or abolished by atropine (Fig. 6).

Neuromuscular Blocking Effects

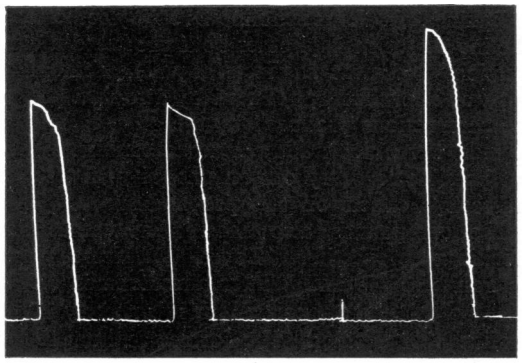
One of the effects noted in mice after intravenous injection of choline 2:6-xylyl ether was muscular weakness and slowed respiration. In the cat under chloralose anaesthesia, doses greater than about 3 mg./kg. caused a brief apnoea. It was therefore decided to examine these compounds for neuromuscular blocking properties.

Effects on Soleus and Tibialis Muscles of the Cat.—The neuromuscular blocking effect seen in the cat under chloralose anaesthesia was one of the most interesting properties of these compounds. With all four compounds the block rapidly reached a maximum and was of short duration.

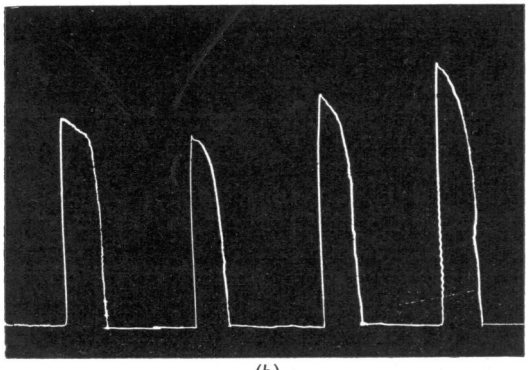
In a dose of 5.0 mg./kg., choline phenyl ether abolished the contractions of both the tibialis and soleus muscles in response to nerve stimulation.

Choline *o*-tolyl ether can block neuromuscular transmission (Exley, 1954). In one preparation, doses of 0.05 and 0.10 mg./kg. caused a brief potentiation followed by a slight reduction of the twitch of soleus and potentiated the twitch of tibialis. The contractions of both muscles were completely inhibited by 0.5 mg./kg. This block lasted for 2.5 min. and recovery was rapid (Fig. 2). Injection of 0.1 mg./kg. neostigmine had little or no effect on the rate of recovery with this compound and in one experiment the relative sensitivity of the two muscles was reversed.

Neuromuscular block appeared with doses of 3 to 5 mg./kg. of choline 2:6-xylyl ether. In the majority of experiments the contractions of soleus were reduced less than were those of tibialis, although the relative sensitivity of the two muscles was sometimes reversed. In no experiment was an initial stimulation of either muscle observed. Fig. 7 shows an experiment in which 7.0 mg./kg. of choline 2:6-xylyl ether



(a)



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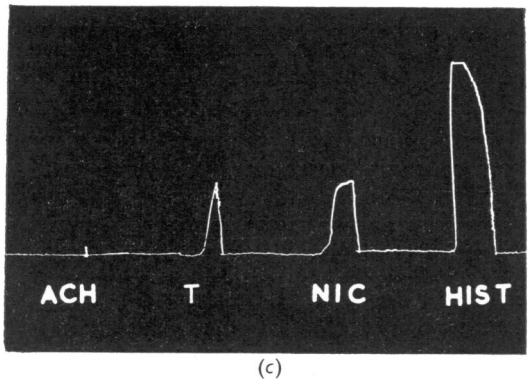

(c)

FIG. 6.—Contractions of isolated guinea-pig ileum. (*a*), 100 μg./ml. hexamethonium present throughout; (*b*), Tyrode solution only; (*c*), 1.0 μg./ml. atropine present throughout. ACh, 0.1 μg. acetylcholine; T, 100 μg. choline 2:6-xylyl ether; NIC, 100 μg. nicotine; HIST, 0.05 μg. histamine. The stimulant drugs were in contact with the ileum for 30 sec. Bath volume, 5 ml.

produced an almost complete inhibition of the contractions of tibialis. The contractions had increased to 50% of the pre-injection height within 6 min. and fully recovered within about 15 min. By comparison, gallamine in a dose of 1.2 mg./kg. produced an almost complete neuro-

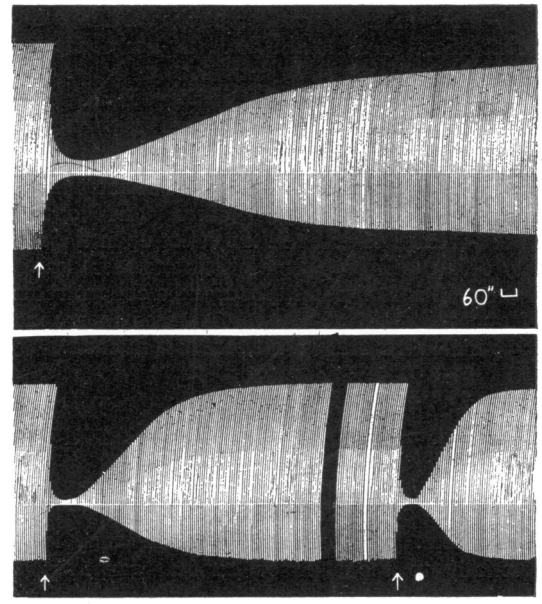

FIG. 7.—Cat, 2.70 kg., chloralose anaesthesia. Effect of 2:6-xylyl ether and gallamine given intravenously on contractions of tibialis muscle in response to stimulation of the sciatic nerve. Top: 1.2 mg./kg. gallamine at arrow. Bottom: 7.0 mg./kg. choline 2:6-xylyl ether at arrows. At ●, 0.15 mg./kg. neostigmine. Note short duration of effect produced by choline 2:6-xylyl ether and increased speed of recovery after injection of neostigmine. Time, 60 sec.

muscular block with 50% recovery in 12 min.; full recovery was not reached after 35 min. Several experiments have shown that choline 2:6-xylyl ether has approximately 0.25 of the potency and duration of action of gallamine. During block due to choline 2:6-xylyl ether, a tetanus was poorly maintained and was followed by partial reversal of the block; neostigmine hastened recovery.

Choline 2:4:6-mesityl ether was much less potent in neuromuscular blocking properties. A dose of 10 mg./kg. was required to produce 20% block of both muscles. 20 mg./kg. produced complete block of short duration, reversible by a tetanus.

Effects on Frog Rectus Abdominis Muscle.—Choline phenyl ether and choline *o*-tolyl ether both caused contracture of the isolated frog rectus abdominis muscle suspended in oxygenated frog Ringer solution. Their activities were about 10% and 35% respectively of the activity of acetylcholine and the contractures were blocked by (+)-tubocurarine.

Choline 2:6-xylyl ether had mixed actions. Concentrations up to 0.2 mg./ml. present in the

bath for 1 min. reduced or abolished the subsequent effect of acetylcholine. Higher concentrations (0.2 to 1 mg./ml.) caused a contracture.

Choline 2:4:6-mesityl ether blocked acetylcholine in concentrations up to 1 mg./ml., but showed no stimulant effect.

Anti-cholinesterase Activity.—Choline phenyl ether, choline 2:6-xylyl ether, and choline 2:4:6-mesityl ether had little effect upon the activity of red cell or plasma cholinesterases derived from defibrinated horse blood, and a concentration of approximately 10^{-2}M was required to produce 50% inhibition using a manometric method.

Discussion

Our results suggest that nicotinic properties occur in this series of compounds. Thus choline phenyl ether and choline *o*-tolyl ether caused a pressor response which could be blocked by hexamethonium. When they were injected intra-arterially to the superior cervical ganglion, all four compounds produced a contraction of the nictitating membrane, even though the external carotid artery was clamped, so that a peripheral action was unlikely. However, results with choline 2:6-xylyl ether and choline 2:4:6-mesityl ether were variable, especially on the superior cervical ganglion. On the isolated guinea-pig ileum, choline phenyl ether and choline *o*-tolyl ether produced a contraction which could be abolished or reduced by hexamethonium. On the isolated frog rectus-abdominis muscle choline 2:4:6-mesityl ether was the only drug which did not produce a contracture.

These nicotinic properties were most pronounced with the unsubstituted compound and decreased with increasing substitution on the ring. It is not clear from the present work why tachyphylaxis of the nicotine-like properties of choline 2:6-xylyl ether, seen both on the blood pressure and on the superior cervical ganglion, should leave responses to choline phenyl ether unaffected. Similar observations have been reported by Willey (1957), who showed that nicotine gave a pressor response when choline 2:6-xylyl ether no longer did so.

Muscarinic properties were also seen in this series. Thus choline phenyl ether and choline 2:6-xylyl ether produced a fall in blood pressure after the nicotinic effects had been eliminated either by hexamethonium or through tachyphylaxis respectively. The depressor response could then be abolished by atropine. Furthermore, on the guinea-pig ileum, choline 2:6-xylyl ether and choline 2:4:6-mesityl ether both produced a contraction which was unaffected by hexamethonium but was abolished or reduced by atropine. Since the contractions produced by the other two compounds were blocked by hexamethonium it was not possible to carry out an unequivocal test for muscarinic effects on this preparation.

The compounds also showed neuromuscular blocking properties. Bovet, Depierre, and Lestrange (1947) and Winter and Lehman (1950) have previously reported this property with choline phenyl ether. The former authors found that 2 to 5 mg. injected intravenously in a rabbit which was receiving artificial respiration caused neuromuscular block which lasted for 20 min. Although we have not examined this compound quantitatively for its neuromuscular blocking action in the cat, evidence from the intravenous toxicity in mice, the relative potency on the frog rectus abdominis muscle, and the doses employed by Bovet *et al.* (1947) to produce neuromuscular paralysis in the rabbit, suggest that this compound is less active than choline *o*-tolyl ether. Both of these compounds were short-acting and predominantly decamethonium-like in action. Choline 2:6-xylyl ether was less active still and its actions were predominantly like those of tubocurarine. This change in mode of action was still allied to a short duration of effect. We have not completely analysed the mode of action of this compound and it is conceivable that neuromuscular paralysis might be due to the prevention of acetylcholine release from the motor nerve endings. However, this possibility seems unlikely in view of the long duration of its local anaesthetic action. A short neuromuscular block was also seen with choline 2:4:6-mesityl ether which had a weak, mainly tubocurarine-like, type of action. This compound also possesses prolonged local anaesthetic properties.

Brown and Hey (1956) have demonstrated inhibition of amine oxidase by compounds in this series; choline phenyl ether was the least active, whereas choline *o*-tolyl ether and choline 2:4:6-mesityl ether possessed high activity and were more active than choline 2:6-xylyl ether. In addition these compounds possess weak anti-adrenaline properties, first described by Hey and Willey (1954).

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