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CHOLINE 2 : 6-XYLYL ETHER BROMIDE; AN ACTIVE QUATERNARY LOCAL ANAESTHETIC

BY

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When examining nuclear substituted choline phenyl ethers for nicotine-like stimulant activity we found that intravenous injection of one of these, choline 2: 6-xylyl ether bromide (TM.10), produced the typical brief rise in the cat's arterial blood pressure which is characteristic of these compounds. Subsequent doses of the same compound, however, failed to produce any further response—even after a lapse of several hours. This rapid and prolonged tachyphylaxis appears to result from a powerful local anaesthetic action of the drug.

METHODS

Cats were used in all the experiments. They were given 1 mg./kg. of atropine sulphate intraperitoneally, after which anaesthesia was induced with ether and maintained by chloralose (100 mg./kg. i.v.). Arterial blood pressure was recorded from a carotid artery by a mercury manometer, and a venous cannula was inserted in the femoral vein. Other experimental procedures are indicated in the description of particular experiments.

RESULTS

The extent of the tachyphylaxis to TM.10 is shown in Fig. 1. This also shows that TM.10 has some slight adrenolytic activity but that the magnitude and duration of this cannot account for the failure of the second dose of TM.10 to produce a pressor effect.

Analogous effects on the nictitating membrane of the cat are shown in Fig. 2. In this experiment the cervical sympathetic nerve was cut on one side and on the other the superior cervical ganglion was removed. On the side with the intact ganglion the response to standard intravenous doses of adrenaline remained fairly constant, whereas the response to doses of TM.10 diminished rapidly. On the side with the ganglion removed, doses of TM.10 regularly produced relaxation, seen also to some extent on the other side following the stimulant effect; it is possible that this is due to a transient adrenolytic action against normally circulating adrenaline.

Further confirmation of the prolonged effect of TM.10 is given by Fig. 3, which shows the contractions of the nictitating membrane on intermittent electrical stimulation of the cervical sympathetic nerve. A single dose of TM.10 abolished all response within a few minutes, and this effect persisted for more than three hours.

From these results it seemed unlikely that the effects of TM.10 could be ascribed to adrenolytic, sympatholytic or ganglion-blocking actions. It therefore seemed possible that the compound might act by suppressing conduction in postganglionic sympathetic fibres.

This hypothesis was tested in an experiment the results of which are shown in Fig. 4. Contractions

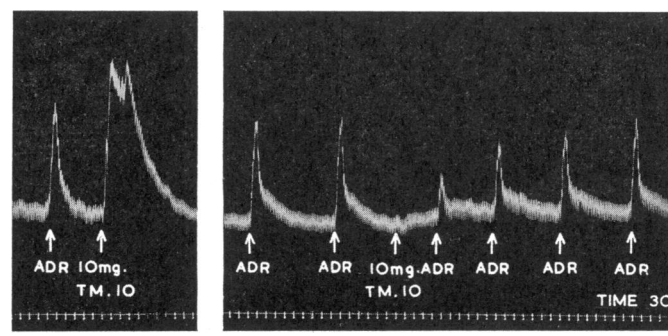


FIG. 1.—Carotid blood pressure of atropinized spinal cat showing tachyphylactic and adrenolytic actions of TM.10 administered intravenously. Adr = adrenaline 3 μg. i.v. Time interval between the two tracings is approximately 1 hr.

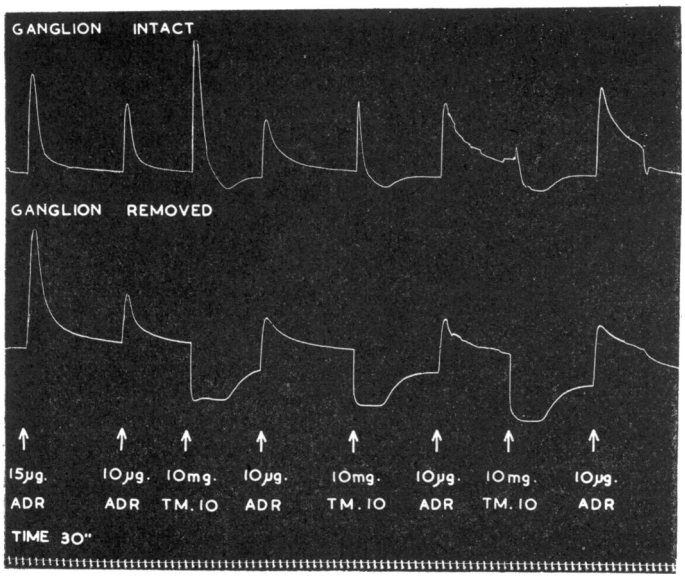


FIG. 2.—Cat, atropinized, chloralose. Responses of nictitating membranes to adrenaline and TM.10. Upper record from side with superior cervical ganglion intact. Lower record from side with ganglion removed at beginning of experiment.

of the nictitating membranes are recorded in response to intermittent electrical stimulation applied on one side to the postganglionic nerve trunk, and on the other to the cervical sympathetic nerve. The cervical sympathetic nerve was cut on both sides. After confirming the correct placing of the electrodes by an intravenous dose of tetraethylammonium iodide, TM.10 was given by the same route. A total dose of 20 mg. of TM.10 completely abolished responses in both membranes. (The residual small irregularities in the trace are due to unavoidable general motor stimulation from the postganglionic electrodes.) This, in conjunction with the other experimental data, made the hypothesis tenable, and, since postganglionic sympathetic nerve fibres resemble sensory fibres in being lightly myelinated, it seemed possible that TM.10 would show local anaesthetic actions.

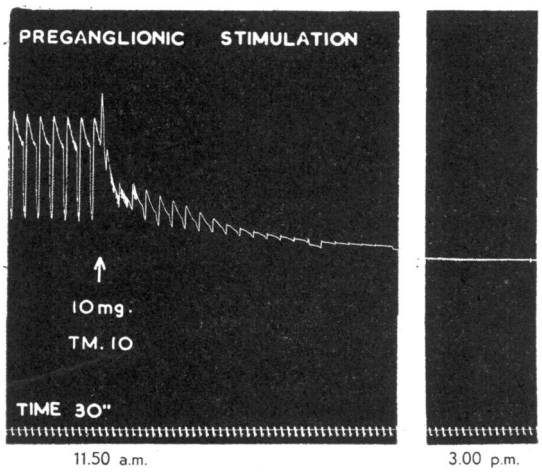


FIG. 3.—Cat, atropinized, chloralose. Nictitating membrane contractions. Periodic preganglionic stimulation (50/sec.) applied for 45 sec. in each min. At the arrow 10 mg. TM.10 injected i.v.

When tested by the cavy intracutaneous weal technique of Bülbring and Wajda (1945), TM.10 had a local anaesthetic activity approximately equal to that of cocaine hydrochloride. There were, however, very marked differences in the durations of the effect; the action of TM.10 lasted 2–10 times as long as that of cocaine, the larger differences being given by the higher concentrations. This difference in the duration of the effects is shown in Fig. 5, where the degree of anaesthesia is plotted, on a linear scale, against time on a logarithmic scale; at 0.3% concentration for both drugs it is evident that the effect of TM.10 lasts 5–6 times as long as does that of cocaine hydrochloride and that there is a detectable delay in the onset of action of the former. In preliminary experiments using a 2% solution of TM.10 the anaesthesia lasted approximately 48 hours.

Following these observations we demonstrated that a sufficiently large intravenous dose of procaine hydrochloride produced the same effects on the response of the nictitating membrane to electrical stimulation, but, as might be expected, these were of shorter duration. This is clearly shown in Fig. 6, in which the experimental conditions were the same as in Fig. 4.

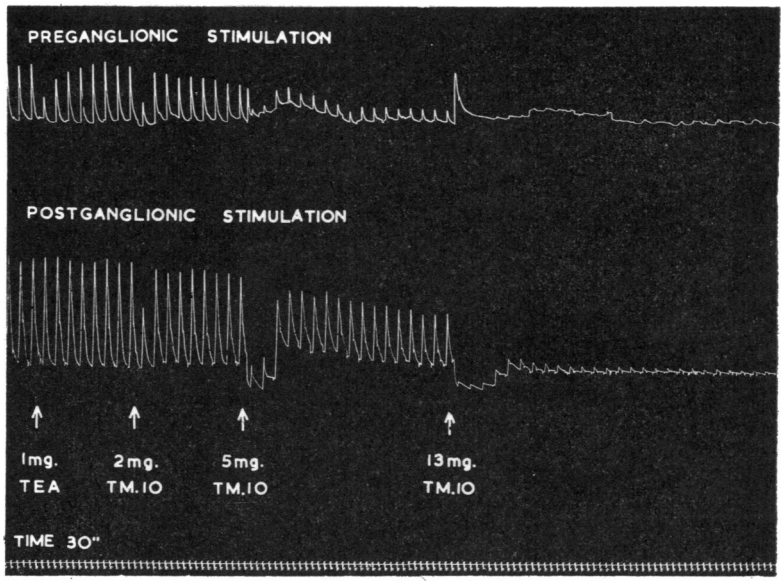


FIG. 4.—Cat, atropinized, chloralose. Contractions of nictitating membranes evoked by periodic stimulation (70 sec.) applied for 2 sec. in each min. to the preganglionic nerve (upper record) and to the postganglionic nerve (lower record). The arrows indicate injection of 1 mg. tetraethylammonium iodide, 2 mg. TM.10, 5 mg. TM.10, and 13 mg. TM.10 respectively.

Pronounced local anaesthetic activity in quaternary ammonium compounds has recently been described by Nádor, Herr, Pataky and Borsy (1953), but, in the compounds they studied, the onset of action was slow, whereas with TM.10 it is rapid.

We subsequently investigated a number of compounds related to TM.10; some data concerning these are presented in Table I. Local anaesthetic activity appears to depend on the presence of at least one suitable group in the ortho position to the side chain and that, given this condition, both quaternary and tertiary compounds are active. Topical activity is shown only by the tertiary compounds, which is in accord with the general conclusion that quaternary cations are poorly absorbed by mucous membranes.

Compound TM.10 has been tested in man by intracutaneous injection and the local anaesthetic properties have been confirmed.

In conclusion it is interesting to note that the more active compounds described show considerable steric similarities to lignocaine hydrochloride (" Xylocaine ").

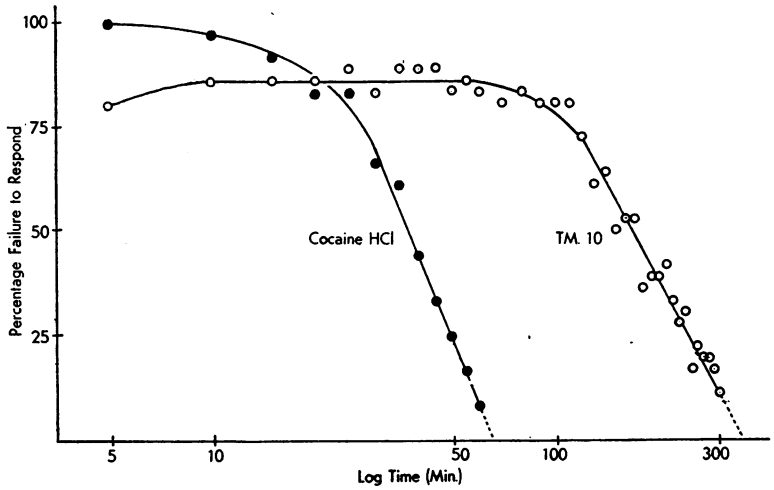


FIG. 5.—Graph showing the relative durations of local anaesthesia produced by intracutaneous injections of 0.3% cocaine hydrochloride and 0.3% TM.10. Ordinate, % failure of the guinea-pig to respond to mechanical stimuli. Abscissa, time (min.) on logarithmic scale.

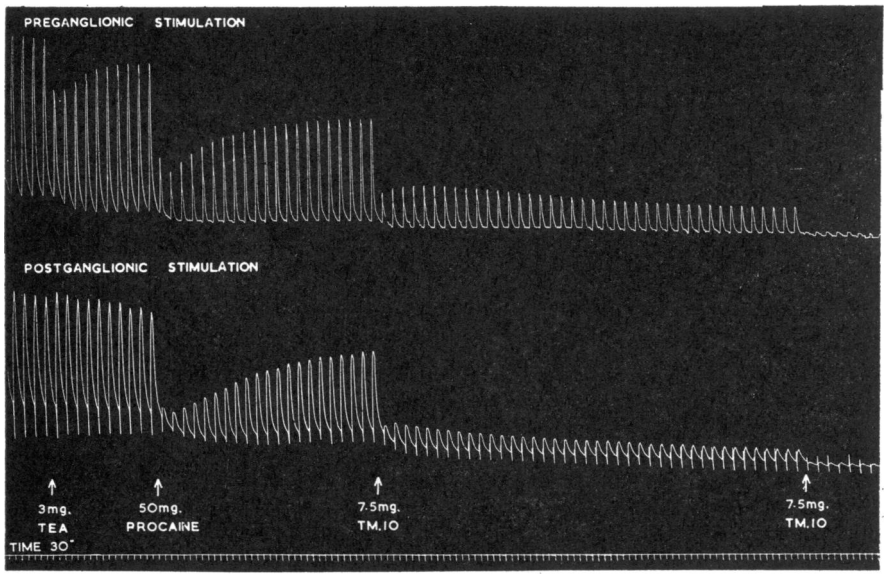
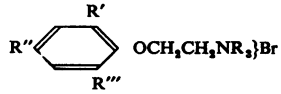
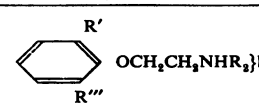


FIG. 6.—Cat, atropinized, chloralose. Contractions of the nictitating membranes in response to periodic stimulation (70/sec.) applied for 15 sec. in each min. The arrows indicate intravenous injections of 3 mg. tetraethylammonium iodide, 50 mg. procaine hydrochloride, 7.5 mg. TM.10, and 7.5 mg. TM.10 respectively.

TABLE I

LOCAL ANAESTHETIC PROPERTIES OF COMPOUNDS RELATED TO TM.10

Code Number	R	R′	R‴	R″	Anaesthetic Activity by Intracutaneous Injection (Cocaine HCl = 1)		Other Comments
					Activity	Duration	
Quaternary ammonium compounds 							
TM.10	CH_3	CH_3	CH_3	H	1	2–10	No topical activity on rabbit cornea. LD50 (i.p. to mice) 95 mg./kg.
TM.1	CH_3	H	H	H	0	0	
TE.10	C_2H_5	CH_3	CH_3	H	1	1–5	No topical activity on rabbit cornea
TM.18	CH_3	CH_3	H	H	—	—	Intracutaneous dose of 1 mg. killed 11/12 cavies; one survivor showed anaesthesia. LD50 (i.p. to mice) 18 mg./kg.*
TM.17	CH_3	CH_3	CH_3	CH_3	1	2–10	
Tertiary amines 							
TTM.10	CH_3	CH_3	CH_3	—	1	1	Shows topical activity on rabbit cornea approximately equal to cocaine
TTE.10	C_2H_5	CH_3	CH_3	—	1	1	Shows topical activity on rabbit cornea approximately equal to cocaine. LD50 (i.p. to mice) 95 mg./kg.

* Dr. K. A. Exley (private communication) has subsequently shown that the toxic effect of TM.18 results from a neuromuscular paralysing activity, approximately one-fifth that of (+)-tubocurarine chloride on a weight basis, and that the difference in toxicity by the intracutaneous and intraperitoneal routes is owing to slow absorption by the latter.

Chemical Section

Melting points and boiling points are uncorrected.

1. β-Aryloxyethyl bromides were prepared by methods similar to those previously described (Hey, 1952). The following are recorded:

(a) *β(2 : 6-Xylyloxy)ethyl bromide.* Liquid, b.p. 123°/10 mm. $[n]_D^{20}$ 1.5391. (Found: C, 53.0; H, 5.9; Br, 32.3. $C_{10}H_{13}OBr$ requires C, 52.4; H, 5.7; Br, 34.8%.)

(b) *β(2 : 4 : 6-Mesityloxy)ethyl bromide.* Liquid, b.p. 148°/15 mm. $[n]_D^{20}$ 1.5348. (Found: C, 54.5; H, 6.1; Br, 32.8. $C_{11}H_{15}OBr$ requires C, 54.3; H, 6.2; Br, 32.9%.)

2. Tertiary amines were prepared by allowing the aryloxy ethyl bromide to react in a sealed ampoule at room temperature with an excess of an ethereal solution of dimethylamine or diethylamine. The following were prepared:

(a) *β(2 : 6-Xylyloxy)ethyldimethylamine.* Liquid, b.p. 124°/10 mm. The hydrobromide (TTM.10) crystallized from methanol in needles, m.p. 166°. (Found: C, 52.3; H, 7.2; N, 5.1; Br, 28.8. $C_{12}H_{20}ONBr$ requires C, 52.6; H, 7.3; N, 5.1; Br, 29.1%.)

(b) *β(2 : 6-Xylyloxy)ethyldiethylamine.* Liquid, b.p. 131°/10 mm. The hydrobromide (TTE.10) crystallized from methanol in needles, m.p. 151°. (Found: C, 55.8; H, 8.1; N, 4.6; Br, 26.3. $C_{14}H_{24}ONBr$ requires C, 55.6; H, 8.0; N, 4.6; Br, 26.4%.)

3. Quaternary ammonium salts were prepared as previously described (Hey, 1952). The following are recorded:

(a) *Choline 2 : 6-xylyl ether bromide* (TM.10). Prisms from acetone-methanol, m.p. 209°. (Found: C, 54.0; H, 7.7; N, 4.6; Br, 27.9. $C_{13}H_{22}ONBr$ requires C, 54.2; H, 7.7; N, 4.9; Br, 27.7%.)

(b) *Choline 2 : 4 : 6-mesitylether bromide* (TM.17). Prisms from acetone-methanol, m.p. 186°. (Found: C, 54.5; H, 8.2; N, 4.5; Br, 26.1. $C_{14}H_{24}ONBr$ requires C, 55.6; H, 8.0; N, 4.6; Br, 26.4%.)

(c) *β(2 : 6-Xylyloxy)ethyltriethylammonium bromide* (TE.10). Prisms from acetone-methanol, m.p. 181°. (Found: C, 57.8; H, 8.6; N, 4.3; Br, 23.8. $C_{16}H_{28}ONBr$ requires C, 58.2; H, 8.5; N, 4.2; Br, 24.2%.)

The choline *o*-tolyl ether (TM.18) was prepared as described by Goldfarb (1941).

Summary

1. Choline 2 : 6-xylyl ether bromide has a powerful and long-lasting local anaesthetic effect. It suppresses conduction for long periods of time in postganglionic fibres arising in the superior cervical ganglion of the cat.

2. Certain related compounds have pronounced local anaesthetic activity.

We are indebted to Mrs. Y. Richards for the microanalyses.

References

Bülbring, E., and Wajda, I. (1945). *J. Pharmacol.*, **85**, 78.

Goldfarb, A. R. (1941). *J. Amer. chem. Soc.*, **63**, 2280.

Hey, P. (1952). *Brit. J. Pharmacol.*, **7**, 117.

Nádor, K., Herr, F., Pataky, Gy., and Borsy, J. (1953). *Nature, Lond.*, **171**, 788.