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INHIBITION OF ACETYLCHOLINE SYNTHESIS IN NERVOUS TISSUE BY SOME QUARTERNARY COMPOUNDS

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Abstract—Investigations of the pharmacological properties of triethylcholine and troxonium (FWH-429) have shown that these agents produce muscular weakness, respiratory paralysis, and failure of transmission at the cholinergic synapses excited repetitively at high frequency. Experiments *in vivo* and *in vitro* show that these effects are antagonized by choline and can be ascribed to their ability specifically to inhibit acetylcholine synthesis. The compounds have no significant inhibitory action on choline acetylase. A quantitative comparison of the inhibitory effect on acetylcholine synthesis in minced mouse brain preparation demonstrated that hemicholinium base (HC-3) is approximately 15 times more potent than FWH-429 and 25 times more potent than triethylcholine. Their action on the release mechanism of acetylcholine appears to be secondary to the capacity to inhibit the formation of bound acetylcholine. In contrast to HC-3, both FWH-429 and triethylcholine exhibit marked postsynaptic blocking action at ganglionic synapses.

HEMICHOLINIUM BASE (HC-3) has been shown to inhibit the synthesis of acetylcholine (ACh) in nervous tissues[1–4] either by preventing access of choline to the intracellular sites of acetylation[5] or by competing with ACh for the storage sites.[6] Recently some tetra-alkylammonium bases have been reported to possess significant hemicholinium-like activity.[7] Tetraethylammonium (TEA) was the most potent of the series tested. Similar effects of TEA on ACh synthesis have been observed in repetitively stimulated perfused superior cervical ganglia of cat.[8] Triethylcholine (TEC), which produces failure of transmission at the neuromuscular junction,[9] has also been shown to inhibit ACh synthesis in "mitochondrial" fractions of rabbit brain and tissue slices obtained from caudate nucleus of rabbit brain.[10]

This communication deals with results obtained with TEC and troxonium tosylate† [FWH-429, triethyl-2(3,4,5-trimethoxybenzoyloxyethylammonium) tosylate] on ACh synthesis in nervous tissue and compares their action with that of HC-3; FWH-429 has previously been reported to possess hypotensive properties[11, 12] and to be of low toxicity in man.[13]

METHODS

Acute toxicity studies in mice

Acute studies were done in female mice (CFW strain, 15 to 20 g) under quiet surroundings at a room temperature of 23°–24°. All compounds were dissolved in

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† Approved British Pharmacopoeia Commission name

saline and administered in a volume equivalent to 10 ml/kg body weight. Each animal received two i.p. injections in rapid succession—one of the test substance followed by a second injection of choline, eserine, or saline—and survival time was noted. The LD_{50} for the compound alone and in combination with choline or eserine at various dosage levels was determined by the method of probits.[14]

Studies of superior cervical ganglion of cat

Cats of either sex, weighing 2 to 3 kg, were anesthetized (chloralose 60 mg/kg and urethane 500 mg/kg, i.p.). The trachea was cannulated, and the animals were maintained on artificial respiration. The preganglionic sympathetic nerve was dissected for 4 to 5 cm and severed caudally. When required, the postganglionic sympathetic nerve was exposed to give easy access to the stimulating electrodes. The preganglionic nerve was stimulated supramaximally with rectangular pulses (0·5 msec duration, 5 to 20 shocks/sec) delivered through platinum electrodes. Contraction of the nictitating membrane was recorded isotonically on smoked paper. The sympathetic nerve was kept immersed in mineral oil, and the stimulating electrodes were occasionally moved closer to the ganglion to maintain maximal excitation. All the compounds were dissolved in saline and administered through the femoral vein unless stated otherwise. The following experiments were then performed.

(*a*) *Ganglionic blocking activity.* Response of the nictitating membrane to preganglionic stimulation at 20 shocks/sec was recorded in the presence of varying doses of test substance. Percentage inhibition of contraction and the time until recovery were calculated. Each animal received only one of the test substances and at a minimal interval of 45 min between successive doses. Occasionally the postganglionic trunk was stimulated at peak of drug action to localize the site of action.

(*b*) *Effect of frequency of stimulation, rest, or choline on ganglionic blockade.* In the atropinized cat (1 mg/kg), contraction of the nictitating membrane was elicited by stimulating the preganglionic nerve at a frequency of 20 shocks/sec at supramaximal strength. The test substance was administered, and the resulting ganglionic blockade was maintained by continuous infusion. After the maximal blockade had been achieved, contraction of the nictitating membrane was recorded in response (i) to reduced rate of stimulation (5 shocks/sec), (ii) to stimulation at a frequency of 20 shocks/sec after resting the ganglion for 10 min, and (iii) to i.v. injection of choline (3 mg/kg).

(*c*) *ACh content of the superior cervical ganglion of cat.* Cats were prepared for recording the contraction of the nictitating membrane and atropinized. Blood vessels in the vicinity of the ganglion, except those supplying the ganglion itself, were carefully tied off to facilitate removal of the ganglion with little bleeding. The ganglion of one side was stimulated supramaximally (20 shocks/sec) for 30 min through the preganglionic sympathetic nerve, and contraction of the nictitating membrane was recorded. It was then rapidly excised and minced in 3 ml of ice-cold 10% trichloroacetic acid (TCA) and left for 90 min to extract the preformed ACh. The opposite ganglion was similarly treated except that stimulation was carried out while the test substance was being infused at a constant rate of 200 μg/kg per min after a priming dose of 2 mg/kg. TCA was removed from each extract by shaking it three or four times with five volumes of water-saturated ether. After evaporation of ether, the pH was adjusted to about 7, and ACh was assayed by the cat blood-pressure method as described by MacIntosh and Perry.[15]

Activity of the samples was ascribed to ACh if it was abolished by atropinization of the cat or by brief contact of the assay material with alkali. Results are expressed in terms of ACh chloride.

Effect of drugs on the response to close intra-arterial injection of ACh

The common carotid artery about 2 to 3 cm below the superior cervical ganglion was exposed in cats. The animal was then injected with atropine (1 mg/kg) and heparin (2 mg/kg). Response of the nictitating membrane to graded doses of ACh, administered through the common carotid artery, was obtained with the external carotid artery occluded at the time of each injection. Then a constant i.v. infusion of the test substance was started, and after the response to injected ACh became constant, the dose response to ACh was redetermined while the test substance was still being infused.

ACh synthesis in minced brain of mice

Albino mice (CFW strain) of either sex, weighing 15 to 20 g, were used. Brains were minced and incubated for 1 hr at 37° in an atmosphere of O_2 and CO_2 mixture (95 : 5, pH 7·4) as described by Bhatnagar and MacIntosh.[7] Incubation medium consisted of NaCl (130 mM), $CaCl_2$ (2 mM), KCl (4 mM), $NaHCO_3$ (25 mM), glucose (10 mM) and eserine sulfate (20 μM). The compound was dissolved in the medium and added to the incubate before incubation. Final concentration of the test substance varied between 0·1 μM and 1 mM and the final volume of each sample was $10 \pm 0{\cdot}1$ ml. Duplicate samples were used. Two control samples, containing no drug, were made up to volume by additional medium. Two samples similarly prepared but not incubated were used for estimating free and bound ACh at zero time. In some experiments choline chloride was added to the incubate in varying concentrations to determine its effectiveness as an antagonist to the inhibitory action of the drug on ACh synthesis.

Free ACh (released into the medium) and bound ACh (extracted from the tissue) were measured as described by Bhatnagar and MacIntosh,[7] and assayed by the cat blood-pressure method immediately after incubation. For estimating free ACh, standards were prepared in the incubation medium containing the appropriate concentrations of the test substance. The presence of ACh was confirmed as described earlier.

ACh synthesis in a system containing choline acetylase in solution

Effect of the compounds on choline acetylase activity was determined by incubating a reaction mixture containing the enzyme (0·1 ml), cysteine (200 μM), EDTA (2 mM), choline chloride (10 μM), acetyl-CoA (150 μM), eserine sulfate (30 μM), and sodium phosphate buffer (167 mM, pH 7) in the presence of the test substance (0·01, 0·1, or 1 mM) at 37° for 30 min. Samples were incubated in duplicate, and final volume of each was 2 ml. Two samples, which contained no drug but were incubated in the usual manner, served as control; two similar samples, not incubated, represented ACh synthesis at zero time.

Choline acetylase was prepared from acetone-dried brain powder of mice as described by Burgen *et al.*[16] and acetyl-CoA by the method of Wilson.[17]

At the end of the incubation period the reaction was terminated by adding 1 ml

of 10% TCA to the mixture. TCA was removed as previously described, and the ACh content was estimated by the cat blood-pressure method.

ACh content of whole brain

Drugs dissolved in saline were administered i.p. to mice in varying doses up to the LD_{50} level; 10 min after injection the animals were killed by decapitation. After rapid dissection and removal of the pituitary, the brain was rinsed in ice-cold saline, wiped clean on a wet filter paper, and weighed. This whole procedure was accomplished within 1 min. Each brain was then minced separately in 5 ml of ice-cold 10% TCA for the extraction of preformed ACh. TCA was removed from the samples after 90 min, and ACh was estimated as previously described.

Modification of hexobarbital-induced sleeping time

Male albino mice (CFW strain), weighing 15 to 20 g, were used. Hexobarbital sodium (100 mg/kg) was injected i.p., and the time from injection until return of the righting reflex was recorded. The test compound, in varying doses, was administered to groups of 20 mice each, either simultaneously with or 5 min prior to the barbiturate. In one experiment the drug was administered i.v. just at the reappearance of the righting reflex. Appropriate controls were included for each experiment.

RESULTS

Acute toxicity studies in mice

In various species of animals, HC-3 has been shown to produce a respiratory paralysis,[18–20] which can be antagonized effectively by choline.[7, 19, 21–25] A similar toxic effect has been reported for TEC[9, 26, 27] and TEA.[6, 7, 28] The specificity of choline as an antidote against compounds resembling HC-3 in their mode of action has been previously demonstrated.[7, 19] While confirming results obtained with TEC, the present investigations show that FWH-429 also produces symptoms in mice that closely resemble those of HC-3 (i.e. at sublethal doses respiratory depression and

TABLE 1. ANTAGONISTIC ACTIVITY OF CHOLINE TO DRUG TOXICITY IN MICE*

Test substance	Dose of choline (mg/kg)					
	Nil	25	50	100	200	300
FWH-429	23 (20–27)	57 (51–63)	61 (55–66)	64 (60–68)	50 (45–55)	
TEC	58 (54–63)	114 (96–134)	150 (142–158)	200 (190–210)	163 (155–171)	
HC-3	0·15 (0·14–0·16)	0·32 (0·27–0·39)	0·36 (0·32–0·41)	0·60 (0·56–0·64)	0·89 (0·82–0·97)	0·18 (0·17–0·20)

* LD_{50} in mg/kg with 95% confidence limits in parentheses; 60 animals for each test.

muscular weakness, and at lethal doses respiratory paralysis and asphyxial convulsions). These effects occurred after a latent period of 10 to 20 min, irrespective of dose. Choline chloride was found to be an effective antidote (Table 1). Against FWH-429 and TEC the optimum protective dose of choline was 100 mg/kg, which increased the value of LD_{50} about threefold. At 200 mg/kg the protective effect of

choline diminished. This might be expected if the toxicity of FWH-429 and TEC with choline were additive. Decrease in the protective effect of choline against HC-3 toxicity, on the other hand, did not occur until the dose of choline approached its own LD_{50} (340 mg/kg).

Greater effectiveness of choline against HC-3 toxicity was further demonstrated by comparing the survival time. At the LD_{50}-dose of FWH-429 the survival time was 13 min; however, when choline (100 mg/kg) was administered with it, death occurred in 7 min ($P < 0{\cdot}05$), but survival time was significantly prolonged when choline was administered with HC-3 (from 16 to 24 min; $P < 0{\cdot}05$). No change in survival time of mice receiving TEC in combination with choline was observed.

Eserine was also an effective antidote against the toxic effects of HC-3 in mice.[7, 19, 23, 25] In similar studies with FWH-429 and TEC no significant protection by eserine (0·25 mg/kg) was noted.

Studies of superior cervical ganglion of cat

(*a*) *Ganglionic blocking activity.* Both TEC and FWH-429 produced ganglionic blockade when the preganglionic sympathetic nerve was stimulated supramaximally at a high frequency (20 shocks/sec). Maximal block occurred within 2 to 3 min; degree and duration of the block was dose dependent. FWH-429 was about 10 time more potent than TEC at the ED_{50} level (ED_{50} being about 0·5 mg/kg, and 5 mg/kg for FWH-429 and TEC respectively). With HC-3, however, no dose response could be obtained. Immediately after the injection of HC-3 (2 mg/kg) the response of the nictitating membrane to preganglionic stimulation either remained unchanged or was slightly diminished. Only after a latent period of 4 to 5 min did the failure of transmission begin to occur, which became maximal 12 to 15 min after injection. Similar effects of HC-3 on the response of the cat nictitating membrane have been reported by Birks and MacIntosh.[4] HC-3 at a dose of 1 mg/kg produced variable effects.

Response of the nictitating membrane to postganglionic nerve stimulation remained unaffected during administration of all drugs, suggesting that their site of action lies within the ganglion.

(*b*) *Effect of frequency of stimulation, rest, or choline on ganglionic blockade.* Figure 1 shows that stimulation at a reduced frequency of 5 shocks/sec, or stimulation at 20 shocks/sec after resting the ganglion for 10 min, or the administration of a dose of choline, by itself too small to excite the ganglion cells or the nictitating membrane,[1]

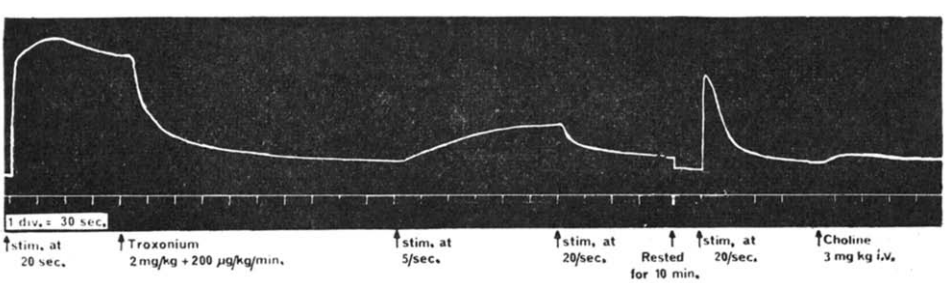

FIG. 1. Effect of frequency of stimulation, rest, or choline on ganglionic blockade produced in atropinized cat by troxonium (FWH-429).

resulted in partial restoration of conduction through the ganglion while FWH-429 was being infused i.v. (2 mg/kg + 200 μg/kg per min). Similar results were obtained with TEC (5 mg/kg + 500 μg/kg per min).

The partial reversal by choline of HC-3-induced blockade was interpreted by MacIntosh[6, cf. 4] as an indication of a presynaptic action of HC-3. Since choline also partially restores conduction through the ganglia treated with FWH-429 and TEC, it appears likely that these agents act like HC-3 at the presynaptic sites. This conclusion is supported by the observation that the ganglionic blockade produced by hexamethonium (0·5 mg/kg + 400 μg/kg per min), which is known to possess purely postsynaptic action, cannot be restored by choline, although reduction of the frequency of stimulation, or stimulation after resting the ganglion for 10 min, did result in partial recovery.

(*c*) *ACh content of the superior cervical ganglion.* Comparison of the ACh content of the ganglia stimulated in the presence of TEC or FWH-429 with those stimulated in their absence showed that both compounds specifically prevented ACh synthesis, as shown in Table 2.

TABLE 2. EFFECT OF THE DRUGS ON ACH CONTENT OF SUPERIOR CERVICAL GANGLION OF CAT*

Expt. no.	Control ganglion		Test ganglion		Reduction %
	Treatment	ACh (mμg/ganglion)	Treatment	ACh (mμg/ganglion)	
1	Stimulated (3)	284 ± 19	TEC + stim.	124 ± 28	56
2	None (3)	241 ± 24	TEC alone	231 ± 18	4
3	Stimulated (4)	275 ± 19	FWH-429 + stim.	104 ± 17	62
4	None (4)	235 ± 21	FWH-429 alone	222 ± 18	4
5	Stimulated (3)	261 ± 26	HC-3 + stim.	72 ± 27	72
6	None (3)	257 ± 21	HC-3 alone	246 ± 19	4
7	None (3)	258 ± 23	Stimulated	249 ± 22	3

* Number of experiments in parentheses. Stimulation of preganglionic sympathetic nerve for 30 min at 20 shocks/sec. Values are means ± S.D. Dose of drugs infused i.v., 2 mg/kg + 200 μg/kg per min.

With TEC, the ACh content of the stimulated ganglion was reduced by 56% (expt. 1) as compared to 62% and 72% in the presence of similar doses of FWH-429 and HC-3 respectively (expt. 3 and 5). Reduction in the ACh content could be effectively prevented by simultaneous administration of choline. The drugs alone (i.e. when the ganglia were not stimulated, expt. 2, 4, and 6) or stimulation alone (expt. 7) produced no significant reduction in ACh content of the ganglia.

Effect of TEC and FWH-429 on the response to close intra-arterial injection of ACh

The dose–response relationship for the ganglion-stimulating effect of ACh determined before and during continuous infusion of FWH-429 (100 μg/kg per min; Fig. 2) and TEC (200 μg/kg per min; Fig. 3) demonstrates that both drugs markedly diminish the ganglion-stimulating effect of ACh. A single injection of FWH-429 (2 mg/kg) or TEC (5 mg/kg) completely abolished the response to the injected ACh, but complete recovery occurred within 15 to 20 min.

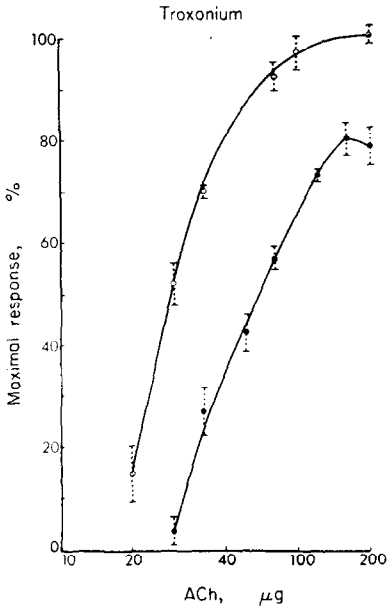

FIG. 2. Influence of the drugs on ganglionic-stimulating effect of ACh in the atropinized cat. Mean of 3 tests ± S.D. Control ○: drugs ●. Troxonium (100 μg/kg per min) infused i.v.

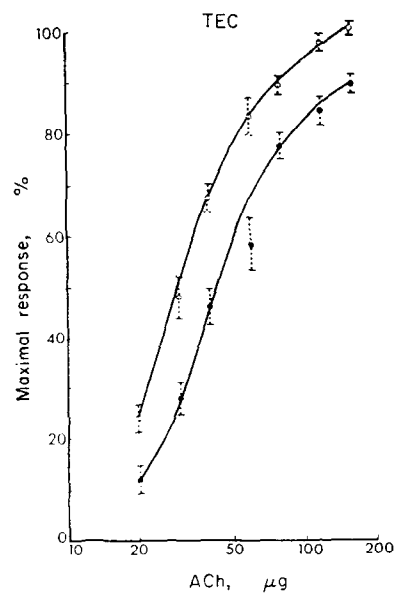

FIG. 3. See Fig. 2; TEC (200 μg/kg per min) infused i.v.

Inhibitory effect on ACh synthesis in minced brain of mice and its reversal by choline

Results summarized in Table 3 express the "total" ACh synthesized in the presence of varying concentrations of test substances as per cent of control. Total ACh synthesized represents the net increase in both free and bound forms of ACh during incubation. These data show that both TEC and FWH-429 strongly inhibit the synthesis of ACh, the ED_{50} being 100 μM and 60 μM respectively. HC-3 (ED_{50} 4μ M)

TABLE 3. EFFECT OF TEC, FWH-429 AND HC-3 ON ACH SYNTHESIS IN MINCED BRAIN OF MICE*

Drug	No. of tests	Concentration of drugs						ED_{50} (μM)
		Nil	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M	
TEC	6	100	99	92	83	51	9	100
FWH-429	6	100	98	90	71	28	7	60
HC-3	6	100	90	63	26	11	11	4

* Total ACh synthesized expressed as % of control. Incubation at 37° for 1 hr in eserinized bicarbonate–Locke's solution (pH 7·4). Gas phase 95% O_2 : 5% CO_2. Each figure represents an average of 6 tests.

was found to be 15 times more potent than FWH-429 and 25 times more potent than TEC.

Figure 4 graphically shows the absolute values for free and bound ACh synthesized (mean of 6 tests ± S.D.). All compounds tested significantly depressed the formation of both free and bound ACh. Free ACh, however, was less readily affected by TEC, causing a slight elevation of the ratio of free to bound ACh (from 0·35 to 0·52; top of Fig. 4). This ratio was not significantly altered by HC-3 or FWH-429 except when

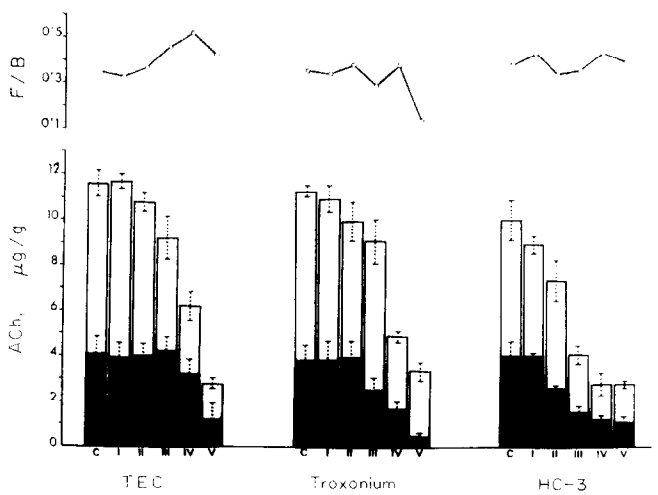

FIG. 4. Effect of TEC, troxonium and HC-3 on ACh synthesis in minced brain of mice. Experimental conditions given in Table 3. Mean of 6 tests ± S.D.

Lower record. Free ■ and bound □ ACh synthetized in 1 hr. Concentration of drug: C, nil; I, 10^{-7} M; II, 10^{-6} M; III, 10^{-5} M; IV, 10^{-4} M; V, 10^{-3} M.

Upper record. Ratio of free to bound ACh, as calculated from the data given in the lower record.

the latter compound was present in high concentration (1 mM) where it was reduced from a control figure of 0·35 to 0·15.

The inhibitory effect of HC-3 and certain other quaternary bases on ACh synthesis in nervous tissue can be reversed effectively by choline.[1–8, 10, 25, 29] In the present studies (Figs. 5–7) the concentration of choline required to completely reverse the

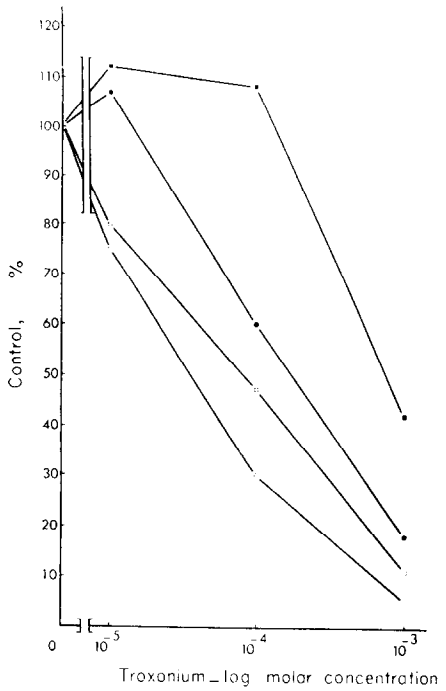

FIG. 5. Influence of choline on inhibitory activity of TEC on ACh synthesis in minced brain of mice. Experimental conditions same as in Table 3. Each point represents an average of two experiments. Concentration of added choline: nil, ○; 10^{-5} M □; 10^{-4} M ● M; 10^{-3} M ■.

inhibitory effect of FWH-429 (Fig. 5) and TEC (Fig. 6) was about 10 times the molar concentration of these compounds. Choline was found to be comparatively less effective against HC-3 (Fig. 7). For example, complete reversal of the inhibitory effect of 10^{-6} M HC-3 could be achieved only when the concentration of choline in the medium was increased to 10^{-3} M. These observations confirm the results obtained by Bhatnagar and MacIntosh[7] where, under similar experimental conditions, even a concentration of 10^{-2} M choline failed to reverse completely the inhibitory activity of 10^{-4} M HC-3. The slow reversal of the inhibitory effect of HC-3 by choline has also been observed in the superior cervical ganglion of cat.[8]

In the presence of TEC and HC-3 the rate of conversion of bound ACh to the free form was significantly accelerated by addition of choline to the incubation medium, as indicated by elevation of the ratio of free to bound ACh (from a control figure of 0·39 to approximately 0·70 for both compounds). No such change was noticed with FWH-429.

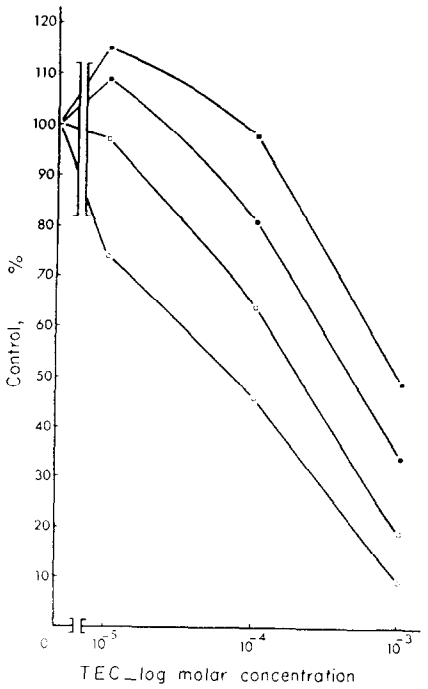

FIG. 6. Influence of choline on inhibitory activity of troxonium. Conditions as in Fig. 5.

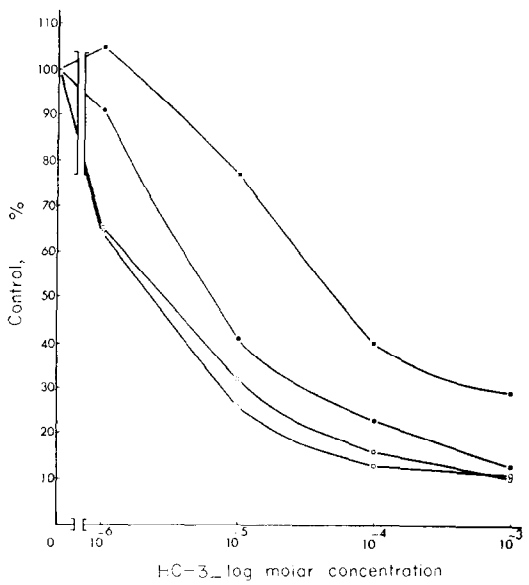

FIG. 7. Influence of choline on inhibitory activity of HC-3. Conditions as in Fig. 5.

ACh synthesis in a system containing choline acetylase in solution

The biological synthesis of ACh in nervous tissue is catalyzed by a specific enzyme, choline acetylase, which transfers the acetyl radical from acetyl-CoA to choline.[30] The possibility that FWH-429 and TEC might prevent formation of ACh by inhibiting acetylation of choline was tested in a system containing choline acetylase, choline, and acetyl-CoA. None of the compounds produced any significant inhibition.

ACh content of whole brain

Table 4 summarizes the absolute values of total cerebral ACh and the per cent

TABLE 4. EFFECT OF THE COMPOUNDS ON ACh CONTENT OF WHOLE BRAIN OF MICE

Drug	No. of animals	Dose (mg/kg)	ACh (μg/g)	% of control	P
Control	25		2·82 ± 0·39		
FWH-429	15	5	2·92 ± 0·35	104	
	15	10	3·20 ± 0·46	113	<0·01
	15	20	3·29 ± 0·31	117	<0·001
Control	15		2·91 ± 0·24		
TEC	10	15	3·03 ± 0·27	104	
	10	30	3·18 ± 0·37	109	<0·05
	5	58	3·14 ± 0·12	108	<0·05
Control	15		2·83 ± 0·28		
HC-3	10	0·05	3·03 ± 0·50	107	
	10	0·10	3·12 ± 0·41	110	<0·05
	5	0·15	3·15 ± 0·14	111	<0·01
Control	25		2·82 ± 0·39		
Choline + FWH-429	10	100 10	3·43 ± 0·21	122	<0·001
Choline	10	100	3·23 ± 0·48	115	<0·05

Values expressed as means ± S.D.

change from control. Results show that at higher doses these compounds produced small but statistically significant increase in cerebral ACh. FWH-429 was the most effective of the three compounds. Treated animals exhibited general depression and reduced motor activity at the time of sacrifice. Choline, which antagonizes the toxic effects of FWH-429 both *in vivo* and *in vitro*, failed to prevent the increase, although animals receiving both choline (100 mg/kg) and FWH-429 (10 mg/kg) showed none of the characteristic toxic symptoms. Elevation in the cerebral level of ACh in this case, however, could be attributed to the specific action of choline, since this increase was not significantly different from that produced by choline alone (100 mg/kg).

Modification of hexobarbital-induced narcosis

None of the three compounds affected sleeping time when injected simultaneously with hexobarbital. When administered 5 min prior to hexobarbital, however, duration of narcosis was significantly ($P < 0·001$) prolonged by FWH-429 and HC-3 (109% and 116% at 10 mg/kg and 0·1 mg/kg respectively) but not by TEC (30 mg/kg). Since the compounds failed to convert the subhypnotic doses of hexobarbital to hypnotic, they could be called prolonging agents rather than true potentiators. Certain

drugs are known to prolong the action of hexobarbital by interfering with its breakdown by the liver.[31–36]

DISCUSSION

Investigations of the pharmacological activity of TEC and FWH-429 have revealed that, like HC-3, these substances cause respiratory depression and muscular weakness at sublethal doses and respiratory paralysis at lethal doses. These effects are produced after a latent period which remains independent of the dose and can be antagonized effectively by choline.

Similarly, the failure of transmission produced by these substances in the rapidly stimulated superior cervical ganglion of cat could be partially counteracted by administration of choline. The reduced ACh content of such ganglia together with *in vitro* studies suggests that these effects can be ascribed in part to their specific inhibitory activity on the ACh-synthesizing mechanism. Since they had no significant inhibitory action on choline acetylase, it is likely that their mode of action is similar to that of HC-3. These experiments provide some support for the conclusions of Bowman and Rand[9] that neuromuscular block, produced by TEC in muscles repetitively excited at high frequency, is caused by lack of the transmitter substance. Preliminary studies (unpublished) on the rat phrenic nerve–diaphragm preparation and the cat tibialis muscle indicate that a similar type of neuromuscular block is produced by FWH-429.

Although the three compounds investigated showed strong ability to inhibit synthesis of ACh *in vivo* and *in vitro*, yet certain dissimilarities between their various pharmacological actions were noticed.

As opposed to the delayed block produced by HC-3 in rapidly stimulated cervical ganglion of cat, both TEC and FWH-429 caused almost immediate failure of transmission; degree and duration of the block was dose related. Reduction in the response of nictitating membrane to intra-arterial injection of ACh following administration of TEC or FWH-429 indicated that these compounds possess some competitive ganglionic blocking action. FWH-429 appeared to be more potent in this respect. It is noteworthy that, in contrast to the purely presynaptic action of HC-3, FWH-429 and TEC possess both pre- and post-synaptic actions in the ganglion. In the present studies no attempt was made to assess the degree to which the presynaptic action of FWH-429 and TEC influences their hypotensive as well as their ganglionic blocking actions.

Comparison of the relative potency of these compounds in minced brain preparation of mouse showed that HC-3 is about 15 times more potent than FWH-429 and 25 times more potent than TEC. In this preparation, TEC shows no detectable influence on the release mechanism of ACh (as indicated by the conversion of bound ACh to the free form) until the level of bound ACh is reduced by at least 50%. Since the amount of ACh released depends upon the level of depot ACh,[4] the fall in free ACh level at high concentrations of TEC could be attributed to the depressed rate of bound ACh formation rather than to specific action of the drug on the release mechanism. In the presence of HC-3 and FWH-429, rate of ACh release closely paralleled the rate of synthesis of bound ACh, except at highest concentration of FWH-429 used (1 mM), where release of ACh was drastically reduced.

Inhibition of ACh synthesis by these compounds could be antagonized effectively

by addition of choline to the incubation medium. Compared to TEC and FWH-429, the inhibitory action of HC-3 was more resistant to the antagonistic effect of choline. This suggests the formation of a more stable drug–receptor complex by HC-3, which wears off at a slower rate as compared to the other two compounds.

The manner in which these compounds inhibit the synthesis of ACh is still elusive and can only be speculated upon. In view of the close resemblances between the action of these compounds and HC-3, it seems reasonable to assume that they may act like HC-3 either by competing with choline for the intracellular sites of acetylation,[5] or by competitively infiltrating into the sites in the presynaptic nerve terminals where ACh is stored.[6] Bowman and Rand[9] have suggested that TEC itself may be transported and acetylated in place of choline, and released as a physiologically inert neurohormone.

In regard to the structure–activity relationship of TEC and FWH-429, despite the common triethylaminoethyl moiety, FWH-429 is a more potent inhibitor of ACh synthesis than is TEC. Recent studies have demonstrated that the pyrrolidinium analogue of FWH-429 (i.e. troxypyrrolidinium) is about six times more potent than FWH-429 as an inhibitor of ACh synthesis in minced brain preparation of mouse.[29] The degree of toxicity *in vivo*, however, appears to bear no correlation to the ability of these compounds to inhibit ACh synthesis.

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