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STIMULANT ACTIVITIES OF QUATERNARY AMMONIUM COMPOUNDS ON MAMMALIAN SKELETAL MUSCLE

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Though much information is available about the nicotine-like stimulant activities of quaternary ammonium compounds on vasomotor ganglia, little quantitative work has been done with such compounds on mammalian skeletal muscle.

Simonart (1932) compared the stimulant activities of some choline esters and ethers on the denervated gastrocnemius muscle of the cat. He used a cannula in the inferior mesenteric artery as a means of introducing the drug close to the muscle. Of the compounds examined he found the esters (acetyl, propionyl, and *n*-butyryl) to be much more active than the ethers (methyl, ethyl, vinyl, and butyl).

On injecting acetylcholine (ACh) into the innervated gastrocnemius muscle, through a cannula in the tibial artery, Brown, Dale and Feldberg (1936) obtained regular contractions. Their technique was also used by Bacq and Brown (1937) to compare the activities of some quaternary ammonium compounds. Brown *et al.* (1936) and Bacq and Brown (1937) concluded that ACh is very active as a stimulant of skeletal muscle, in contrast to its low stimulant activity on the blood pressure.

The present work was undertaken to extend and confirm these observations—especially for various new nicotine-like stimulant compounds prepared in this laboratory by Hey (1952, 1954) and previously investigated by him from the point of view of the blood pressure, on which many are extremely active.

As distinct differences have been noted between " red " and " white " muscles in their responses to stimulant drugs (Riesser, 1921 ; Zaimis, 1951) and to neuromuscular blocking agents (Paton and Zaimis, 1949, 1951 ; Zaimis, 1951), a method was devised to record simultaneously the contractions caused by the close-arterial injection of the stimulant compounds to both " white " (gastrocnemius) and " red " (soleus) muscle.

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METHODS

The following compounds were investigated : propionylcholine, *n*-butyrylcholine, *n*-valerylcholine, *iso*-butyrylcholine, trimethylacetylcholine, 4-keto-amyltrimethylammonium, *n*-amyltrimethylammonium, choline ethyl ether, choline butyl ether, choline phenyl ether, choline *p*-chlorophenyl ether, choline *m*-bromophenyl ether, and nicotine.

Cats anaesthetized with chloralose (100 mg./kg.) and treated with intraperitoneal atropine sulphate (2 mg./kg.) were used throughout.

Activity of Nicotine-like Stimulant Compounds on Skeletal Muscle

The drugs were administered by a method similar to that of Brown, Dale, and Feldberg (1936). Muscle contractions were recorded with a Brown-Schuster mammalian muscle myograph, provision having been made for the simultaneous recording of the contractions of both gastrocnemius and soleus muscles. The sciatic nerve was stimulated by periodic condenser discharges (4/min.) through shielded platinum electrodes. During chemical stimulation of the muscle one electrical impulse was omitted. Stimulant drugs were injected retrogradely into the cannulated stump of the cut anterior tibial artery ; during the injection the popliteal artery was occluded. A constant injection volume of 1 ml. was used throughout. All drugs were made up in saline with a *p*H of about 5. The doses were measured in micromoles. Before each injection the cannula in the tibial artery was filled, using a fine hypodermic needle, with a solution of the drug in the same concentration as that about to be administered.

The response of the muscle was estimated as the ratio of the increased tension produced by the injection of the stimulant drug to that produced by the electrical stimulus. The initial tension in all experiments was maintained at 50 g.

Some of the compounds produced a paralysis of the muscles. The activities of the compounds which caused little or no paralysis were estimated by a (2 + 2) assay technique. Acetylcholine was used as the standard drug throughout the assays ; two doses of ACh were compared with two doses of the drug under test. These four doses were injected in random order ; this series of four injections was repeated, again in random order.

The activities of the compounds which caused a long-lasting or permanent paralysis were compared with ACh by a (2 + 1) assay technique. Two doses of ACh were compared with one dose of the test drug, which, on the basis of previous observations, would be expected to produce a response lying between the two responses to ACh.

Inhibition of True and Pseudo-Cholinesterases

Two specific anticholinesterase drugs were used to inhibit cholinesterase activity—iso-ompa (Austin and Berry, 1953a, b) and 284C51 (Austin and Berry, 1953a, b; Fulton and Mogey, 1954). Using the enzymes of human blood, Austin and Berry found that iso-ompa irreversibly inactivated the plasma pseudo-cholinesterase. Fulton and Mogey showed that 284C51 produced a reversible inhibition of true cholinesterase and had little or no effect on pseudo-cholinesterase.

Iso-ompa (25 mg./kg.) was injected via the femoral vein at the beginning of the experiment. 284C51 (0.25 mg./kg.) was also injected by this route at the beginning of each assay—that is, about every 30 min. —since Fulton and Mogey showed that intraperitoneal doses of this order produced an anticholinesterase action in mice which lasted at least half an hour.

Estimation of Cholinesterase Levels. — Cholinesterase activity was determined manometrically at 37.5° C. using open manometers and Warburg manometer flasks, with a bicarbonate-Ringer solution as the medium, equilibrated with 5% carbon dioxide and 95% nitrogen. Gastrocnemius muscle, homogenized in bicarbonate-Ringer so that 5 ml. of final suspension was equivalent to 1 g. of the original muscle, was used as the source of true cholinesterase, and oxalated plasma as the source of pseudo-cholinesterase. The substrates were acetylcholine, acetyl-β-methylcholine, and butyrylcholine, the final concentrations being 0.02M, 0.03M, and 0.02M respectively. The following quantities were placed in each flask : substrate 0.3 ml. ; muscle homogenate 2.0 ml. ; oxalated plasma 0.2 ml. with ACh and butyrylcholine as substrates, or 1.5 ml. with acetyl-β-methylcholine as substrate. The total volume was always adjusted to 3.0 ml. with bicarbonate-Ringer.

For the determination of the initial cholinesterase levels in plasma and in muscle, after the administration of atropine and chloralose, the tissues were dealt with in the following way : A sample of blood from the carotid artery was oxalated, centrifuged, and the plasma removed. The right gastrocnemius muscle was removed from the animal, washed with saline, lightly dried with filter paper, and weighed. Both plasma and muscle were stored overnight in a refrigerator at −5° C. The same procedure was adopted with blood and with the left gastrocnemius muscle after the injection of the two anticholinesterase drugs. The estimation of the initial and final cholinesterase levels was made on the following day.

RESULTS

Nature of the Relation between the Dose of Acetylcholine (ACh) and the Response of Skeletal Muscle

There has been little quantitative work on the response of skeletal muscle to nicotine-like stimulant drugs ; the first point investigated, therefore, was the nature of the relation between the dose and response of skeletal muscle to repeated injections of ACh.

Four different doses of ACh were given via the anterior tibial artery, in 4 series of randomized blocks, and the resultant contractions of the soleus and gastrocnemius muscles recorded. Data from this experiment are tabulated in Tables I and II. Statistical analyses, shown in Tables Ia and IIa, indicate that the regression of response on log. dose is highly significant ($P<0.001$), and

TABLE I

RELATIONSHIP BETWEEN DOSE OF ACh AND RESPONSE OF THE SOLEUS MUSCLE

Dose of ACh (μg.)	10	20	30	40	
Log dose = x ..	1·0	1·301	1·477	1·602	$\bar{x}$ = 1·345
	Response = y				Totals
Series 1	0·562	0·781	0·976	1·073	3·392
,, 2	0·415	0·658	0·775	0·850	2·698
,, 3	0·333	0·425	0·606	0·768	2·132
,, 4	0·200	0·389	0·526	0·677	1·792
Totals	1·510	2·253	2·883	3·368	$\bar{y}$ = 0·626

Regression coefficient b = 0·765

TABLE Ia

ANALYSIS OF THE DATA IN TABLE I

Source of Variation	Sum of Squares	d.f.	Mean Square	Variance Ratio	P
Regression ..	0·47930	1	0·47930	352·43	< 0·001
Linearity ..	0·00599	2	0·00299	2·20	0·2
Between doses ..	0·48529	3	0·16176	118·94	< 0·001
,, expts. ..	0·36787	3	0·12262	90·16	< 0·001
Residual error ..	0·01225	9	0·00136		
Total	0·86541	15			

TABLE II

RELATIONSHIP BETWEEN DOSE OF ACh AND RESPONSE OF GASTROCNEMIUS MUSCLE

Dose of ACh (μg.)	10	20	30	40	
Log dose = x ..	1·0	1·301	1·477	1·602	$\bar{x}$ = 1·345
	Response = y				Totals
Series 1	0·438	0·688	1·188	1·375	3·689
,, 2	0·286	0·760	1·000	1·136	3·182
,, 3	0·569	0·569	0·863	1·118	3·119
,, 4	0·200	0·600	0·706	1·020	2·526
Totals	1·493	2·617	3·757	4·649	$\bar{y}$ = 0·782

Regression coefficient b = 1·297

TABLE IIa
ANALYSIS OF THE DATA IN TABLE II

Source of Variation	Sum of Squares	d.f.	Mean Square	Variance Ratio	P
Regression ..	1·37528	1	1·37528	97·82	<0·001
Linearity ..	0·03557	2	0·01778	1·26	>0·2
Between doses ..	1·41085	3	0·47028	33·45	<0·001
,, expts. ..	0·17003	3	0·05667	4·03	0·05
Residual error ..	0·12658	9	0·01406		
Total	1·70746	15			

is also linear (P>0.2) for both the soleus and gastrocnemius muscles, taking P=0.05 as the level of significance in these and in all subsequent analyses. Fig. 1 shows the dose-response relation obtained for the soleus muscle; this illustrates a significant (P<0.001) reduction in the sensitivity to the chemical stimulus as the experiment proceeds.

It is of interest that the slopes of the regressions for ACh on the gastrocnemius and soleus muscles are significantly different (P<0.001); in this experiment the dose-response graph for ACh on the gastrocnemius muscle is steeper than that on the soleus muscle. This, however, does not always hold. In some subsequent experiments this state of affairs was reversed, and in others there was no significant difference between the regressions.

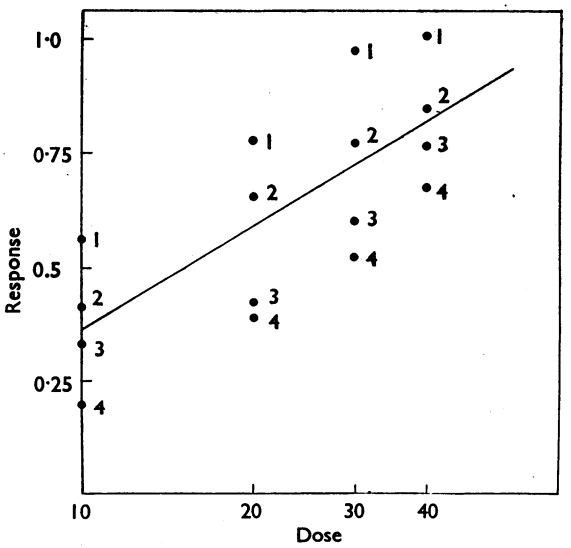

FIG. 1.—Dose-response curve to acetylcholine bromide on soleus muscle of a cat under chloralose anaesthesia. The numerals 1 to 4 refer to the successive series of doses. Injections were given into the anterior tibial artery. Electrical stimulation was by periodic condenser discharges (4/min.). Abscissa: dose of AChBr in μg. (log scale). Ordinate: response of muscle, estimated as the ratio of the increased tension produced by injection of the drug to that produced by the electrical stimulus. Note the decreased sensitivity to the chemical stimulus as the experiment proceeds.

Activity of Nicotine-like Stimulant Compounds on Skeletal Muscle

The (2+2) assay technique was used for the following compounds: propionylcholine, *n*-butyrylcholine, *iso*butyrylcholine, *n*-valerylcholine, trimethylacetylcholine and 4-keto-amyltrimethylammonium. Over the dose-range used both trimethylacetylcholine and 4-keto-amyltrimethylammonium produce a paralysis lasting approximately 30 min.; but, in contrast to those compounds estimated by the (2+1) assay technique, the muscles show complete recovery. Fig. 2 shows a typical record in which ACh was compared with *n*-valerylcholine. This record also demonstrates the transient paralysis produced by *n*-valerylcholine, a feature shown to a lesser extent by *iso*butyrylcholine, *n*-butyrylcholine and propionylcholine. Data from these assays were analysed, and the slope and parallelism of the regressions tested. Only in occasional assays was there any significant deviation from parallelism; such assays were discarded.

The (2+1) assay technique was used for the remaining compounds tested—that is, *n*-amyltrimethylammonium, choline ethyl ether, choline butyl ether, choline phenyl ether, choline *p*-chlorophenyl ether, choline *m*-bromophenyl ether, and nicotine. Fig. 3 shows a typical result, obtained with this technique, in which ACh is compared with choline phenyl ether. All these compounds cause a paralysis of approximately the same duration in the doses used, indicating a clear correlation between stimulating and paralysing activities.

From each assay the logarithm of the potency relative to ACh was determined. For those nicotine-like stimulant drugs whose activity was estimated by the (2+2) assay technique, the following formula (Burn, Finney, and Goodwin, 1950) was used to determine the logarithm of the relative potency:

$$M = \bar{x}_S - \bar{x}_T - \frac{\bar{y}_S - \bar{y}_T}{b}$$

where M is the logarithm of the relative potency, $\bar{x}_S$ and x_T are the mean values for the log dose of standard and test drug respectively, $\bar{y}_S$ and $\bar{y}_T$ are the mean responses, and b is the common regression coefficient. In the analysis of the (2+1) assays the assumption was made that the dose-response lines are parallel—an assumption not unfounded, since analysis of the data from the (2+2) assays shows that in most of the experiments there is no significant deviation from parallelism (P>0.05).

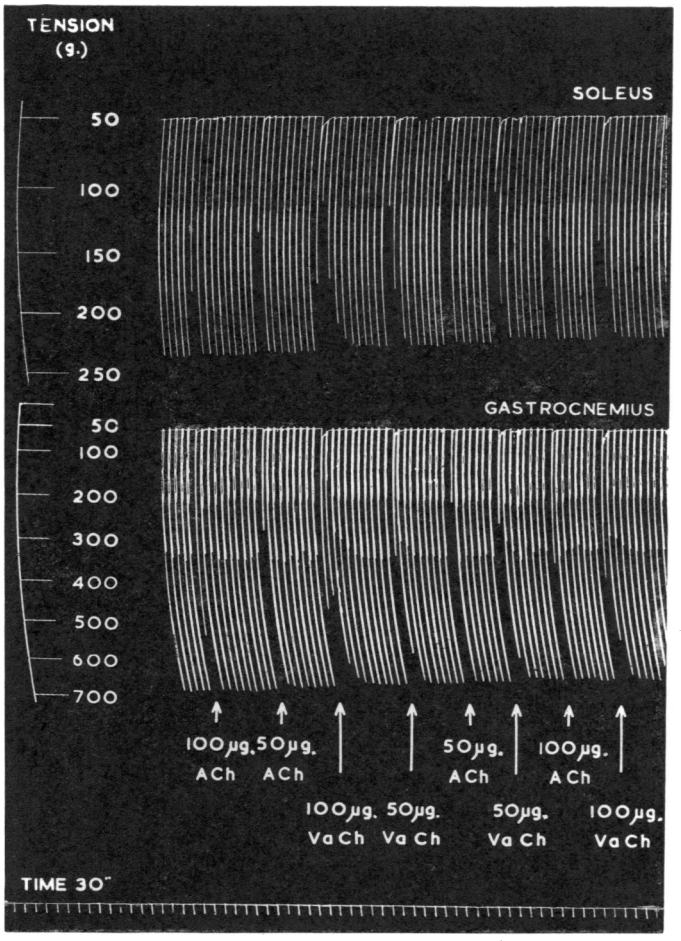

FIG. 2.—Comparison of acetylcholine and *n*-valerylcholine using the (2+2) assay technique. Cat, 3·2 kg., chloralose, atropine. Injections made into the anterior tibial artery during cessation of the supramaximal electrical stimuli applied to the sciatic nerve.

A complete list of the individual estimated potencies is given in Table III; Table IIIa shows the analysis of these results. Table IV shows the mean relative potencies and the limits of error (P=0.95) calculated from the residual mean square in Table IIIa. It should be noted that the last seven drugs in Table III were all estimated by a (2+1) assay, the test drug being injected after the two ACh injections, and as the response decreases as the experiment proceeds (Tables Ia and IIa), the estimates may all be too low; for this reason the limits of error have not been described as fiducial limits.

The analysis in Table IIIa reveals that there is no significant difference between the log relative potencies of these compounds when determined on either the soleus or the gastrocnemius muscle, and so the estimates of potency on the two muscles were grouped together.

Effect of Anticholinesterase Drugs on Relative Potencies

Before these results are discussed it must be remembered that the standard drug (ACh) undergoes enzymatic hydrolysis *in vivo*, whereas some of the test drugs do not. Hence the potency of the test drug may be altered if enzymatic hydrolysis is prevented. The experimental results recorded in this section show that prevention of the hydrolysis does not alter the relative potencies.

The drugs selected for this investigation were: propionylcholine, an ester rapidly hydrolysed by cholinesterases; trimethylacetylcholine, an ester causing paralysis of the muscles following preliminary stimulation; choline ethyl ether, a straight-chain ether; choline phenyl ether, and 4-keto-amyltrimethylammonium. The individual potencies were estimated graphically and are recorded in Table V.

A series of *t* tests was performed to determine whether there was any significant difference between the values of *M* for normal animals and for those treated with the two anticholinesterases. Taking P=0.05 as defining the level of significance, there is no significant difference between the mean relative potencies on the treated and untreated animals in four of the five tests of significance; the fifth test, on trimethylacetylcholine, borders on significance (P=0.04). Too much weight should not, however, be attached to this last test, as there is no reason to expect a significant difference between treated and untreated animals for trimethylacetylcholine and not for the other compounds. A joint probability was therefore derived from the results of the individual tests of significance, using the method described by Fisher (1944); this gives P=0.2–0.3.

There is thus no significant difference between the relative potencies estimated on the skeletal muscles of normal animals and on animals treated with anticholinesterase drugs.

Manometric Estimation of the Cholinesterase Levels in Skeletal Muscle and Oxalated Plasma.—In every experiment the dose of iso-ompa used

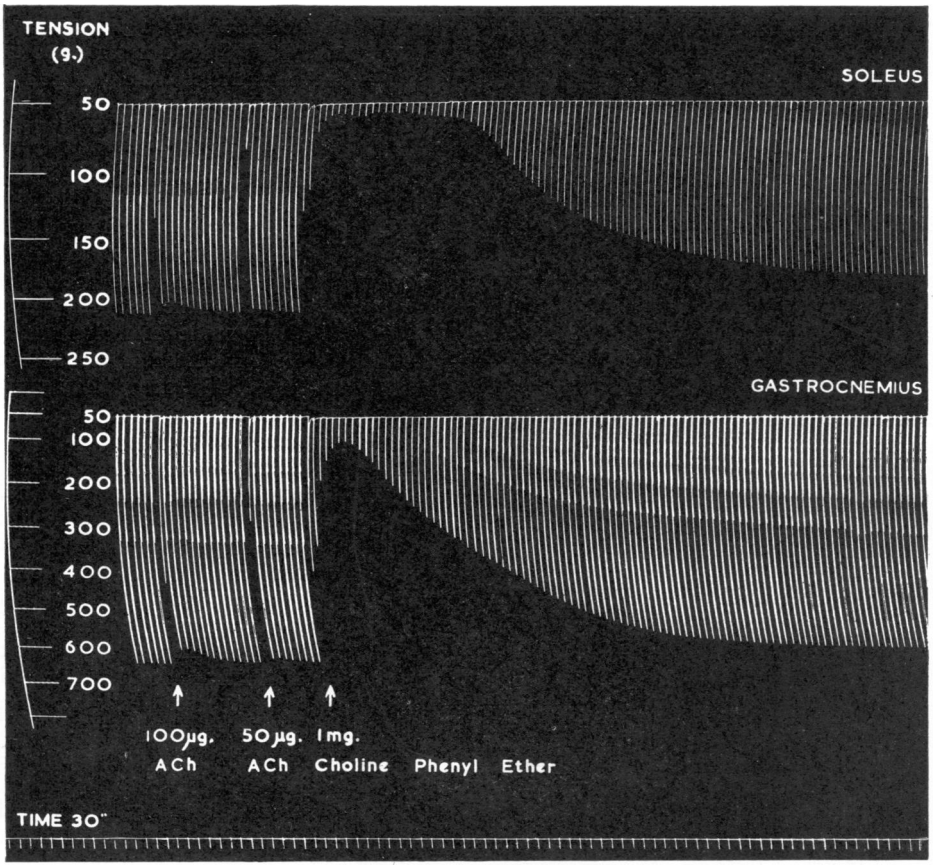

FIG. 3.—Comparison of acetylcholine and choline phenyl ether using the (2+1) assay technique. Cat, 3·2 kg., chloralose, atropine. Injections made into the anterior tibial artery during cessation of the supramaximal electrical stimuli applied to the sciatic nerve.

TABLE III

LOG POTENCIES RELATIVE TO ACh OF ALL THE NICOTINE-LIKE STIMULANT COMPOUNDS INVESTIGATED, DETERMINED ON THE SOLEUS AND GASTROCNEMIUS MUSCLES

Drug	Muscle	Log Individual Estimates of Potency					
Propionylcholine iodide	Soleus	+0·117	+0·131	+0·088	−0·008	−0·012	
	Gastrocnemius	+0·173	+0·136	+0·097	−0·039	−0·026	
n-Butyrylcholine ,,	Soleus	+0·097	−0·225	−0·078	+0·006		
	Gastrocnemius	+0·188	−0·008	−0·077	−0·016		
n-Valerylcholine ,,	Soleus	+0·048	+0·127	−0·043	−0·107		
	Gastrocnemius	−0·045	+0·189	−0·061	+0·070		
*iso*Butyrylcholine ,,	Soleus	−0·468	−0·357	−0·396			
	Gastrocnemius	−0·330	−0·399	−0·393			
Trimethylacetylcholine iodide	Soleus	−0·778	−0·918	−0·772			
	Gastrocnemius	−0·918	−0·797	−0·852			
4-Keto-amyltrimethylammonium iodide	Soleus	−0·979	−0·970	−0·775			
	Gastrocnemius	−0·655	−1·002	−0·727			
n-Amyltrimethylammonium iodide	Soleus	−0·796	−0·987	−0·758	−0·939	−0·704	
	Gastrocnemius	−0·604	−1·355	−0·758	−0 959	−0·815	
Choline ethyl ether bromide	Soleus	−1·448	−1·182	−1·117	−1·049	−1·340	
	Gastrocnemius	−1·157	−1·133	−1·230	−0·982	−1·322	
Choline butyl ,, ,,	Soleus	−1·893	−1·453	−1·658	−1·721	−1·544	
	Gastrocnemius	−1·890	−1·554	−1·442	−1·570	−1·431	
Choline phenyl ,, ,,	Soleus	−0·978	−1·263	−1·261	−1·103	−1·375	
	Gastrocnemius	−0·992	−1·301	−1·090	−0·954	−1·379	
Choline *p*-chlorophenyl ether bromide	Soleus	−2·251	−3·016	−3·120	−3·164	−3·104	−2·962
	Gastrocnemius	−2·222	−3·050	−3·012	−3·041	−3·161	−2·824
Choline *m*-bromophenyl ,, ,,	Soleus	−0·598	−0·669	−0·722	−0·936	−0·919	
	Gastrocnemius	−0·763	−0·574	−0·861	−0·706	−0·758	
Nicotine acid tartrate	Soleus	−1·255	−1·778				
	Gastrocnemius	−1·100	−1·372				

TABLE IIIa

ANALYSIS OF LOG RELATIVE POTENCIES OF TABLE III

Source of Variation	Sum of Squares	d.f.	Mean Square	Variance Ratio	P
Between drugs ..	79·61094	12	6·63424	213·05	<0·001
,, muscles	0·05588	1	0·05588	1·79	0·1–0·2
Residual error ..	2·98986	96	0·03114		
Total	82·65668	109			

TABLE IV

POTENCIES RELATIVE TO ACh=1, AND APPROXIMATE LIMITS (P=0·95), OF THE NICOTINE-LIKE STIMULANT COMPOUNDS ON CAT SKELETAL MUSCLE

Drug	Mean Relative Potency (ACh=1)	Approximate Limits
Propionylcholine iodide	1·164	0·901 to 1·503
n-Butyrylcholine ,, ...	0·967	0·727 ,, 1·288
n-Valerylcholine ,, ...	1·052	0·791 ,, 1·401
*iso*Butyrylcholine ,, ...	0·407	0·293 ,, 0·566
Trimethylacetylcholine iodide ..	0·145	0·104 ,, 0·202
4-Keto-amyltrimethylammonium iodide	0·141	0·101 ,, 0·196
n-Amyltrimethylammonium iodide	0·136	0·105 ,, 0·175
Choline ethyl ether bromide ..	0·0637	0·0493 ,, 0·0822
,, butyl ,, ,, ..	0·0242	0·0188 ,, 0·0313
,, phenyl ether bromide ..	0·0677	0·0524 ,, 0·0874
,, *p*-chlorophenyl ether bromide	0·00123	0·000973 ,, 0·00155
Choline *m*-bromophenyl ether bromide	0·178	0·138 ,, 0·229
Nicotine acid tartrate	0·0420	0·0281 ,, 0·0630

TABLE V

RELATIVE POTENCIES, AND APPROXIMATE LIMITS (P=0·95), OF CERTAIN NICOTINE-LIKE STIMULANT COMPOUNDS ON SKELETAL MUSCLE OF CATS PRETREATED WITH ISO-OMPA AND 284C51

Drug	Mean Relative Potency (ACh=1)	Approximate Limits
4-Keto-amyltrimethylammonium iodide	0·137	0·107 to 0·177
Trimethylacetylcholine iodide ..	0·222	0·173 ,, 0·286
Propionylcholine iodide ..	0·890	0·692 ,, 1·145
Choline ethyl ether bromide ..	0·054	0·042 ,, 0·070
,, phenyl ,, ,, ..	0·070	0·054 ,, 0·090

TABLE VI

INHIBITION OF CAT SKELETAL MUSCLE AND OXALATED PLASMA CHOLINESTERASES BY 284C51 AND ISO-OMPA

μl. CO_2/30 min./0·4 g. muscle or 0·2 ml. plasma

Expt. No.	Enzyme Source	Acetylcholine		Acetyl-β-methylcholine		Butyrylcholine	
		Normal	Treated	Normal	Treated	Normal	Treated
1	Plasma	114·9	20·2	101·9	56·3	247·0	−5·6
	Muscle	51·9	43·9	44·7	34·7	7·6	0·8
2	Plasma	82·1	26·2	95·6	63·0	176·3	−13·0
	Muscle	71·5	53·5	46·0	31·0	19·5	3·4
3	Plasma	87·1	−0·6	66·8	−1·9	149·5	−4·2
	Muscle	61·1	29·9	30·8	14·1	26·2	−0·4

produced complete inhibition of the pseudocholinesterase of blood plasma, indicated by the non-hydrolysis of butyrylcholine (Table VI). In two of the three experiments the true cholinesterase of blood plasma was only partially inhibited, as indicated by the reduced rate of hydrolysis of acetyl-β-methylcholine. Inhibition of the true cholinesterase of muscle was also incomplete. However, the inhibition with 284C51 is reversible, and, as the concentration of substrate in the manometric determinations was many times greater than the *in vivo* concentration, inhibition *in vivo* is expected to be greater than in the manometric estimations.

DISCUSSION

It is interesting to compare the nicotine-like stimulant activities of these quaternary ammonium compounds on cat skeletal muscle with their activities on the blood pressure of the spinal cat. These comparisons are shown in Table VII. Hey's (1952, 1954) estimates on the blood pressure were

TABLE VII

POTENCIES, RELATING TO ACh=1, OF NICOTINE-LIKE STIMULANT COMPOUNDS ON CAT BLOOD PRESSURE AND SKELETAL MUSCLE

Drug	Activity on Blood Pressure	Activity on Skeletal Muscle
Acetylcholine bromide	1·00	1·000
Propionylcholine iodide	1·55*	1·164
n-Butyrylcholine ,, ...	2·10*	0·968
n-Valerylcholine ,, ...	2·25*	1·052
*iso*Butyrylcholine ,, ...	3·80*	0·407
Trimethylacetylcholine iodide	10·1*	0·145
Choline phenyl ether bromide	82·9	0·068
Choline *p*-chlorophenyl ether bromide	7·16	0·001
Choline *m*-bromophenyl ,, ,, ...	319	0·178
Choline butyl ether bromide	1·88	0·024
Choline ethyl ,, ,, ...	0·56	0·064
4-Keto-amyltrimethylammonium iodide ..	24·2	0·141
n-Amyltrimethylammonium bromide	8·55	0·136
Nicotine acid tartrate	7·96	0·042

* Activity estimations obtained by Hey (1952; 1954).

obtained from cats under chloralose anaesthesia. I determined the pressor activities of the other compounds on spinal cats.

None of the straight-chain choline esters investigated differs significantly from ACh in its stimulant activity on skeletal muscle. However, the mean values for the activity on the blood pressure show a gradual increase as the chain length is increased.

Successive replacement of the hydrogen atoms in the acetyl group of ACh has a very marked effect. Whereas replacement of one of the hydrogen atoms—as in propionylcholine—does not produce a significant difference in activity on skeletal muscle, the replacement of a second hydrogen

atom—*iso*butyrylcholine—increases the activity on the blood pressure but diminishes that on skeletal muscle to a highly significant extent ($P<0.001$). Replacement of all the hydrogen atoms—trimethylacetylcholine—again increases the activity on the blood pressure while diminishing that on skeletal muscle ($P<0.001$).

Although substituted choline phenyl ethers are many times more active than ACh on the blood pressure and much less active on skeletal muscle, these activities are related. Choline *m*-bromophenyl ether is most active on the blood pressure and skeletal muscle; choline phenyl ether is next in order of activity, and choline *p*-chlorophenyl ether is least active on both effectors. Moreover, if the activity of choline *m*-bromophenyl ether on both the blood pressure and skeletal muscle is given an arbitrary value of 1, then the activity of choline phenyl ether relative to choline *m*-bromophenyl ether on the blood pressure becomes similar to its relative activity on skeletal muscle. An analogous relation exists between choline *m*-bromophenyl ether and choline *p*-chlorophenyl ether.

When the effect of chain structure on the activities of these compounds as stimulants of blood pressure and of skeletal muscle contraction is considered, little apparent relation exists between the activities on the two effector structures. Whereas 4-keto-amyltrimethylammonium and *n*-amyltrimethylammonium do not differ significantly in their activities on skeletal muscle, the stimulant activity of 4-keto-amyltrimethylammonium on the blood pressure is much greater than that of *n*-amyltrimethylammonium. Choline butyl ether and choline ethyl ether do, however, show a type of relation similar to that of the series of compounds representing the successive replacement of the hydrogen atoms in the acetyl group of ACh. Choline butyl ether is more active than choline ethyl ether on the blood pressure, but is significantly less active ($P<0.001$) on skeletal muscle.

These results are thus extremely complex. Some of the compounds within a series show certain relations between their stimulant activities on skeletal muscle and on blood pressure. Other series, such as those having the same chain length as ACh (4-keto-amyltrimethylammonium, *n*-amyltrimethylammonium and choline ethyl ether), show no apparent relation.

Even where there is a relation—as in the substituted choline phenyl ethers, and in the series of compounds representing the replacement of the hydrogen atoms in the acetyl group of ACh by methyl groups—this is not straightforward. The substituted choline phenyl ethers show a corresponding increase in stimulant activity on both the blood pressure and skeletal muscle, whereas the effect of hydrogen atom replacement in ACh is to increase the stimulant activity on the blood pressure but to diminish the activity on skeletal muscle.

It appears that the chain length of the molecule is of little importance to blood-pressure stimulating activity. One possible factor influencing this activity may be the electron density on the oxygen atom of choline compounds—as suggested by Hey (1952)—the activity increasing as the electron density is reduced.

Certain series of the compounds described do show increasing activity as the chain length is increased—for example, the straight-chain esters and ethers. This effect may possibly be caused by an increase in the lipoid solubility of the molecule, consequent on the increase in size of the alkyl groups, resulting in the "active spots" or "receptors" being made more easily accessible. The theory of Hey (1952) does not account for the difference in activity between choline butyl ether and choline ethyl ether, since the electron density on the oxygen atom is not expected to be much different in these two compounds; the lipoid solubility hypothesis would, however, account for this difference. Similarly, the lipoid solubility of the group attached to the ether oxygen atom can explain the increase in activity on the blood pressure and the decrease in activity on skeletal muscle shown by the two series consisting of propionylcholine, *iso*butyrylcholine and trimethylacetylcholine on the one hand, and choline ethyl ether and choline butyl ether on the other. The activity on the blood pressure increases in each series as the size of the alkyl groups is increased, whereas the activity on skeletal muscle—which contains little lipoid material in comparison with vasomotor ganglia—is diminished as the size of the alkyl groups is increased.

These concluding remarks are, however, largely speculative, and further work alone can determine their worth.

Summary

1. The nicotine-like stimulant activities of some quaternary ammonium compounds have been investigated on (*a*) the blood pressure and (*b*) the gastrocnemius ("white") and soleus ("red") muscles of the cat.

2. The potencies of these compounds, relative to acetylcholine, do not differ significantly when determined on either the soleus or the gastro-

cnemius muscle ; nor are these potencies affected by previous injection of anticholinesterase drugs.

3. The straight-chain choline esters—acetylcholine, propionylcholine, *n*-butyrylcholine and *n*-valerylcholine—do not differ significantly in their activities on skeletal muscle, but the pressor activity increases as the chain length is increased.

4. Successive replacement of the hydrogen atoms in the acetyl group of acetylcholine diminishes the activity on skeletal muscle while increasing that on the blood pressure.

5. Nuclear substitution in choline phenyl ether leads to changes in activity on skeletal muscle which parallel the changes in activity on the blood pressure. Choline *m*-bromophenyl ether has the highest activity, choline *p*-chlorophenyl ether the least, and that of choline phenyl ether is intermediate.

6. Compounds with the same chain length as acetylcholine—choline ethyl ether, 4-keto-amyltrimethylammonium and *n*-amyltrimethylammonium—show no apparent relation between their activities on blood pressure and on skeletal muscle.

7. The possible inferences from these results are discussed.

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