Br. J. Pharmac. (1972), **46**, 300–314.

A comparison of the affinities of antagonists for acetylcholine receptors in the ileum, bronchial muscle and iris of the guinea-pig

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with an appendix on

The effects of different recording conditions on the estimates of affinity constants of antagonists for acetylcholine receptors in the guinea-pig ileum

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Summary

1. Isolated preparations of bronchial strip and of intact iris from the guinea-pig have been adapted for the measurement of affinity constants of substances which block post-ganglionic acetylcholine receptors.

2. The affinity constants of 28 compounds on bronchial muscle and of 8 compounds on the iris have been compared with values measured on the guinea-pig ileum.

3. Although the compounds differed up to a million-fold in affinity, most of the estimates of log affinity constant for the bronchial muscle and iris differed only slightly from those on the ileum.

4. Some of the differences could be attributed to the actions of hexamethonium, used in the tests on the ileum but not, initially, in the tests with the bronchial strip and iris. Hexamethonium reduced most of the estimates of log K for the receptors in the bronchial strip by a variable but significant amount, which could be due, at least in part, to a weak post-ganglionic blocking (atropine-like) action. On average, hexamethonium had little effect on the estimates made with the ileum, appreciably decreasing estimates with some compounds and increasing those with others.

5. The results indicate that, although there may be differences between the acetylcholine receptors in the three types of tissue, there is no conclusive evidence, because the differences in affinity which we have observed could have arisen from differences in the experimental conditions. This is illustrated by results obtained with the guinea-pig ileum recorded with the same technique as was used for the bronchial strip, which are presented as an appendix.

6. Such differences as may exist between these three types of acetylcholine receptor are likely to be limited to the replacement of one aminoacid in the receptor protein by a homologue.

Introduction

Blocking agents have been widely used for classifying pharmacological receptors. Receptors which are blocked by the same drug are likely to be similar in structure and accurate measurements of the affinity of the drug for the receptors are likely to indicate the extent of the similarity between them. Arunlakshana & Schild (1959), for example, found that values of the pA_2 (which is equal to log K) of diphenhydramine for histamine receptors in the guinea-pig ileum, perfused lung, and trachea, were 8·0, 7·8, and 7·8 respectively, and that those of mepyramine were 9·3, 9·4, and 9·1 respectively. This suggests that the histamine receptors in these tissues are all similar in structure and this similarity is likely to extend to histamine receptors in human bronchi, for which Hawkins & Schild (1951) obtained a value of 9·3 for the pA_2 of mepyramine.

We have been interested to see how far 'muscarine-sensitive' acetylcholine receptors in various tissues may be similar in structure and this paper describes the results of measurements of the affinity constants of a number of antagonists of acetylcholine for receptors in guinea-pig ileum, bronchial muscle and iris. The tissues selected can all be used in automated apparatus and the iris, unlike the other two, does not contain parasympathetic ganglia. The responses to the agonist have different time-courses. The effects on bronchial muscle require several minutes before they are fully established, whereas those on the ileum require 10–20 seconds and those on the iris 20–40 seconds.

The antagonists chosen include many for which results on the ileum had already been obtained by Abramson, Barlow, Mustafa & Stephenson (1969) but we have also included some of the resolved optical isomers of esters of phenylcyclohexylglycollic acid. We have used as wide a variety of compounds as we could because it is possible that receptors may resemble enzymes in that they may exist in groups of closely related but not actually identical species.

Methods

The affinity constants were determined by measuring the dose-ratios produced by concentrations of the antagonists. In all the experiments carbachol was used as the agonist and each antagonist was tested in at least two concentrations. These usually differed by a factor of 10 and the dose-ratios measured were between 20 and 800. With the two weakest compounds, however, and some dicyclohexyl compounds, it was only possible to use concentration ranges of 2-fold and 4-fold and the dose ratios were between 2·5 and 20. Each compound was tested on at least four preparations of each of the three tissues and the results are presented as mean values of log K, as in previous work (Barlow, Scott & Stephenson, 1963).

Guinea-pig ileum. Results for many of the compounds on this preparation were obtained by Abramson *et al.* (1969). Additional measurements, including those for compounds not previously tested, were made in exactly the same conditions, with the tissue suspended in aerated Tyrode solution at 37° C in the presence of $2{\cdot}76 \times 10^{-4}$M hexamethonium, and carbachol, allowed to act for 30 s, given once every 90 seconds. Measurements have also been made with some of the compounds without hexamethonium present.

Bronchial spiral strip. Continuous spiral strips of circular bronchial muscle were made from the bronchi of guinea-pigs weighing 200–300 g. Two preparations

could often be obtained, one from each bronchus, but with smaller animals a single preparation was obtained by leaving the lower end of the trachea joining the two bronchi. The tissue was suspended in Krebs solution, aerated with oxygen and 5% carbon dioxide, at 37° C.

Responses to the agonist were recorded isometrically and, with a base-line tension of about 0·5 g, suitable increases in tension were obtained with 10^{-7} and 4×10^{-7}M carbachol. Automated apparatus, similar to that used with the ileum, was used for applying the drug solutions. The carbachol was allowed to act for 5 min, the tissue was then washed, and again, 5 min later, and a second concentration of agonist was applied 15 min after the first. A specimen record is shown in Figure 1.

In the first group of experiments hexamethonium was omitted because, after a few tests, we concluded that it did not have any effect. Subsequently, however, we found that this was incorrect and measurements were repeated with several of the compounds in the presence of hexamethonium, $2{\cdot}76 \times 10^{-4}$M.

Isolated iris. In the method described by Quilliam (1949) the eyes were removed from cats killed with chloralose, put into cold Krebs solution and the intact iris was dissected out. We have followed the same procedure except that we used guinea-pigs with dark eyes, killed by a blow on the head and cutting the throat. We were therefore able to obtain two iris preparations, two bronchial strips and a large supply of ileum from one animal.

The preparation was pinned through the sclera over a hole in the centre of a PTFE diaphragm and the excess flaps of sclera were trimmed away (Fig. 2). Several other holes in this diaphragm allowed the fluid to wash through the organ bath by passing round the edge of the preparation, rather than only through the pupil.

The diaphragm, with the preparation mounted on it, was clamped into the organ bath, which consisted of a glass flat-flange joint with optically flat windows and narrow bore tubes sealed into each half (Fig. 2). Light was passed from a 14 V pre-focus bulb fixed in position behind one window, through the pupil, onto a cadmium sulphide photoelectric cell (ORP 12) fixed behind the other window. A rubber washer provided a seal between the two halves when these were clamped together. The outside of the bath was painted black to exclude any stray light. The volume of the organ bath was about 5 ml and the bathing fluid was Krebs solution, aerated with oxygen and 5% carbon dioxide. The solution was bubbled

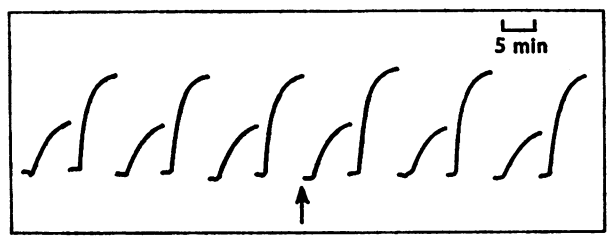


FIG. 1. Isolated guinea-pig bronchial strip preparation. Responses, recorded isometrically, are shown to 1 and 4×10^{-7}M carbachol; after the arrow *cyclo*hexylacetoxyethyltriethylammonium iodide, 4×10^{-5}M, was present throughout the rest of the experiment and the carbachol concentrations were increased to 2 and 8×10^{-6}M. The record was interrupted for 7·5 min during wash and recovery.

at room temperature and then warmed to 37° C; T-junction bubble traps were placed between each warming coil and the organ bath to prevent bubbles interfering with the light path through the organ bath.

A dry battery was connected across the photocell and changes in resistance produced by changes in the light falling on it were detected with a potentiometric recorder. The light bulb was powered by a Coutant stabilized 24 volt supply.

The drug solutions were applied to the preparation by automated apparatus, similar to that used for the ileum and bronchial strip. Carbachol, 2×10^{-7} and 4×10^{-7}M, was allowed to act for 1 min, the tissue was then washed at intervals of 2·5 min and a second concentration of carbachol was applied 10 min after the first. A specimen record is shown in Figure 3. Hexamethonium was not present in these experiments on the iris.

Compounds. Twenty of the compounds tested are representatives of series for which analyses have already been published (Abramson *et al.*, 1969). Values for the other eight compounds are shown in Table 1. Carbachol was obtained from British Drug Houses Ltd.

Results

Estimates of the values of log K for the compounds on the various preparations are listed in Table 2. They are arranged in order of increasing affinity for receptors in the guinea-pig ileum, and for many of them the values shown for this

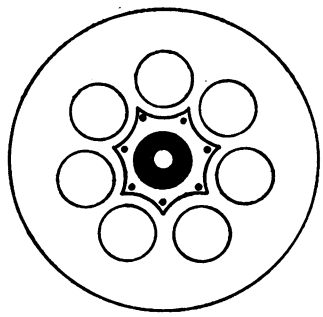


Fig. 2A

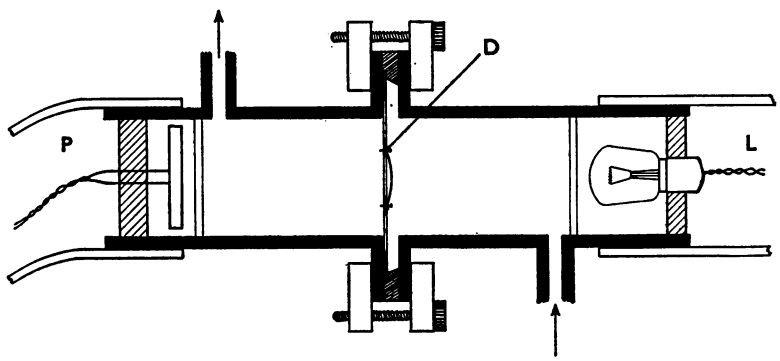


Fig. 2B

FIG. 2. A. Iris pinned to PTFE diaphragm. The holes round the preparation allow the passage of bath fluid which would otherwise only be able to flow through the pupil. B. Organ bath showing diaphragm (D), light (L) and photocell (P) with inlet and outlet for bath fluid.

tissue are those obtained by Abramson *et al.* (1969). Initially the experiments on the ileum were all performed in the presence of hexamethonium. This substance was used by Stephenson (1956) in his studies with alkyltrimethylammonium salts to limit the observations to actions at postganglionic receptors. It was also used in the estimation of affinity constants by Barlow *et al.* (1963). It is possible that when high concentrations of carbachol are used the results may be affected by ganglionic effects (possibly involving circular muscle). We were doubtful of the need to use it on the bronchial strip preparation and a few tests made with *cyclo*hexylacetoxyethyltriethylammonium suggested that we obtained the same

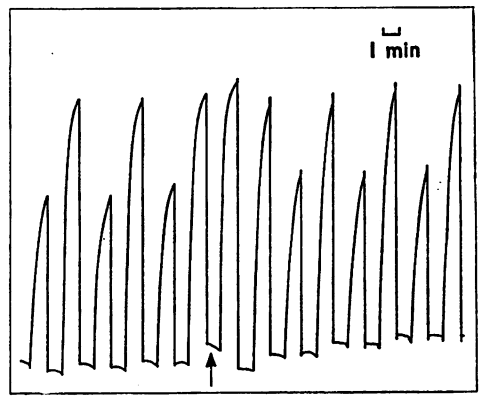


FIG. 3. Isolated guinea-pig intact iris preparation. Responses, recorded as the change in output from the photocell system, are shown to 2 and 4×10^{-7}M carbachol; after the arrow R-phenyl*cyclo*hexylglycollylethyltriethylammonium iodide, 10^{-8}M, was present throughout the rest of the experiment and the carbachol concentrations were increased to 2·8 and $5{\cdot}6\times10^{-5}$M. The record was interrupted for 8 min during wash and recovery.

TABLE 1. *Compounds tested for their affinity for acetylcholine receptors in the ileum, bronchial muscle and iris of the guinea-pig*

$Ph(C_6H_{11})C(OH)COOCH_2CH_2$-		M.P.	Solvent	M_D	I⁻% Found	Theory
$\overset{+}{N}Me_3$	(R)	141·6–142·4	B+C	+23·6	28·20	28·35
	(S)	140·8–142·0	B+C	−23·2	28·05	
	(R)	186·9–187·3	B+A	+27·2	26·00	26·05
	(S)	186·5–187·4	B+A	−28·4	25·85	
$\overset{+}{N}Et_3$	(R)	196·4–196·7 dec	B+A	+28·0	25·85	25·95
	(S)	197·2–197·4 dec	B+A	−29·2	25·95	
Hyoscine ethiodide						
	(R)	182·0–183·0 dec	A+C	+104		
	(S)	185·7–186·1 dec	A+C	−108	27·60	27·65

The molar rotations of the isomers of hyoscine ethiodide were measured in water ($c=2\times10^{-2}$M); values for the other compounds were measured in methanol ($c=5\times10^{-3}$M). Note that with the esters of phenylcyclohexylglycollic acid the sign of rotation at 300 nm is the same as that of the parent acid (the R acid is (−) and the S is (+)) but at the D line (589 nm) the rotations are of the opposite sign. These measurements were made with a Bellingham and Stanley Model B spectropolarimeter. Solvents used for recrystallization were ethanol (A), ethylmethylketone (B) and ethylacetate (C). Melting points were measured with a Mettler FP1 instrument coupled to a potentiometric recorder. Analyses for iodide were gravimetric with samples of 50–100 mg but there was insufficient of the R-form of hyoscine ethiodide for this.

TABLE 2. *Mean values of log K±standard error of antagonists for acetylcholine receptors; the number of estimates is shown in parentheses. An asterisk indicates that the value is significantly different from that on the ileum at a level of probability of* 0·05. *Measurements made in the presence of hexamethonium,* $2{\cdot}76\times10^{-4}$M, *are indicated by* (+*Hex*). *The symbol* † *indicates pooled results from more than one group of experiments* (*see Table* 3)

	Ileum (+Hex)	Bronchial strip	Iris
$CH_3CH_2OCH_2CH_2\overset{+}{N}Et_3$	3·974±0·019 (12)	4·295±0·041* (4)	4·419±0·063* (3)
$CH_3(CH_2)_4\overset{+}{N}Et_3$	4·588±0·011 (15)	4·718±0·087 (4)	
$C_6H_{11}CH_2COOCH_2CH_2\overset{+}{N}Et_3$	5·630±0·017 (8)	5·673±0·028 (4)	5·692±0·038 (6)
$PhCH_2CH_2OCH_2CH_2\overset{+}{N}Et_3$	5·758±0·028 (8)	5·812±0·075 (4)	
$PhCH_2COOCH_2CH_2\overset{+}{N}Et_3$	5·785±0·008 (7)	5·780±0·048 (5)	
$Ph(CH_2)_5\overset{+}{N}Et_3$	5·808±0·015† (26)	5·805±0·100 (4)	
$C_6H_{11}CH_2CH_2OCH_2CH_2\overset{+}{N}Et_3$	5·912±0·024 (6)	5·966±0·102 (4)	
$C_6H_{11}(CH_2)_5\overset{+}{N}Et_3$	5·921±0·037 (8)	5·757±0·043*† (9)	5·831±0·052 (5)
$Ph_2CHCH_2OCH_2CH_2\overset{+}{N}Et_3$	6·374±0·024 (7)	6·352±0·020 (4)	
$Ph_2CH(CH_2)_4\overset{+}{N}Et_3$	6·712±0·015 (5)	6·606±0·081 (6)	
$Ph_2CHCOOCH_2CH_2\overset{+}{N}Me_3$	7·159±0·025 (4)	7·283±0·036* (4)	
$Ph_2CHCOOCH_2CH_2\overset{+}{N}(Me)$ (ring)	7·260±0·039 (4)	7·138±0·070 (6)	
$(C_6H_{11})_2CHCH_2OCH_2CH_2\overset{+}{N}Et_3$	7·354±0·030 (6)	6·985±0·038* (4)	6·408±0·070* (4)
$Ph_2CHCOOCH_2CH_2\overset{+}{N}Et_3$	7·367±0·021 (4)	7·283±0·036 (4)	
$Ph_2C(OH)COOCH_2CH_2\overset{+}{N}(Me)$ (ring)	8·034±0·014 (16)	8·144±0·048*† (9)	
$(C_6H_{11})_2CHCOOCH_2CH_2\overset{+}{N}Et_3$	8·068±0·022 (7)	7·891±0·077 (4)	7·976±0·052 (6)
$Ph_2C(OH)COOCH_2CH_2\overset{+}{N}Me_3$	8·511±0·008 (4)	8·597±0·064 (4)	
(±) Ph, C_6H_{11}, $CHCOOCH_2CH_2\overset{+}{N}Et_3$	8·566±0·019 (8)	8·552±0·115 (4)	
$Ph_2C(OH)COOCH_2CH_2\overset{+}{N}Et_3$	8·682±0·006 (4)	8·566±0·057 (5)	
(±) Ph, C_6H_{11}, $C(OH)COOCH_2CH_2\overset{+}{N}Et_3$	9·482±0·030 (8)	9·675±0·031* (4)	
Ph, C_6H_{11}, $C(OH)COOCH_2CH_2\overset{+}{N}Me_3$			
S-	7·257±0·021† (13)	7·284±0·023 (4)	
R-	9·647±0·073 (11)	9·935±0·046* (5)	
Ph, C_6H_{11}, $C(OH)COOCH_2CH_2\overset{+}{N}(Me)$ (ring)			
S-	7·334±0·033 (6)	7·413±0·051 (6)	
R-	9·474±0·041 (6)	9·973±0·024* (5)	

TABLE 2 (*continued*)

	Ileum (+Hex)		Bronchial strip		Iris	
Ph \ C(OH)COOCH$_2$CH$_2$$\overset{+}{N}Et_3$ / C_6H_{11}						
S-	7·989±0·035	(7)	7·992±0·023	(4)	8·006±0·051	(7)
R-	9·600±0·068	(8)	10·001±0·055*	(7)	10·148±0·044*	(6)
Hyoscine ethiodide						
R-	7·153±0·036	(7)	7·268±0·072	(4)		
S-	8·603±0·055	(7)	8·793±0·069	(4)	8·889±0·068*	(4)

result whether hexamethonium were present or not. We therefore tested all the compounds on this preparation without hexamethonium.

Although most of the results on the bronchial strip were not significantly different from those on the ileum, there were some exceptions. These can be seen in Fig. 4, in which the estimates of log K for the receptors in the ileum are plotted against those of log K for the receptors in the bronchial strip. Most of the points lie along the straight line which would be obtained if the values of log K were the same in the two types of experiment. Some points lie off the line, however, and correspond to differences which are statistically significant (Table 2).

The results might, therefore, indicate that the receptors in the two tissues are different in structure but they might also merely indicate that the statistical assessment of the variance is an underestimate of the real error. It is possible that there are systematic errors in the experiments even with a single type of preparation, and there certainly are systematic differences between the experiments with different types of tissue, such as differences in the length of time for which the agonist was allowed to act, in the method of recording the response of the tissue, and particularly in the presence or absence of hexamethonium.

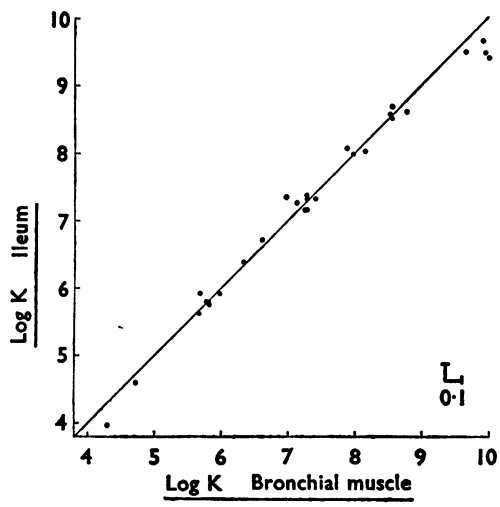


FIG. 4. Values of log K for the compounds on the ileum are plotted against values for the bronchial muscle. If they are identical, the points should lie along the straight line. Those less than 0·1 log units from the line (see mark at bottom right) can be regarded as not lying off it though the situation is complicated by possible effects of hexamethonium, which was not present in the tests with bronchial muscle (see text).

With some compounds estimates of mean values of log K were made on more than one occasion and the results (Table 3) make it possible to obtain some idea of the reproducibility of these mean values obtained from experiments which were performed in conditions which were nominally identical. The first entries for phenylpentyltrimethylammonium and for phenylpentyltriethylammonium on the ileum are values given by Abramson *et al.* (1969). Our subsequent estimates and the other results in Table 3 confirm the impression which these workers obtained, from similar tests on the reproducibility of estimates of log K, that the variance is an underestimate of the real error, that it depends upon the compound, but that in general ' differences of the order of 0·1 log units were likely to indicate real differences ' between the affinities of compounds for receptors. Our results with bronchial muscle indicate that the same is likely to be true for estimates with this preparation.

TABLE 3. *Reproducibility of estimates of log K of antagonists for acetylcholine receptors; mean values are shown with the standard error and number of estimates. The greatest difference between mean values is shown in the column marked* M_1–M_2

	Guinea-pig ileum (Tyrode solution; $2{\cdot}76\times10^{-4}$ hexamethonium)			
				M_1–M_2
$Ph(CH_2)\overset{+}{N}Me_3$	5·180 ±0·016 (10)	5·198 ±0·013 (8)	5·134 ±0·050 (8)	0·064
$Ph(CH_2)_5\overset{+}{N}Et_3$	5·849 ±0·022 (12)	5·767 ±0·013 (6)	5·779 ±0·030 (8)	0·082
				
S-	7·316 ±0·024 (6)	7·207 ±0·019 (7)		0·109
				
R-	9·600 ±0·068 (8)	9·652 (Krebs) ±0·059 (7)		0·052

The estimate marked (Krebs) was obtained from experiments in which this solution was used in place of Tyrode solution.

	Bronchial muscle (Krebs solution)		
$C_6H_{11}(CH_2)_5\overset{+}{N}Et_3$	No hexamethonium		
	5·709 ±0·087 (4)	5·796 ±0·035 (5)	0·087
	No hexamethonium		
	8·169 ±0·046 (4)	8·124 ±0·083 (5)	0·045
	$2{\cdot}76\times10^{-4}$ M hexamethonium		
	7·781 ±0·091 (4)	7·694 ±0·070 (8)	0·087

From tests made with the guinea-pig ileum in Krebs solution instead of in Tyrode solution (Table 3) it does not seem likely that differences between the estimates of log K for the bronchial muscle and for the ileum are simply due to the different bathing fluid used in the two types of experiment. It was clear, however, that contrary to our first impressions (see above) the estimates of log K for some compounds on bronchial muscle were affected by hexamethonium. We therefore re-tested all the compounds whose affinity on the bronchial muscle appeared to be different from that on the ileum by 0·15 log units or more, making estimates in the presence of hexamethonium on the bronchial muscle and omitting the hexamethonium in the experiments with the ileum. The results are shown in Table 4.

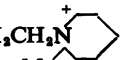
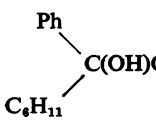
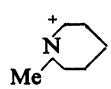
Although there was a considerable variation between the results for individual compounds, hexamethonium reduced nearly all the estimates of log K for the receptors on bronchial muscle and the average decrease was 0·19 log units (statistically significant, $P=0{\cdot}05$). This suggests that hexamethonium may have some slight blocking activity itself at postganglionic receptors in this preparation. The effects of one competitive antagonist on results obtained with another have been discussed by Paton & Rang (1965) and Abramson *et al.* (1969) and in an experiment in which hexamethonium is present throughout, the estimate of log K for an antagonist will be less than the true value by an amount equal to the logarithm of the dose-ratio produced by the hexamethonium. With a hexamethonium concentration of $2{\cdot}76\times10^{-4}$M apparently producing a dose-ratio of around 1·5, the affinity constant of hexamethonium for the postganglionic acetylcholine receptors of bronchial muscle should be about $1{\cdot}8\times10^{3}$, which is about 0·5% of the affinity constant for the ganglionic receptors in the guinea-pig ileum ($2{\cdot}6\times10^{5}$; Barlow & Franks, 1971).

We have tried to measure the affinity constant of hexamethonium for postganglionic receptors in bronchial muscle directly but could not obtain consistent results. Concentrations exceeding 10^{-3}M were needed to produce dose-ratios greater than 2, and it was not really satisfactory to use such strong solutions or to attempt to measure such small dose-ratios. We found, however, that hexamethonium also reduced the values of log K of ethoxyethyltriethylammonium on the iris preparation by 0·208 log units (to $4{\cdot}201\pm0{\cdot}034$, 3 estimates). This tissue does not contain ganglia, and the simplest explanation for the difference is that the hexamethonium is acting as an antagonist at the acetylcholine receptors on the circular muscle of the iris.

On average, hexamethonium had little effect on the values of log K for the ileum because although there were decreases, these were offset by increases (Table 4). The effects of hexamethonium on the affinities of some compounds, e.g. di*cyclo*hexylacetoxyethyltriethylammonium and R-*cyclo*hexylphenylglycolloylethyltriethylammonium, appear statistically to be significantly different from those on the majority, and this applies also to some individual compounds, not necessarily the same ones, in the tests with the bronchial strip. We have no explanation for this, and indeed it is particularly difficult to see why hexamethonium should apparently increase the estimates of log K. If it is dismissed simply as experimental error then these must be considerably greater than our estimates of the error based on the results shown in Table 3. The concentration of hexamethonium used is

high ($2{\cdot}76 \times 10^{-4}$M, producing a dose-ratio for ganglionic receptors of about 70) and it would certainly seem desirable to work in a situation where it is not necessary to use it.

TABLE 4. *Mean values of log K of antagonists of acetylcholine receptors; the standard errors and number of estimates are shown except when they have already been given in Table 2. Measurements made in the presence of hexamethonium, $2{\cdot}76 \times 10^{-4}$M, are indicated by (hex.) and the effects of hexamethonium on the value of log K are shown in the column headed Δ*

	Ileum		Bronchial strip		Δ	
	No hex.	Hex.	No hex.	Hex.	Ileum	Bronchial strip
$CH_3CH_2OCH_2CH_2\overset{+}{N}Et_3$	4·062 ±0·033 (4)	3·974	4·295	4·249 ±0·084 (5)	−0·088	−0·046
$C_6H_{11}CHCOOCH_2CH_2\overset{+}{N}Et_3$	5·602 ±0·023 (8)	5·630	5·673	5·523 ±0·033 (4)	+0·028	−0·150
$C_6H_{11}(CH_2)_5\overset{+}{N}Et_3$	5·868 ±0·012 (7)	5·921	5·757	5·832 ±0·059 (4)	+0·053	+0·075
$(C_6H_{11})_2CHCH_2OCH_2CH_2\overset{+}{N}Et_3$	7·224 ±0·041 (9)	7·354	6·985	6·403 ±0·115 (6)	+0·130	−0·582
$Ph_2C(OH)COOCH_2CH_2\overset{+}{N}(Me)$ 	8·032 ±0·025 (6)	8·034	8·144	7·723 ±0·054 (12)	+0·002	−0·421
$(C_6H_{11})_2CHCOOCH_2CH_2\overset{+}{N}Et_3$	7·675 ±0·013 (8)	8·068	7·891	7·902 ±0·057 (6)	+0·393	+0·011
 $\overset{+}{N}Me_3$; S-	7·348 ±0·033 (6)	7·257	7·284		−0·091	
R-	9·695 ±0·052 (9)	9·647	9·935	9·818 ±0·035 (4)	−0·048	−0·117
 ; S-	7·378 ±0·016 (8)	7·334	7·413		−0·044	
R-	9·560 ±0·040 (6)	9·474	9·973	9·449 ±0·028 (4)	−0·086	−0·524
$\overset{+}{N}Et_3$; S-	7·979 ±0·025 (6)	7·989	7·992	7·845 ±0·042 (6)	+0·010	−0·147
R-	9·843 ±0·047 (7)	9·600	10·001	9·891 ±0·024 (6)	−0·243	−0·110
Hyoscine ethiodide S-	8·692 ±0·016 (6)	8·603	8·793	8·708 ±0·059 (5)	−0·089	−0·085
				Average	−0·006 ±0·041 (13)	−0·191 ±0·066 (11)

Discussion

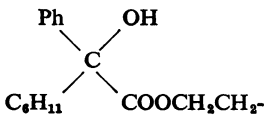
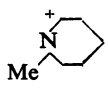
Our results with 28 compounds show that there is a striking similarity between the values of log K for the acetylcholine receptors on the ileum and values for the receptors on bronchial muscle. From tests with 8 compounds it seems that this similarity extends to the receptors on the circular muscle of the iris. With some compounds there were differences which were statistically significant ($P=0{\cdot}05$) and these are summarized in Table 5; it is arguable whether these indicate real differences in affinity and therefore differences in receptor structure. There are some values which are much bigger than the expected experimental error (Table 3) but it is necessary to consider how variable the effects of hexamethonium are (Table 4), also that the responses to the agonist are recorded in different ways and that the agonist is in contact with the tissue for different lengths of time with the three types of preparation. Some estimates of log K obtained with the guinea-pig ileum recorded isometrically and with the agonist acting for different periods of time are presented as an appendix to this paper.

There is no conclusive proof, therefore, that the acetylcholine receptors in the three tissues are not identical in structure, but there are some suggestions that they are different. The effects of hexamethonium on the estimates obtained with the bronchial strip do not seem to be the same as those with the ileum (Table 4), and a further indication of a difference between them is obtained by studying the stereospecific index (the ratio of the biological activities) of the four enantiomeric

TABLE 5. *Differences in mean values of log affinity constant of antagonists for acetycholine receptors*

	Bronchi-ileum		Bronchi-iris
	No hex.	Hex.	No hex.
$CH_3CH_2OCH_2CH_2\overset{+}{N}Et_3$	+0·233	+0·275	−0·124
$C_6H_{11}CHCOOCH_2CH_2\overset{+}{N}Et_3$	+0·071	−0·107	−0·019
$C_6H_{11}(CH_2)_5\overset{+}{N}Et_3$	−0·111	−0·089	−0·074
$(C_6H_{11})_2CHCH_2OCH_2CH_2\overset{+}{N}Et_3$	−0·239	−0·951	+0·577
$Ph_2C(OH)COOCH_2CH_2\overset{+}{N}$(Me)	+0·112	−0·311	
$(C_6H_{11})_2CHCOOCH_2CH_2\overset{+}{N}Et_3$	+0·216	−0·166	−0·085
Ph, $C(OH)COOCH_2CH_2$-, C_6H_{11}			
$\overset{+}{N}Me_3$; S-	−0·064	—	
R-	+0·240	+0·171	
$\overset{+}{N}$(Me); S-	+0·035	—	
R-	+0·413	−0·025	
$\overset{+}{N}Et_3$; S-	+0·013	−0·144	−0·014
R-	+0·158	+0·291	−0·147
Hyoscine ethiodide			
S-	+0·101	+0·105	−0·096

TABLE 6. *Stereospecific index*

	Ileum		Bronchial strip		
	No hex.	Hex.	No hex.	Hex.	Iris
					
$\overset{+}{N}Me_3$	(315)	(345)	(607)		
	222	246	448		
	(157)	(175)	(330)		
$\overset{+}{N}Et_3$	(97)	(61)	(152)	(142)	(197)
	73	41	102	111	139
	(55)	(27)	(69)	(87)	(98)
	(185)	(181)	(497)		
	152	138	363		
	(125)	(105)	(266)		
Hyoscine ethiodide		(39)	(59)		
		28	33		
		(20)	(19)		

Values are the ratios of the affinity constants for the enantiomers: the 95% confidence limits are shown in parentheses.

pairs tested (Table 6). Appreciably lower values were obtained with the ileum than with the other two preparations, and though some of the differences may not be outside the limits of experimental error, they appear to be consistent.

What is clear from our results is that any differences between the structure of these receptors must be small. The biggest differences in affinities for the preparations shown in Table 5 could be accounted for by the replacement of one amino-acid in the receptor protein by a homologue, for example, valine by leucine or isoleucine. The additional binding which could be achieved by a suitable molecule as a result of such a change could be of the order of 1·8 kJ (400 cal)/mole, which would increase log K by 0·3 units. This change in structure may reduce the binding of other compounds so the differences in affinity will be greater than 0·3 log units and might even be as big as 0·9 log units, the largest difference which we observed (Table 5).

Our results, therefore, do not rule out the possibility that acetylcholine receptors in different tissues may not be identical but they show that in these particular preparations the family resemblance between the receptors must be very close indeed.

We wish to thank Miss Margaret Harrison for her highly competent and enthusiastic technical assistance, Dr. N. Ambache for advice about the iris preparation, Mr. John Noble for devising a photoelectric recording method for use with it, Mr. R. P. Stephenson for advice about automation, and Dr. B. L. Ginsborg for his extremely valuable comments on the work during its progress and on the manuscript.

We thank the Faculty of Medicine for the award of a scholarship (to J. D. M. Pearson) and the Medical Research Council for their generous support.

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(*Received April* 25, 1972)

Appendix

The effects of different recording conditions on the estimates of affinity constants of antagonists for acetylcholine receptors in the guinea-pig ileum

ALISON A. BUTT

Summary

The mean of the estimates of log K for one compound recorded isometrically was not significantly different from that of estimates recorded isotonically but with the other compound it was appreciably higher. With both compounds the estimates of log K were bigger when they were based on comparisons in which the agonist had acted for a longer period.

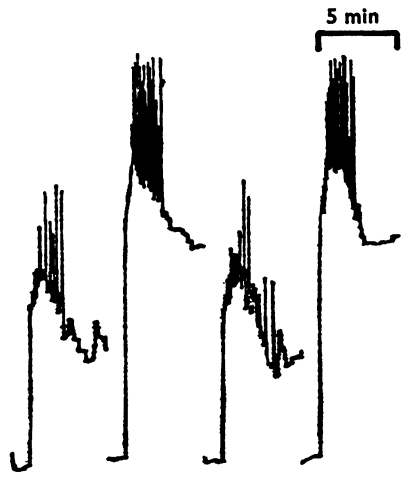

FIG. 1. Isolated guinea-pig ileum recorded isometrically. Responses are shown to 2 and 4×10^{-7}M carbachol. The time intervals were the same as with the bronchial strip preparation (main text, Fig. 1) and the record was interrupted for 7·5 min. during wash and recovery.