The significance of differences in the potency of enantiomers of anti-acetylcholine* drugs

R. W. BRIMBLECOMBE, D. M. GREEN. T. D. INCH AND PAMELA B. J. THOMPSON

Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire, U.K.

The anti-acetylcholine potencies of the dimethylaminoethyl and *N*-methyl piperidin-4-yl esters of *R* and *S* 2-cyclohexyl-2-hydroxy-2-phenylacetic acid and their quaternary derivatives have been measured by *in vitro* and *in vivo* procedures. The *R*-enantiomer of dimethyl-aminoethyl-2-cyclohexyl-2-hydroxy-2-phenylacetate was approximately 100 times more active than the corresponding *S*-enantiomer both *in vivo* and *in vitro*. In contrast, the differences in potencies of the enantiomers of the other compounds were smaller *in vivo* than *in vitro* and moreover, the *in vivo* differences in potency decreased as the potency of the racemates increased. The relevance of these results to general studies of enantiomeric differences is discussed.

The results from pharmacological comparisons of enantiomers which differ in potency are normally used to draw conclusions concerning geometrical requirements for efficient interaction between drug and receptor (Casy, 1970). In this connection it has been suggested that the high ratio between the potencies of enantiomers for highly active drugs compared with the lower enantiomeric potency ratios observed for less potent drugs, reflects the better geometrical fit of the former for the receptor (Pfeiffer, 1956). However, although it is usually convenient and attractive, and probably correct, to invoke differences in the nature of the drug–receptor interaction as the principal reason for differences in the potency of enantiomers, the possible importance of other factors in contributing to these differences have long been recognized. For example, the following factors have been listed (Pfeiffer, 1956): (a) differences in the rates of absorption, destruction and excretion of isomers, (b) competitive inhibition between isomers, (c) differential penetration of enantiomers to the site of drug action and (d) the possible racemization *in vivo* of one or both of the enantiomers. Despite general recognition of many of these and other factors, few detailed discussions have been published concerning the type and extent of information that pharmacological comparisons of enantiomers can be expected to contribute to our understanding of drug action, or of the experimental conditions necessary for maximum information to be obtained. In this paper some results from pharmacological comparisons of the enantiomers and racemates of the hydrochlorides and methiodides of dimethylaminoethyl 2-cyclohexyl-2-hydroxy-2-phenylacetate and *N*-methyl piperidin-4-yl-2-cyclohexyl-2-hydroxy-2-phenylacetate are given.

Enantiomeric pairs of anti-acetylcholine drugs were chosen for study because it was apparent from previously reported studies (Ariëns, 1966; Casy, 1970) that drugs of this type fulfilled most of the requirements that appeared to be necessary to obtain the maximum information possible. Some of the requirements were that the enantiomers of a number of drugs within a series should be available with known

---

* Editorial policy has dictated the use of the term anti-acetylcholine drugs for the drugs used in the experiments described in this paper.
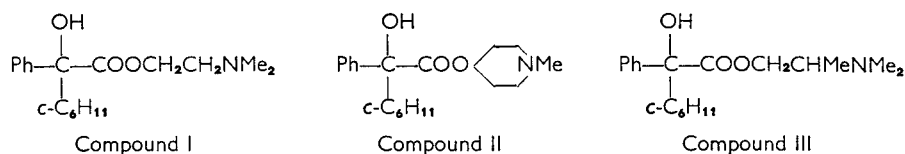
configuration and with appreciable differences in potency. Additionally there should be a variety of pharmacological test procedures sufficiently accurate to indicate whether the potency of the more active enantiomer was twice that of the racemate. Although the enantiomers of atropine and hyoscine fulfilled the above requirements (Buckett & Haining, 1965), they were not available in optically pure forms, which was another necessary prerequisite if maximum information was to be obtained. In compounds like atropine and hyoscine where the enantiomeric potency ratio approaches or exceeds 100, the presence as impurity of 1 % or less of the most active isomer can account for most (or all) of the apparent activity of the less active (or inactive) isomer leading to possible misinterpretation of the pharmacological results. The enantiomers of atropine and hyoscine have only been obtained by classical resolution methods and no techniques are available that permit unequivocal determinations of the optical purity of resolved enantiomers* (Eliel, 1962). For enantiomers prepared in this way even the tedious and time-consuming procedure of purifying enantiomers to states of constant biological activity (Long, Luduena & others, 1956) although providing a good indication, is no guarantee of optical purity. Fortunately, any complications which could have arisen from the use of resolved enantiomeric pairs were avoided by the choice of dimethylaminoethyl- and N-methylpiperidin-4-yl 2-cyclohexyl-2-hydroxy-2-phenylacetates as the anti-acetylcholine drugs for detailed study. The methyl esters of R- and S-2-cyclohexyl-2-hydroxy-2-phenylacetic acid were prepared by a stereospecific synthetic procedure that guaranteed the optical purity of the enantiomers and permitted unequivocal configurational assignments to be made (Inch, Ley & Rich, 1968). Transesterification of the methyl esters with N-methylpiperidin-4-ol or dimethylaminoethanol afforded the optically pure R and S N-methylpiperidin-4-yl and dimethylaminoethyl-2-cyclohexyl-2-hydroxy-2-phenylacetates which were converted into their respective hydrochlorides and methiodides. Dimethylaminoethyl-2-cyclohexyl-2-hydroxy-2-phenylacetate was chosen because of the fact that the enantiomers of this highly potent anti-acetylcholine compound had already been established as having appreciably different potencies (Ellenbroek, Nivard & others, 1965). N-Methylpiperidin-4-yl-2-cyclohexyl-2-hydroxy-2-phenylacetate was chosen because it was known that glycollic acid esters of N-methylpiperidin-4-ol are amongst the most potent anti-acetylcholine drugs available (Abood, 1968).

The four tests for anti-acetylcholine potency we used were chosen because they have proved to be reliable both in our laboratory and elsewhere (Abramson, Barlow & others, 1967). Further, two of the tests, production of mydriasis in mice and antagonism of carbachol-induced contractions of the guinea-pig isolated ileum, may be used to follow the time course of drug action. It will be shown subsequently that the value of enantiomeric potency ratios in providing information about modes of action of drugs is of limited value unless due allowance, where necessary, is made for differences in the time course of action of the enantiomers.

Drugs

The following drugs were examined for anti-acetylcholine activity. (Hydrochlorides are designated by a number and a configurational notation. Methiodides are designated by a number, the letter M, and a configurational notation.)

---

* Since this paper was submitted a detailed treatment of the biological consequences of optical impurities has appeared [Barlow, R. B. (1971), *J. Pharm. Pharmac.*, **23**, 90].

OH
|
Ph—C—COOCH₂CH₂NMe₂
|
c-C₆H₁₁

Compound I

OH
|
Ph—C—COO⟨NMe⟩
|
c-C₆H₁₁

Compound II

OH
|
Ph—C—COOCH₂CHMeNMe₂
|
c-C₆H₁₁

Compound III

Compound I. Dimethylaminoethyl(R)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (IR) and methiodide (IMR). Dimethylaminoethyl 2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (I Rac) and methiodide (IM Rac). Dimethylaminoethyl(S)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (IS) and methiodide (IMS).

Compound II. N-Methyl-piperidin-4-yl(R)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (IIR) and methiodide (IIMR). N-Methyl-piperidin-4-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (II Rac) and methiodide (IIM Rac). N-Methyl-piperidin-4-yl(S)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (IIS) and methiodide (IIMS).

Compound III. 2(S)-Dimethylaminopropan-1-yl(R)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (IIIRS) and methiodide (IIIMRS). 2(R)-dimethylaminopropan-1-yl(R)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (IIIRR) and methiodide (IIIMRR). The first configurational term (IIIRS) refers to the configuration of the acid moiety and the second configurational term (IIIRS) refers to the configuration of the aminoalcohol.

The enantiomeric dimethylaminoethyl- and N-methylpiperidin-4-yl 2-cyclohexyl-2-hydroxy-2-phenylacetates were prepared by base catalysed transesterification of the optically pure enantiomers of methyl 2-cyclohexyl-2-hydroxy-2-phenylacetate (Inch & others, 1968) with dimethylaminoethanol and N-methylpiperidin-4-ol. The optically pure 2(S) and 2(R)-dimethylaminopropan-1-ols which were transesterified with methyl (R)-2-cyclohexyl-2-hydroxy-2-phenylacetate were prepared as described by Inch & Lewis (1971). Racemic compounds were prepared by standard procedures.

METHODS AND RESULTS

Method 1

Determination of affinity constants. The terminal 2–3 cm of ileum from a guinea-pig was suspended in a bath (2·5 ml) of Ringer Tyrode solution at 37°. The ileum was attached to a Devices 2LDO1 optical wedge transducer and subjected to a load of 0·5 g and contractions were recorded on a direct writing pen recorder. The bath was connected to reservoirs and overflow so that the fluid in the bath could be changed by upward displacement either by Tyrode alone or by Tyrode containing drugs at predetermined concentrations. Events in the bath were controlled automatically by an apparatus similar to that described by Schild (1947). The method used for determining the affinity constants of the anti-acetylcholine drugs was similar to that described by Barlow, Scott & Stephenson (1963) except that carbachol instead of acetylcholine was used as agonist. The agonist was in contact with the tissue for 15 s and the interval between doses was 1·5 min. All solutions contained hexamethonium bromide ($1·1 \times 10^{-4}$M). After regular responses were obtained for two different concentrations of carbachol, the Tyrode solution in which the ileum was suspended was replaced by Tyrode solution containing the antagonist and the concentration of the

agonist was increased to maintain comparable contractions. For drugs with low affinity constants (log k < 9) the antagonist and the ileum came into equilibrium in less than 10 min but for drugs with higher affinity constants, e.g. IMR, IIR, II Rac, IIS, IIMR, IIM Rac and IIMS much longer periods were required before a steady response to the agonist in the presence of the antagonist was obtained.

The affinity constant of the antagonist using the four point assay procedure was calculated by the method of the Edinburgh group (Pharmacological Experiments on Isolated Preparations, Staff of the Department of Pharmacology, F. & S. Livingstone, 1968).

In Table 1 the logarithms of the affinity constants are expressed as a mean of four to ten determinations using two to five different concentrations of antagonist. One preparation was used for only one concentration of antagonist. For compounds of affinity constant Log k < 10·0 dose ratios in the range 10–400 were used whereas dose ratios in the range 50–1000 were used for compounds of affinity constant log k > 10·0. It will be seen from Table 1 that with the exception of compounds IIM Rac the standard error associated with the measurements of affinity constants was small. Plots of dose ratio against concentration for IMR and IMS and for IIMR and IIMS are shown in Fig. 1. The times taken for compounds II Rac, IIM Rac
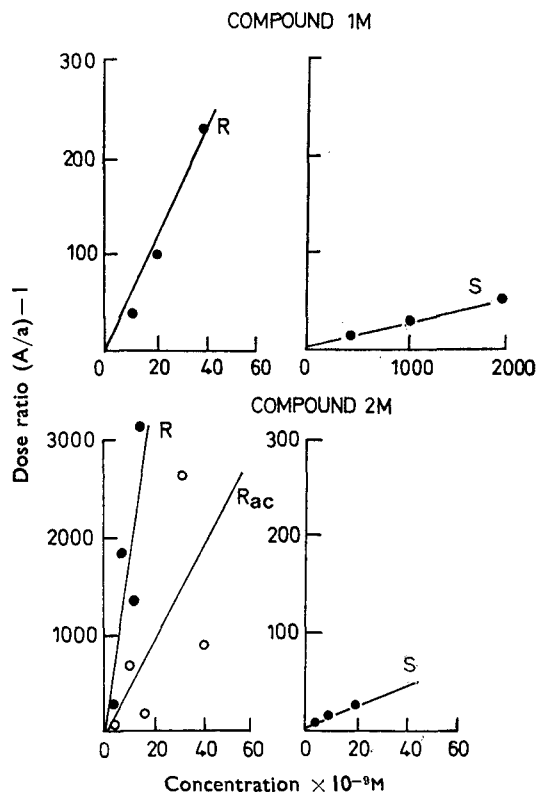

FIG. 1. Effect of isomers of compound 1M and isomers and racemate of compound 2M in antagonizing carbachol induced contractions on the guinea-pig isolated ileum. Each point on the graph represents the mean dose ratio obtained from two determinations. A linear relation between dose ratio $\left(\frac{A}{a}\right) - 1$ and antagonist concentration demonstrates that the compounds are competitive antagonists.

Table 1. *Summary of pharmacological results.*

Compound	Method 1 log k ± s.e. (n results)	Method 2. Production of mydriasis in mice Potency relative to atropine with 95% confidence limits in brackets	Time to peak response (min)	Duration (min) ± s.e.	Method 3 (Salivation) ED50 μmol/kg with 95% confidence limits in brackets	Method 4 (Tremors) ED50 μmol/kg with 95% confidence limits in brackets
IR	*9·06 ± 0·01 (5)	0·76 (0·56–0·96)	10	73 ± 3	0·76 (0·44–1·29)	5·07 (3·87–6·9)
I Rac	*8·73 ± 0·02 (4)	0·34 (0·28–0·41)	10	58 ± 5	1·40 (1·11–3·33)	22·7 (7·39–41·4)
IS	*7·07 ± 0·01 (5)	0·006 (0·004–0·008)	<5	65 ± 6	>100	>100
IMR	9·66 ± 0·08 (6)	2·81 (2·34–3·44)	10	70 ± 10	0·06 (0·02–0·07)	> 50
IM Rac	*9·36 ± 0·03 (7)	1·67 (1·29–2·1)	20	90 ± 12	0·20 (0·08–0·52)	> 50
IMS	7·38 ± 0·02 (6)	0·074 (0·06–0·09)	5	34 ± 3	8·84 (5·15–15·2)	> 25
IIR	10·92 ± 0·10 (4)	2·36 (2·04–2·72)	80	901 ± 85	0·18 (0·04–0·31)	0·56 (0·30–1·3)
II Rac	10·53 ± 0·07 (4)	1·17 (1·02–1·36)	90	552 ± 45	0·31 (0·18–0·54)	3·29 (1·35–8·44)
IIS	8·48 ± 0·04 (4)	0·12 (0·10–0·14)	5	32 ± 4	7·75 (3·8–16·2)	9·42 (5·54–16·6)
IIMR	11·08 ± 0·10 (8)	2·76 (2·33–3·32)	80	1024 ± 48	0·06 (0·03–0·13)	24·32 (13·8–46·9)
IIM Rac	10·39 ± 0·17 (10)	2·1 (1·68–2·60)	10	780 ± 68	0·05 (0·03–0·11)	> 50
IIMS	9·08 ± 0·05 (6)	1·17 (0·99–1·39)	5	41 ± 2	1·05 (0·52–1·88)	73·8 (39·9–91·4)
IIIRS	10·00 ± 0·04 (6)	1·63 (1·35–1·99)	20	143 ± 10	0·55 (0·31–0·98)	0·72 (0·14–3·62)
IIIRR	9·88 ± 0·05 (6)	2·78 (2·29–3·40)	20	102 ± 7	0·55 (0·31–0·96)	2·52 (1·46–4·39)
IIIMRS	10·04 ± 0·03 (6)	3·16 (2·47–4·19)	40	645 ± 110	0·27 (0·15–0·49)	> 50
IIIMRR	10·08 ± 0·05 (6)	4·25 (3·4–5·24)	40	267 ± 28	0·20 (0·10–0·40)	> 25

* Determinations made by Dr. R. B. Barlow, Department of Pharmacology, University of Edinburgh.

and their enantiomers and compound IMR to reach equilibrium at different concentrations are listed in Table 4. The time to reach equilibrium was determined when contractions of the ileum elicited by the agonist in the presence of the antagonist had reached a constant height.

Method 2

Production of mydriasis in mice. Male mice (18–25 g) were used. Drugs were injected into a tail vein. Preliminary experiments were made on single animals to obtain an indication of suitable dose levels. Then, using groups of 10 mice at each of three dose levels, the pupil diameter was measured at different times after injection, to cover as far as possible the total period of action of the drug. The eyes were held 20 cm from a Watson microscope lamp and the measurement was made using an eyepiece graticule in a ×20 microscope. The mean pupil diameter from the two eyes were used and the mice were kept in the dark before and between readings. Graphs were plotted of pupil diameter against time. The time to peak effect varied with dose so in calculations of potency relative to atropine the maximum mean pupil diameter reached at each dose was used, irrespective of time and the results calculated on the basis of a six-point assay. Estimates of the duration of action were obtained from the graphs and were the times from injection, through peak pupil diameter, to 50% maximum pupil diameter. Estimates of the time to reach peak pupil diameter were also made. The results are listed in Table 1 and results for compounds IR and IS and IIR and IIS are shown in Fig. 2.

Methods 3 and 4

Antagonism of oxotremorine effects in mice. Male mice (18–25 g) were used. The drugs were injected intraperitoneally 15 min before the injection into a tail vein of 100 μg/kg oxotremorine. Animals were examined at 5, 10 and 15 min after the oxotremorine injection for the presence of salivation or tremors. No attempt was made to grade the severity of either response, it was noted as being present or absent. Four groups each containing five mice were used and ED50s for block of salivation (Method 3) and of tremors (Method 4) were calculated by probit analysis.
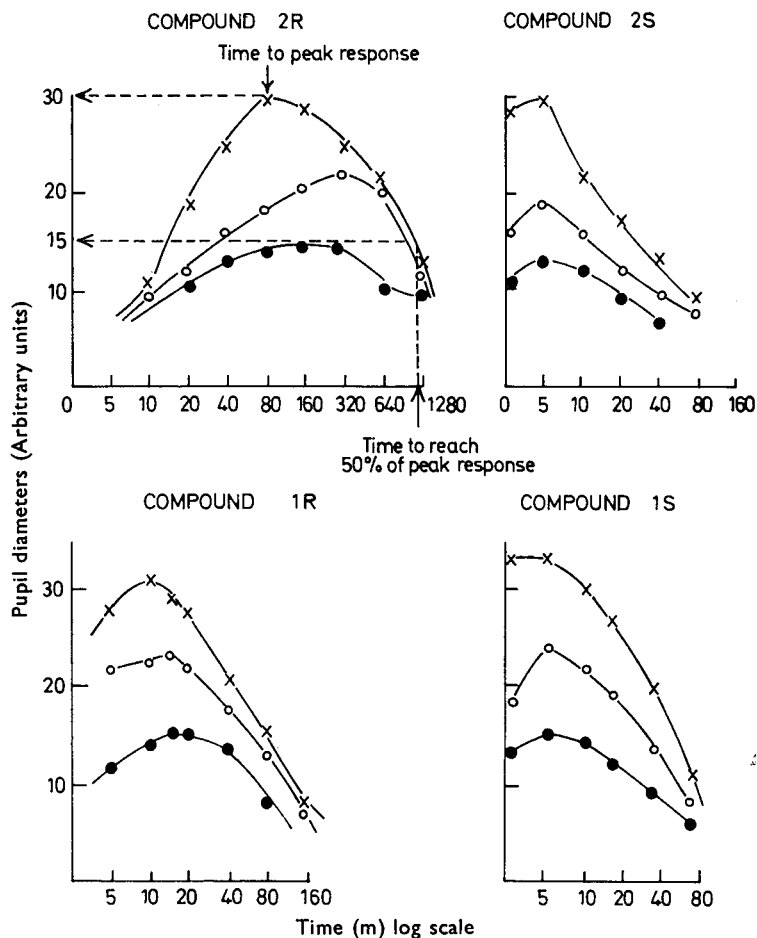
FIG. 2. Graphs showing production and duration of action of mydriasis induced by isomers of compounds 1 and 2. Compounds 1R 2R: $\times$ = 0·1, $\bigcirc$ = 0·05, $\bullet$ = 0·025 μmol/kg. Compound 2S: $\times$ = 2, $\bigcirc$ = 1, $\bullet$ = 0·5 μmol/kg. Compound 1S: $\times$ = 4, $\bigcirc$ = 2, $\bullet$ = 1 μmol/kg.

DISCUSSION

The results for I Rac and its enantiomers and the corresponding methiodides (Table 1) showed that the three tests used for assessing peripheral anti-acetylcholine potency (measurement of affinity constants on the guinea-pig isolated ileum, blockade of oxotremorine-induced salivation in mice and production of mydriasis in mice) were consistently accurate. For example, the R-enantiomers IR and IMR were approximately twice as active as their respective racemates I Rac and IM Rac in all three tests (Table 2) as required for compounds where the inactive isomer is appreciably less potent than the active isomer. Also, the IR:IS potency ratio (Table 3) was very similar in the three tests, thereby providing evidence for the dependability of the three tests. Reasons for the greater variation in the enantiomeric potency ratios of IMR and IMS will be discussed later. The consistent results obtained from the three peripheral anti-acetylcholine assay procedures for compounds I Rac and IM Rac and their enantiomers made an interesting contrast with the much more variable

results obtained for compounds II Rac, IIM Rac and their enantiomers. Thus although IIR was approximately twice as active as its racemate in these three tests, IIMR was five times more active than its racemate in antagonizing the effects of carbachol-induced contractions of guinea-pig ileum but only of similar potency to its racemate in producing mydriasis in mice or in antagonizing the production of oxotremorine-induced salivation (Table 2). Even more striking variations were exhibited by the IIR:IIS and IIMR:IIMS potency ratios (Table 3). The *in vitro* ratio was 272 for IIR:IIS, 20 in the mydriasis test and 43 in the test for antagonism of oxotremorine-induced salivation. Similarly the *in vitro* ratio was 100 for IIMR:IIMS and 2·3 and 17·6 in the *in vivo* tests. The following conclusions may be drawn from the above results.

It was probable that the differences in potency of IR and IS, and IMR and IMS, as determined by the guinea-pig ileum assay reflected accurately the differences in affinity of the drugs for the receptor because the straight line dose ratio–concentration plots obtained for the enantiomers and the racemates (Abramson & others, 1969) indicated that they elicited their effects by a similar mechanism. Under the experimental conditions which were used, differences in rates of destruction (if any) of enantiomers were unlikely to make any significant contribution to differences in enantiomeric potency and the fact that the R-enantiomers were twice as active as their racemates showed that no racemization of the enantiomers occurred. If the conclusion is correct that the observed *in vitro* differences in enantiomeric potency for IR and IS and IMR and IMS resulted from factors associated only with the drug–receptor interaction, a similar conclusion about the *in vivo* differences in the potencies of IR and IS can be drawn from the facts that the enantiomeric potency ratio for IR:IS was similar *in vivo* and *in vitro* and that *in vivo*, as *in vitro*, compound IR was approximately twice as active as the racemate I Rac. Further, the consistency of the IR:IS enantiomeric potency ratios provide an indication that the anti-acetylcholine receptors in the guinea-pig ileum, mouse eye and mouse salivary gland are essentially identical.

Table 2. *Relative anti-acetylcholine activities of* R *and* S *enantiomers compared with racemate.*

Compound	Method 1	Method 2	Method 3	Method 4
IR	2·14	2·23	1·84	4·47
IS	0·022	0·018	<0·01	<0·2
IMR	2·00	1·68	3·33	—
IMS	0·01	0·044	0·022	—
IIR	2·45	2·01	1·72	5·86
IIS	0·009	0·10	0·04	0·607
IIMR	5·0	1·31	0·83	—
IIMS	0·05	0·55	—	—

Table 3. *Enantiomeric potency ratios.*

Enantiomers	Method 1	Method 2	Method 3	Method 4
IR:IS	100	123	>100	>22
IMR:IMS	200	38	147	—
IIR:IIS	272	20	43	38
IIMR:IIMS	100	2·3	17·6	—

It must be pointed out that conclusions, such as those reached above, will usually only be valid if the time profiles of pharmacological action of the enantiomers and their racemate are taken into account. This is important because the law of mass action requires that the rate of drug action be proportional to the concentration of the drug and thus where the differences in potency of enantiomers are large, concentration effects can be exceedingly important where comparisons are made at the ED50 level. The time–activity profiles for IR, I Rac and IS were found to be similar both in Method 1 (affinity constants) and Method 2 (mydriasis) and consequently the fact that possible differences in time–activity profiles were not taken into account in Method 3 was unlikely to affect seriously the IR:IS enantiomeric potency ratio obtained using this method. However, as will be demonstrated subsequently, differences in the time profile of compounds such as IIMR and IIMS are large and have a pronounced effect on the pharmacological results.

The results that the enantiomeric pairs IMR and IMS, IIR and IIS, and IIMR and IIMS exhibited considerable variation in enantiomeric potency ratios, and that compound IIMR was not twice as active as its racemate (IIM Rac) in any of the three tests for peripheral anti-acetylcholine activity, seem at first sight to contradict the conclusions reached above. We feel that an explanation can be offered which provides interesting and important new information about the behaviour of anti-acetylcholine drugs. Examination of the changes in potency and changes in enantiomeric potency ratios caused by quaternization of IR and IS, and IIR and IIS reveals an explanation for the facts that *only* the IR:IS potency ratio was similar *in vivo* and *in vitro* and the enantiomeric potency ratios of the other pairs were much smaller *in vivo* than *in vitro*. Quaternization of IIS increased mydriatic potency (Method 2) from 0·12 to 1·17 and the potency of antagonism of salivation from an ED50 of 7·75 to an ED50 of 1·05 μmol/kg (Method 3), whereas quaternization of IIR caused corresponding increases from 2·36 to 2·76 and 0·18 to 0·06. Thus the changes produced by quaternization of IIR were much smaller than the changes which resulted from the quaternization of IIS. In contrast, the increases in potency caused by quaternization of IR [0·76 to 2·81 (Method 2) and 0·76 to 0·06 μmol/kg (Method 3)] and IS [0·006 to 0·074 (Method 2) and >100 to 8·84 μmol/kg (Method 3)] were similar. These results, and the observations that IR was much less potent than IIR whereas the quaternary derivatives of IR and IIR have equivalent *in vivo* potencies even though the affinity constant of IIMR (log k = 11·08) was greater than IMR (log k = 9·66), suggest that there is a minimum dosage below which maximum anti-acetylcholine effects cannot be obtained *in vivo*, and that the potencies of IMR, IIR and IIMR all approached this minimum value. The fact that IIMR was only slightly more potent than IIMS in causing mydriasis also favoured the concept that a minimum dose is required to produce a maximum biological effect for anti-acetylcholine drugs.

As mentioned previously, the rates of onset of anti-acetylcholine effects *in vivo* and the rates at which the drugs equilibrate with the guinea-pig ileum are considerably influenced by dose or concentration of the drug. Thus Fig. 2 illustrates that, with the possible exception of compound IIR, the times to onset of mydriatic effects decrease as drug dosage is increased and similarly the results in Table 4 show that drugs equilibrate more rapidly with the guinea-pig ileum at high concentration than at low concentration. However the results also show that concentration effects alone do not control the rate at which anti-acetylcholine drugs act. Thus, although

Table 4. *Equilibration times with the guinea-pig ileum.*

Compound	Bath concentration (mol)	Time to equilibrium* (Plateau time) min	Mean log k
IMR	1×10^{-8}	24	9·66
	2×10^{-8}	15	
	4×10^{-8}	12	
IIR	2×10^{-9}	60	10·92
	4×10^{-9}	44	
	8×10^{-9}	40	
II Rac	4×10^{-9}	58	10·53
	8×10^{-9}	44	
IIS	2×10^{-7}	18	8·41
	5×10^{-7}	15	
IIMR	4×10^{-9}	107	11·08
	8×10^{-9}	77	
	$1·2 \times 10^{-8}$	40	
	$1·6 \times 10^{-8}$	59	
IIM Rac	4×10^{-9}	67	10·32
	$1·2 \times 10^{-8}$	65	
	$1·6 \times 10^{-8}$	75	
	$3·2 \times 10^{-8}$	70	
	4×10^{-8}	56	
IIMS	4×10^{-9}	28	9·10
	$8·0 \times 10^{-9}$	28	
	2×10^{-8}	24	

* Each time represents the mean obtained from two determinations.

compounds IR and IIR both produce maximum mydriatic effects at a dose of $0·1$ μmol/kg, the effects appear much more rapidly for IR than for IIR (Fig. 2). Also, at a concentration of 4×10^{-9}M, compound IIMR required 107 min to equilibrate with guinea-pig ileum, whereas IIMS equilibrated in 28 min. From the results in Tables 1 and 4 there is some indication that drugs with high affinity constants take longer to produce mydriatic effects and longer to reach equilibrium with the ileum than compounds with lower affinity constants, and also that drugs with high affinity constants have a more prolonged effect. The result that IIMS, which has a similar affinity constant and *in vivo* potencies to compound IMR, also has a similar time–activity profile, indicated that the factors which control the time course of drug action are not critically dependent on drug stereochemistry. Also some evidence is available which suggests that the time–activity profiles are not significantly influenced by metabolic factors. For example, the affinity constants for IIMR are the same (Fig. 1) whether measured at low concentration with a long period of time for equilibrium to be reached or at a high concentration with a short period of time to equilibrium (Table 4). The reason why anti-acetylcholine drugs with high affinity constants require longer to produce mydriatic effects than anti-acetylcholine drugs with lower affinity constants even where equimolar doses are administered is not understood.

The obvious importance of the differences in the time–activity profiles for the pairs of enantiomers IMR and IMS, IIR and IIS, and IIMR and IIMS is possibly reflected by the results that the enantiomeric potency ratios for these pairs were smaller in the mydriasis tests (Method 2), where time effects were considered, than in the salivation tests (Method 3), where a set time schedule was adhered to for the injection of drugs and the making of observations. The higher enantiomeric potency ratios in the salivation test (Method 3) compared with the mydriasis test can be explained if the

less active isomers produced their maximum anti-acetylcholine effects *before* the oxo-tremorine was administered, and if there was little difference between the observed potency of the R-enantiomers and their maximum potency at the optimum time.　In preliminary experiments, the time–activity profile of IIR and IIS in antagonizing oxotremorine-induced salivation has been measured by modifying Method 3 so that in separate experiments oxotremorine was administered 5, 10, 15, 20, 80, and 160 min after the administration of IIR or IIS.　It was found that IIR was most potent (ED50, 0·09 μmol/kg) 80 min after its administration and that IIS showed a peak effect (ED50, 2·5 μmol/kg) after 10 min.　Thus the potency ratio IIR : IIS fell from 43 to 28, the latter value being close to the IIR : IIS ratio observed in Method 2.　However, it must be pointed out that in Method 3 some error may result because of the mode of administration (intraperitoneal injection) of the drugs in the salivation experiments.　The preliminary experiments showed that whereas IIR had similar time–activity profiles in the mydriasis and salivation experiments, IIS had a sharp peak of activity in the mydriasis test but showed no such obvious peak of activity in the salivation test.　These results may indicate that whereas for IIR the rate of diffusion within the body to the receptor is the controlling feature, for IIS the rate of diffusion from the peritoneum into the body is the rate controlling step.　As a general screening procedure and for comparisons of compounds with similar time–activity profiles, such as IR and IS, Method 3 is perfectly adequate.　However for compounds with different time–activity profiles it is clear that intravenous admini-stration of the anti-acetylcholine drugs would be more appropriate than intraperitoneal injection and that measurements of ED50s should be made at the optimum times.　Method 4 (antagonism of oxotremorine induced tremors) suffers from the same deficiencies as Method 3.

Another consequence of large differences in the time–activity profiles of enantiomers is that measurements of the affinity constant of their racemate may be made more difficult.　For example, there was a far greater random error in the experimental measurements of the affinity constant of IIM Rac (Fig. 1) than for any other com-pound and it was for the enantiomers for IIM Rac that the largest difference in equilibration times was observed (Table 4).　It is not unreasonable to suppose that, under the conditions used for measuring the affinity constant of IIM Rac, the rate of equilibration between IIMR, IIMS (both highly potent drugs) and the ileum was so slow that the equilibrium situation was difficult to assess experimentally, with the consequence that reproducible results were difficult to obtain.　Under these circum-stances the result that the ratio (5 : 1) of the affinity constants of IIMR and IIM Rac was particularly high must be interpreted with caution.

The general implications of the above discussion are that comparisons of enantio-meric potency ratios can provide evidence concerning the similarity or otherwise of receptors at different sites in the body or in different species providing that due allowance is made for differences in the time course of the enantiomers and that the potency of the more active isomer does not approach the minimum dose that is necessary to cause maximum biological effects.　Nevertheless, if the concentration requirements cause a uniform decrease in *in vivo* enantiomeric potency ratios com-pared with *in vitro* ratios, it is possible that comparisons of these *in vivo* ratios can still provide evidence in favour of receptor similarity, if not of receptor differences.　For example, the similarity of the IR : IS peripheral and central enantiomeric potency ratios provides evidence to suggest that the central receptors with which anti-acetylcholine

drugs interact are similar to those in the peripheral nervous system. This conclusion is supported by the similarity of the IIR : IIS *in vivo* peripheral and central enantiomeric potency ratios and also by the *in vivo* central and peripheral ratios obtained for *dextro* and *laevo* hyoscyamine (Buckett & Haining, 1968).

Conclusions that were reached from previous studies of enantiomers obviously require reappraisal in terms of the results obtained above. For example, it was suggested by Pfeiffer in 1956 that the enantiomeric potency ratios for highly active drugs should be greater than the enantiomeric potency ratios for less active drugs. This suggestion has subsequently been alluded to as Pfeiffer's Rule (Ariëns, 1965). It has also been claimed that where the ratio of the potency of the more active enantiomer to the racemate falls appreciably below 2 there is a lack of receptor selectivity and that in the limiting case where this ratio is 1 and the enantiomers are equiactive, there is a complete lack of stereochemical sensitivity (Casy, 1970). The results which have been described in this paper show that these generalizations are not valid, at least for anti-acetylcholine drugs, in *in vivo* experiments although they may possibly be justified for some isolated tissue experiments.

Ariëns and his coworkers (Ariëns, 1966) modified Pfeiffer's rule to the effect that the enantiomers of highly active drugs only have large differences in potency if the asymmetric centre is in a region of the molecule which is essential to the drug–receptor interaction. Ariëns' proposition was based on the values of the affinity constants (pA$_2$ values) for the rat jejunum of the choline and β-methylcholine esters of benzilic acid and 2-cyclohexyl-2-hydroxy-2-phenylacetic acid. It was found that the activity of these esters depended little on the configuration of the β-methylcholine moiety and that the β-methylcholine esters were less potent than the corresponding choline esters. These results were considered to imply that the β-methyl region of the β-methylcholine anti-acetylcholine esters contributed little to the potency of the drugs. The results in Table 1, which showed that the α-methylcholine esters IIIMRR and IIIMRS [and the tertiary analogues IIIRR and IIIRS] of R2-cyclohexyl-2-hydroxy-2-phenylacetate had similar affinity constants and were equally potent in antagonizing the effects of oxotremorine induced salivation (Method 3), and moreover were more potent than the corresponding choline derivative (IMR) [and the tertiary analogue IR] do not support Ariëns, modification of Pfeiffer's rule. There can be little doubt that the α-methyl group in IIIRR, IIIRS, IIIMRR and IIIMS makes a considerable contribution to the observed anti-acetylcholine potency of these compounds which is independent of stereochemistry. The conclusion follows from these results that Pfeiffer's rule (or Ariëns' modification) does not apply generally to *in vitro* or *in vivo* experiments.

In a recent review (Abood, 1970) and elsewhere (Abood, 1968; Gabel & Abood, 1965), Abood and his coworkers have made a number of suggestions concerning the mode of action of anti-acetylcholine drugs. Some of these suggestions are invalidated by the results that are summarized in Table 1. For example, it was suggested that these drugs may act by a carbonium ion mechanism, e.g.
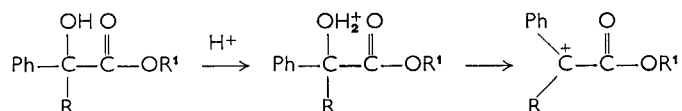
Since carbonium ion formation necessitates destruction of asymmetry it cannot be consistent with the results that enantiomers have different potencies and different

durations of action. Also the suggestion that the duration of action of the drugs is related to their ease of acidic hydrolysis cannot be valid since pairs of enantiomers which are hydrolysed at the same rate have quite different durations of action.

It has been suggested by Abood, in detailed discussions of the relation between anti-acetylcholine activity and molecular conformation that the activity of glycollic acid esters of tertiary aminoalcohols was very dependent on the availability of the lone pair of electrons on nitrogen, particularly in cyclic aminoalcohols. It was also stated that in reaching this conclusion, little distinction was made between central and peripheral anti-acetylcholine effects. Since in this paper and elsewhere it has been shown that quaternary compounds are usually more potent peripherally than tertiary compounds, the contribution of the lone pair of electrons of nitrogen to the peripheral anti-acetylcholine activity of the glycollate esters is clearly of little consequence. This evidence must also detract from the possibility of there being any connection between the lone pair availability and the effects of glycollate esters in the central nervous system.

In summary it appears that, even without knowledge of the configuration of enantiomers, the results from studies of enantiomers and their racemates can provide critical tests of certain aspects of drug action and indicate whether or not receptors in different species and in different parts of the body are similar. Other workers have demonstrated the utility of this approach previously with experiments with (+)- and (−)-amphetamine (Taylor & Snyder, 1970) and with (+)- and (−)-noradrenaline (Patel, 1969) and provided that due consideration is given to time–activity profiles and minimum dose requirements, this approach should be of potential relevance to many active types of drug. Where the absolute configuration of the enantiomers are known, studies of enantiomers are able to provide additional information. For example, since $S(−)$hyoscyamine, dimethylaminoethyl-R-2-cyclo-hexyl-2-hydroxy-2-phenylacetate, 2-cyclohexyl-2-phenyl-4-dimethylaminomethyl-1,3-dioxolan (Brimblecombe & Inch, 1970) and 4-dimethylaminomethyl-2-(1-cyclohexyl-1-hydroxy-1-phenyl)methyl-1,3-dioxolan (Brimblecombe, Inch & others, 1971) are configurationally related, it can be assumed that they act in the same way. In contrast, since the stereochemical requirements of acetylcholine-like and anti-acetyl-choline drugs differ in some cases (Brimblecombe, Green & Inch, 1970) it is possible that both kinds of drugs do not share points of attachment with a common receptor. Knowledge of the precise stereochemistry of drugs is also important from the viewpoint of design of new drugs. Hopefully in the future it is possible that knowledge of the absolute stereochemical requirements for high potency will help in the elucidation of receptor structure.

REFERENCES

ABOOD, L. G. (1968). In *Drugs Affecting the Central Nervous System*. Editor: Burger, A. New York: Marcel Dekker.

ABOOD, L. G. (1970). In *Psychotomimetic Drugs*, pp. 67–74. Editor: Efron, D. H. New York: Raven Press.

ABRAMSON, F. B., BARLOW, R. B., MUSTAFA, M. G. & STEPHENSON, R. P. (1969). *Br. J. Pharmac.*, 37, 207–233.

ARIËNS, E. J. (1966). *Adv. Drug. Res.*, 3, 325–285.

BARLOW, R. B., SCOTT, K. A. & STEPHENSON, R. P. (1963). *Br. J. Pharmac. Chemother.*, 21, 509–522.

BRIMBLECOMBE, R. W., GREEN, D. M. & INCH, T. D. (1970). *J. Pharm. Pharmac.*, 22, 951–953.

BRIMBLECOMBE, R. W. & INCH, T. D. (1970). *Ibid.*, 22, 881–888.

BRIMBLECOMBE, R. W., INCH, T. D., WETHERELL, J. & WILLIAMS, N. (1971). *Ibid.*, 23, 649–661.

BUCKETT, W. R. & HAINING, C. G. (1965). *Br. J. Pharmac. Chemother.*, 24, 138–146.

CASY, A. F. (1970). In *Medicinal Chemistry*, p. 81. Editor: Burger, A. New York: Wiley-Interscience, 1970.

ELLENBROEK, B. W. J., NIVARD, R. J. F., VAN ROSSUM, J. M. & ARIËNS, E. J. (1965). *J. Pharm. Pharmac.*, 17, 393–404.

ELIEL, E. L. (1962). *Stereochemistry of Carbon Compounds*, p. 83. McGraw Hill.

GABEL, N. & ABOOD, L. G. (1965). *J. mednl Chem.*, 8, 616–619.

INCH, T. D. & LEWIS, G. J. (1971). *Carbohydrate Res.*, 16, 455–458.

INCH, T. D., LEY, R. V. & RICH, P. (1968). *J. chem. Soc.* (C), 1693–1699.

LONG, J. P., LUDUENA, F. P., TULLAR, B. F. & LANDS, A. M. (1956). *J. Pharmac. exp. Ther.*, 117, 29–38.

PATEL, P. N. (1969). *J. Pharm. Pharmac.*, 21, 628–629.

PFEIFFER, C. C. (1956). *Science, N.Y.*, 124, 29–31.

SCHILD, H. O. (1947). *Br. J. Pharmac. Chemother.*, 2, 189–206.

TAYLOR, K. M. & SNYDER, S, H, (1970), *Science, N.Y.*, 168, 1487–1489.