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The central and peripheral activities of anti-acetylcholine drugs. Some concepts of practical relevance

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Five procedures have been used to compare the pharmacological effects and time–activity profiles of anti-acetylcholine drugs in the central and peripheral nervous system (cns, pns). In particular, the potencies of optically pure enantiomers of anti-acetylcholine drugs which contain an asymmetric centre have been determined. The following four observations are made which are of relevance to all studies of anti-acetylcholine drugs. (1) Times to onset of activity of anti-acetylcholine drugs in three *in vivo* tests and the duration of action in one *in vivo* test are shown to increase as affinity constants, determined *in vitro*, increase. (2) Below a dose of *ca* 0·03 μmol kg^{-1} no anti-acetylcholine drug produces maximum mydriatic effects and all anti-acetylcholine drugs which have log affinity constant values $>$9·49 produce maximal effects at approximately this dose. (3) The receptor with which anti-acetylcholine drugs interact is essentially the same in the eye, salivary gland and cns of the mouse, the guinea-pig ileum, and in the cat cns. (4) For any anti-acetylcholine drug the time to onset of effects in the cns is similar to the time of onset of effects in the pns and appears to depend on the affinity constant rather than on the partition properties of the drug. The practical and theoretical significance of these and other observations are discussed.

There have been many attempts to synthesize drugs that are more potent than atropine or in which some of its properties are retained or enhanced while others are reduced or eliminated. Most of the observed effects, which include antispasmodic, antisecretory and mydriatic effects and central effects which cause behavioural disturbance, are now recognized to result from the ability of atropine and related compounds to antagonize the effects of acetylcholine in the peripheral and central nervous systems. Rama Sastry (1970) concluded from a survey of anti-acetylcholine drugs that, despite claims to the contrary, none of the newly introduced synthetic drugs possesses outstanding potency and freedom from side-effects when compared clinically with atropine and hyoscine. Further, amongst the compounds investigated there had been no dissociation of the three types of anti-acetylcholine activity (i.e. antisecretory, antispasmodic and mydriatic activity). The results of a recent comparison (Brimblecombe, Green & others, 1971b) of the enantiomers of a series of anti-acetylcholine drugs provide a rationale for the conclusions reached by Rama Sastry. It was found that a minimum dose (0·03 μmol kg^{-1}) of an anti-acetylcholine drug was necessary if maximum anti-acetylcholine effects were to be observed *in vivo* and that atropine and hyoscine were active at almost this minimum dose. Thus it is not surprising that drugs significantly more potent than atropine and hyoscine *in vivo* in the pns have not been found. Also the observation was made that, under controlled conditions, the ratio

of the potencies of the enantiomers of dimethylaminoethyl 2-cyclohexyl-2-hydroxy-2-phenylacetate was essentially constant when calculated from affinity constants determined on the guinea-pig ileum, from mydriatic potencies in mice, and from potencies for blockade of oxotremorine-induced salivation and tremor in mice. These results indicate that the receptors with which anti-acetylcholine drugs interact were essentially the same on the guinea-pig ileum, and the iris, salivary gland and the cns of mice. Since these results carry the very significant practical implication that little is to be gained from further attempts to synthesize more potent anti-acetylcholine drugs, or drugs that retain only certain specified anti-acetylcholine properties, it was considered essential to substantiate the above conclusions. In this paper the procedures for measuring antagonism of oxotremorine-induced salivation and tremor which are respectively tests for anti-acetylcholine activity in the pns and cns are re-evaluated and pairs of enantiomers are compared in an additional test for central anti-acetylcholine activity, i.e. the ability to cause changes in the eeg arousal threshold of the cat encéphale isolé preparation. Particular attention is focussed on relative potencies and comparisons of time–activity profiles in the cns and pns.

Drugs

The compounds used are listed in Table 1. Atropine sulphate, atropine methylnitrate and hyoscine hydrobromide were purchased from British Drug Houses

Table 1. *List of compounds studied.*

Compound No.	Name
1	Atropine sulphate
2	Methyl atropine (nitrate)
3	Hyoscine hydrobromide
4	*N*-Methyl piperidin-3-yl benzilate hydrochloride
5	*N*-Ethyl piperidin-3-yl benzilate hydrochloride
6	*N*-Methyl piperidin-3-yl 2-cyclopentyl-2-hydroxy-2-phenylacetate hydrochloride
7	*N*-Ethyl piperidin-3-yl 2-cyclopentyl-2-hydroxy 2-phenylacetate hydrochloride
8	(*N*-Ethylpyrollidene-2) methyl 2-cyclopentyl-2-hydroxy-2-phenylacetate hydrochloride
9	Ditran
10 (Rac)	*N*-Methyl piperidin-4-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride
10R	*R*-Enantiomer of 10 Rac
10S	*S*-Enantiomer of 10 Rac
10M (Rac)	*N*-Methyl piperidin-4-yl 2-cyclohexyl-2-hydroxy 2-phenylacetate methiodide
10MR	*R*-Enantiomer of 10M Rac
10MS	*S*-Enantiomer of 10M Rac
11 (Rac)	2′-Dimethylaminoethyl 2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride
11R	*R*-Enantiomer of 11 Rac
11S	*S*-Enantiomer of 11 Rac
11M (Rac)	2′-Dimethylaminoethyl 2-cyclohexyl-2-hydroxy-2-phenylacetate methiodide
11M R	*R*-Enantiomer of 11M Rac
11M S	*S*-Enantiomer of 11M Rac
12RS	2*S*-Dimethylaminoprop-1-yl 2*R*-2-cyclohexyl-2-hydroxy-2-phenyl-acetate hydrochloride
12M RS	Methiodide of 12RS
12RR	2*R*-Dimethylaminoprop-1-yl 2*R*-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride
12MRR	Methiodide of 12RR
13	*N*-Methylpiperidin-4-yl 1-phenylcyclopentane-carboxylate hydrochloride (G3063)
14	2-Diethylaminoethyl 1-phenyl-cyclopentyl-carboxylate hydrochloride. (Caramiphen)
15	*N*-Ethyl-*N*-propyl-aminoethyl benzilate hydrochloride (BRL 1288)
16	Pyrrolino-*N*-ethyl benzilate hydrochloride
17 (Rac)	1-Benzyl-4(2,6-dioxo-3-phenyl-3-piperidyl)-piperidine hydrochloride (Benzetimide)
17S	Dexetimide (*S*-Enantiomer of 17 Rac)
18RR	3*R*-Quinuclidin-3-yl 2*R*-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride
18M RR	Methiodide of 18 RR

Limited, Evans Medical Limited and McFarlan Smith Limited respectively. Benzetimide and dexetimide were kindly donated by Janssen Pharmaceuticals Limited. All the other compounds were synthesized in these laboratories and had spectral properties and analytical data consistent with the assigned structures.

METHODS AND RESULTS

Determination of affinity constants on guinea-pig isolated ileum and production of mydriasis in mice. Affinity constants and estimates of mydriatic activity of the compounds were determined according to Brimblecombe & others (1971b). The logarithms of the affinity constants (log K), mydriatic potency relative to atropine, and estimate of time to peak and duration of action of mydriasis are listed in Table 2.

Antagonism of oxotremorine-induced tremors and salivation in mice

(a) *Antagonism by intravenous (i.v.) injection at different time intervals*

(i) *Tremors.* The compounds were injected into mice, 18–25 g, at a selected time before injection of a dose of oxotremorine (0·49 μmol kg^{-1}, 100 μg kg^{-1}, i.v.) which was 1·5 × ED90 for production of tremors in untreated mice. Animals were examined 10 min after oxotremorine for the occurrence of tremors. The mice were placed on their backs and the hind limbs were observed for the presence or absence of tremors that occurred before whole body tremors became apparent; no attempt was made to grade their severity. Four groups, each containing five mice, were used and the ED50

Table 2. *Results from guinea-pig ileum and mouse mydriasis experiments.*

Compound No.	Antagonism of carbachol induced contractions in guinea-pig ileum log K ± s.e.	Production of mydriasis: Potency relative to atropine (95% confidence limits)	Production of mydriasis: Dose μmol kg^{-1} to produce a mean pupil diameter of 20 arbitrary units	Onset of mydriasis: Time to peak response min	Duration of mydriasis: Time (descending) to reach 50% of peak response min ± s.e.
18 (RR)	11·67 ± 0·05	3·11 (2·6 - 3·8)	0·042	80	1080 ± 34
18M (RR)	11·28 ± 0·18	3·37 (2·6 - 4·7)	0·039	80	1046 ± 48
*10M (R)	11·08 ± 0·1	2·76 (2·33–3·32)	0·047	80	1024 ± 48
*10 (R)	10·92 ± 0·1	2·36 (2·04–2·72)	0·055	80	901 ± 85
*10 (Rac)	10·52 ± 0·07	1·17 (1·02–1·36)	0·11	90	552 ± 45
*10M (Rac)	10·39 ± 0·17	2·10 (1·68–2·60)	0·062	10	780 ± 68
17 +	10·25 ± 0·07	1·65 (1·36–2·06)	0·079	80	285 ± 16
12M (RR)	10·08 ± 0·03	4·25 (3·4–5·24)	0·031	40	267 ± 28
12M (RS)	10·04 ± 0·1	3·16 (2·47–4·19)	0·041	40	645 ± 110
12 (RS)	10·00 ± 0·04	1·63 (1·35–1·99)	0·08	20	143 ± 10
17 (Rac)	9·95 ± 0·08	0·89 (0·73–1·09)	0·145	80	299 ± 13
12 (RR)	9·88 ± 0·05	2·78 (2·29–3·40)	0·047	20	102 ± 7
*11M (R)	9·66 ± 0·08	2·81 (2·34–3·49)	0·046	10	70 ± 10
2	9·50 ± 0·04	2·11 (1·76–2·60)	0·061	10	76 ± 9
3	9·49 ± 0·02	5·0 (3·90–6·40)	0·026	10	89 ± 5
6	9·41 ± 0·07	0·39 (0·35–0·42)	0·33	30	161 ± 11
9	9·40 ± 0·07	0·63 (0·50–0·83)	0·21	<5	79 ± 8
*11M (Rac)	9·36 ± 0·03	1·67 (1·29–2·1)	0·078	20	90 ± 12
7	9·24 ± 0·04	0·25 (0·22–0·27)	0·54	16	188 ± 18
8	9·20 ± 0·04	1·00 (0·82–1·2)	0·13	<5	90 ± 7
*10M (S)	9·08 ± 0·05	1·17 (0·99–1·39)	0·11	5	41 ± 2
*11 (R)	9·06 ± 0·01	0·76 (0·56–0·96)	0·17	10	73 ± 3
* 1	8·95 ± 0·04	1·00	0·13	<5	75 ± 3
13	8·75 ± 0·05	0·33 (0·31–0·35)	0·39	5	60 ± 7
*11 (Rac)	8·73 ± 0·02	0·34 (0·28–0·41)	0·38	10	58 ± 5
*10 (S)	8·48 ± 0·04	0·12 (0·10–0·14)	1·08	4	32 ± 4
4	8·48 ± 0·04	0·20 (0·16–0·24)	0·65	3	25 ± 2
5	8·24 ± 0·07	0·12 (0·1–0·14)	1·08	2	18 ± 2
*11M (S)	7·38 ± 0·02	0·074 (0·06–0·09)	1·75	4	34 ± 3
14	7·34 ± 0·02	0·02 (0·01–0·03)	6·5	3	34 + 4
16	7·32 ± 0·04	0·044 (0·037–0·055)	2·96	1	13 ± 1
*11 (S)	7·07 ± 0·01	0·006 (0·004–0·008)	21·6	<5	65 ± 6
15	6·75 ± 0·03	0·008 (0·007–0·01)	16·3	1	9 ± 1

* Results previously reported by Brimblecombe & others, 1971b.

Table 3. *Results of oxotremorine experiments.*

Compound	Time† min	Central: Antagonism of oxotremorine induced tremors in mice ED50 μmol kg⁻¹ i.v. (95% confidence limits)	Peripheral: Antagonism of oxotremorine induced salivation in mice ED50 μmol kg⁻¹ i.v. (95% confidence limits)	Peripheral: Central potency ratio‡ (95% confidence limits)
		16·2 (10·0 –26·6)*	0·44 (0·30 –0·66)*	36·8 i.p.
1	1	<22·5 >15	0·156 (0·111–0·218)	
	2·5	9·65 (6·87 –13·49)	0·145 (0·10 – 0·20)	66·7 (50·0 –100·6)
	5	12·26 (8·92 –17·07)	0·156 (0·11 – 0·218)	
	10	12·26 (8·92 –17·07)	ca. 0·225	
	20	16·88 (11·8 –24·95)	—	—
	40	21·7 (15·45 –30·36)	—	—
		1·1 (0·6 –2·5)*	0·05 (0·02 –0·08)*	22·0 i.p.
3	2·5	0·57 (0·33 – 0·98)	0·019 (0·096– 0·035)	22·7 (11·25–45·9)
	5	0·24 (0·14 – 0·43)	0·011 (0·006– 0·019)	
	10	0·28 (0·16 – 0·49)	0·019 (0·01 – 0·033)	
	20	1·13 (0·66 – 1·99)	0·048 (0·03 – 0·087)	
		2·7 (1·4 – 5·3)*	7·1 (4·1 –12·10)*	0·38 i.p.
5	1	>2		
	2	0·707 (0·41 – 1·23)	4·29 (3·04 – 6·02)	0·193 (0·12 – 0·31)
	4	0·615 (0·466– 0·82)	3·22 (1·88 – 5·59)	
	8	0·915 (0·53 – 1·66)	>4·0	
		0·56 (0·3 – 1·3)*	0·18 (0·04 – 0·31)*	3·11 i.p.
10 (R)	20	0·22 (0·127– 0·44)	0·086 (0·061– 0·12)	
	40	0·124 (0·071– 0·213)	0·065 (0·049– 0·086)	2·84 (1·57 – 5·15)
	80	0·141 (0·08 – 0·246)	0·05 (0·029– 0·085)	
	160	>0·4	0·057 (0·033– 0·098)	
		2·29 (1·47 – 3·55)*	0·31 (0·18 – 0·54)*	10·6 i.p.
10 (Rac)	22·5	—	0·161 (0·094– 0·280)	
	45	0·403 (0·23 – 0·699)	0·141 (0·081– 0·250)	2·5 (1·41 – 4·44)
	90	0·354 (0·203– 0·615)	0·160 (0·12 – 0·21)	
	180	>2·0		
		9·42 (5·14 –16·6)*	7·75 (3·8 –10·2)*	1·21 i.p.
10S	1	>4·0 <8·0	>4·0	
	2	2·83 (1·63 – 4·92)	1·61 (0·89 – 3·1)	
	4	3·22 (1·88 – 5·99)	2·83 (1·63 – 4·92)	2·02 (1·04– 3·92)
	8	4·29 (3·06 – 6·01)	1·87 (1·06 – 2·62)	
	16	>8·0	>4·0	
		5·07 (3·07 – 6·9)*	0·76 (0·44 – 1·29)*	6·7 i.p.
11 (R)	5	0·915 (0·53 – 1·66)	0·123 (0·092– 0·161)	5·85 (3·24 –10·7)
	10	0·707 (0·407– 1·23)	0·121 (0·07 – 0·21)	
	20	1·61 (0·94 – 2·8)	0·222 (0·158– 0·312)	
		15·4 (9·9 –23·9)*	1·4 (1·11– 3·33)*	11·0 i.p.
11 (Rac)	1	—	0·81 (0·44 – 1·55)	
	2·5	2·83 (1·59 – 5·03)	0·55 (0·3 – 0·95)	2·57 (1·44– 4·72)
	5	1·41 (0·81 – 2·46)	0·55 (0·3 – 0·95)	
	10	1·61 (0·94 – 2·8)	0·81 (0·47 – 1·4)	
	20	>2·0	0·81 (0·47 – 1·4)	
		>100*	>100*	—
11 (S)	1	21·21 (12·2 – 36·9)	18·63 (10·73 –32·0)	1·4 (0·86– 2·33)
	2	>30 <60	15·11 (10·31–21·44)	
	4	>30 <60	18·63 (10·73–32·00)	
	8	*ca* 60		
		70·7 (47·8 –104·6)*	123 (74·1 –162·4)*	0·58 i.p.
15	1	8·97 (7·77 – 9·91)	>20 toxic at 40 μmol kg⁻¹	<0·5
	2	>10 <20	" " " "	
	4	*ca* 20		
16		100*	*ca* 100*	>1 i.p.
		2·25 (1·58 – 3·33)*	0·66 (0·48 – 0·96)*	3·38 i.p.
17 (S)	40	0·74 (0·52 – 1·04)	0·065 (0·044–0·091)	
	80	0·45 (0·31 – 0·64)	0·047 (0·033–0·066)	9·52 (6·17–14·17)
	160	>1·0	0·067 (0·048– 0·101)	
		2·64 (1·13 – 3·62)*	1·01 (0·66 – 1·49)*	2·60 i.p.
17 (Rac)	20	3·25 (2·31 – 4·54)	0·11 (0·081 – 0·156)	33·3 (19·2–46·5)
	40	2·33 (1·67 – 3·27)	0·076 (0·053–0·107)	
	80	2·59 (1·73 – 4·23)	0·089 (0·061– 0·133)	
	160	>7·57		

* ED50 values obtained when oxotremorine was given i.v., 15 min after i.p. injection of anti-acetylcholine compound. (Brimblecombe & others, 1971b).

† Time at which oxotremorine was given i.v., after i.v. injection of anti-acetylcholine compound.

‡ Obtained by dividing peak ED50 for blockade of tremors by peak ED50 for blockade of salivation. For comparison potency ratios by i.p. procedure are also listed.

values for prevention of tremors were calculated by probit analysis (Finney, 1947). The ED50 values obtained for the anti-acetylcholine compounds injected at various times before the injection of oxotremorine are listed in Table 3. The lowest ED50 values were obtained when the anti-acetylcholine drug was administered at the optimal time before oxotremorine. This time was considered to be the time to peak effect.

(ii) *Salivation.* A procedure similar to that described in section (a) (i) above was used except that 0·24 μmol kg^{-1} (i.v.) oxotremorine (48 μg kg^{-1}, 1·5 $\times$ ED90 for production of salivation in untreated mice) was used. (Reasons for the choice of dose levels of oxotremorine are given in the Discussion.) Any dampness of the lips was taken to indicate presence of salivation. ED50 values for blockade of salivation were calculated and listed in Table 3.

(b) *Antagonism by intraperitoneal (i.p.) injection at a fixed time interval*

Tremors and salivation. The methods were those of Brimblecombe, Green & others (1971a). The compounds were injected (i.p.) 15 min before injection of 0·49 μmol kg^{-1} (100 μg kg^{-1}) oxotremorine (i.v.). Mice were examined 10 min after the oxotremorine injection for the presence of salivation or tremors and ED50 values for blockade of salivation and tremors were calculated. These are listed in Table 3 together with the values obtained by the intravenous test procedure in order to facilitate comparison of results obtained by the different methods.

Elevation of eeg arousal threshold in cat encéphale isolé *preparations.* Cats, 1·8–2·5 kg, were prepared under halothane anaesthesia using the operative procedures of Bradley & Key (1958). The dose of anti-acetylcholine compound required to raise the eeg arousal threshold by 100% (eeg arousal being produced by electrical stimulation of the mesencephalic reticular formation) was determined as described by Brimblecombe & others (1971a).

Three cat preparations were used for each compound studied. The compounds were administered by the femoral vein in incremental doses every 20 min. Eeg arousal thresholds were determined 20 min after each dose of compound. Curves were plotted relating dose of compound to the threshold for eeg arousal, and from these it was possible to read off the dose required to raise the eeg arousal threshold by 100%. These doses (mean of three determinations) are listed in Table 4.

Table 4. *Results of cat encéphale isolé experiments.*

Compound	Elevation of eeg arousal threshold by 100% in cat encéphale isolé μmol kg^{-1}
10 (*R*)	0·11
10 (Rac)	0·20
10 (*S*)	4·4
11 (*R*)	0·55
11 (Rac)	1·04
11 (*S*)	64·4

DISCUSSION

The results in Table 2 show that the time to onset of peak mydriatic effects and the duration of these effects are related to the affinity constants of the anti-acetylcholine drugs. The duration of mydriatic effects clearly increases as the affinity constants

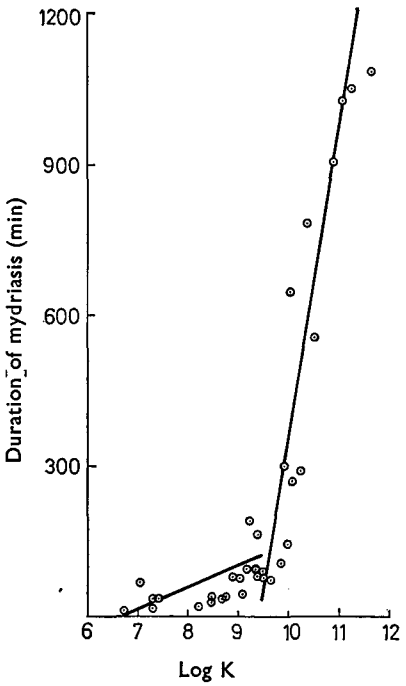

FIG. 1. Graph showing relation between Log K and duration of mydriasis for 33 anti-acetylcholine compounds.

increase. The relation between log K and duration of mydriasis is illustrated in Fig. 1. There is a highly significant ($P < 0{\cdot}001$) linear regression of duration of mydriasis on log K for compounds with log K $\geqslant 9{\cdot}49$ (regression coefficient, b = 548 $\pm$ standard error (s.e.) 61) and there was also a significant linear regression for compounds with log K $< 9{\cdot}49$ (b = 33 $\pm$ s.e. 10·5). It is not known whether the differences in the linear regressions above and below log K = 9·49 indicate differences in mechanisms. It may be relevant however that all drugs with log K $\geqslant 9{\cdot}49$ are approximately equiactive (at *ca* 0·03 μmol kg^{-1}) *in vivo* whereas for drugs with log K $< 9{\cdot}49$ drug potency tends to increase as log K increases (see later for further discussion).

No statistical analysis of the relation between log K and time to onset of effects was possible since no precise measurements of times to onset were made. Nevertheless, the results listed in Table 2 show the clear trend that times to onset of mydriatic effects increase as affinity constants increase.*

It was clear from the results of the mydriasis experiments that if similar time–activity profiles occurred in the tests for antagonism of oxotremorine-induced

* The relation between duration of action and affinity constants can be demonstrated only if great care is taken to ensure equilibrium conditions during measurements of anti-acetylcholine activity using the guinea-pig ileum. For example, the published pA_{10} values of benzetimide and dexetimide (Janssen, Niemegeers & others, 1971) were 7·56 and 8·10 respectively. [These values are equivalent to log K values of 8·51 and 9·05 (cf. Barlow, 1964)]. If the relation between duration of mydriasis and affinity constants shown by anti-acetylcholine esters is followed by these relatively long acting drugs (chemically unrelated to anti-acetylcholine esters) the published mydriasis data is consistent with affinity constants of approximately log K = 10 and 10·3 respectively and not to the lower reported data which relate to short acting drugs. We have derived log K value of 9·95 and 10·25 for benzetimide and dexetimide, thereby providing some evidence that the relation between affinity constants and duration of action is independent of chemical structure.

tremors and salivation, the results obtained by Brimblecombe & others (1971b) from experiments in which oxotremorine was administered 15 min after the anti-acetylcholine drug (i.p.) were suspect particularly for those drugs with very short or very long times to onset and durations of effects. Consequently the previous salivation and tremor procedures have been modified so that some estimate of the time–activity profile of this antagonism could be obtained. Although insufficient measurements were made by the modified procedures to allow meaningful estimates of the duration of effects, the time required for the onset of peak activity was measured and from a comparison of the data in Tables 2 and 3 it is clear that drugs with low affinity constants act more rapidly than drugs with high affinity constants in antagonizing oxotremorine-induced tremors and salivation as well as in producing mydriasis.

The results in Table 3 also show that for any drug the ED50 values for antagonism of oxotremorine-induced tremors and salivation, which were obtained by the modified procedure where the anti-acetylcholine drug was administered i.v., were much lower than when the previous fixed time (i.p.) procedure was used. However, there were only small random variations between procedures in the ratio of the activity of enantiomers to that of their racemate (Table 5) and in Table 6 it is shown that the ratio of the potencies of the enantiomers of *N*-methyl piperid-4-yl-and 2′-dimethylaminoethyl 2-cyclohexyl-2-hydroxy-2-phenylacetate (compounds 10 and 11 respectively) remained similar in the two procedures. Thus the previous conclusions about receptor similarity were justified (Brimblecombe & others, 1971b).

To obtain evidence that the receptors were similar in the cns of cats to those in the cns and pns of mice and the pns of guinea-pigs, it was decided to compare doses of enantiomers of anti-acetylcholine drugs which elevated the eeg arousal threshold in

Table 5. *Ratio of activities of R and S enantiomers compared with racemate.*

		Peripheral		Central	
Compound	Log K	Production of mydriasis	Antagonism of salivation	Antagonism of tremors	Elevation of eeg arousal threshold
10 (R)	2·45	2·01	2·86 (1·72*)	2·5 (4·09)	1·82
10 (S)	0·009	0·10	0·088 (0·04)	0·11 (0·24)	0·046
11 (R)	2·14	2·23	4·57 (1·84)	2·0 (3·04)	1·89
11 (S)	0·022	0·018	0·03(<0·01)	0·07 (<0·15)	0·016
17 (S)	2·00	1·85	1·67 (1·53)	5·2 (1·17)	—

* Figures in brackets denote results obtained by i.p. injection.

Table 6. *Enantiomeric potency ratios.*

		Peripheral		Central	
Compound	Log K	Production of mydriasis	Antagonism of salivation	Antagonism of tremors	Elevation of eeg arousal threshold
10 (R):10 (S)	272	20	32·3 (43)*	22·7 (17)	40
11 (R):11 (S)	100	123	125 (>100)	30·0 (>22)	117

* Figures in brackets denote results obtained by i.p. injection.

cat encéphale isolé preparations (Brimblecombe & Green, 1968). The results are given in Table 4. The potency ratio of compounds 10R:10S proved similar when measured by the elevation of eeg arousal threshold in the cat experiments, to that measured by the other three *in vivo* procedures listed in Table 6. Further, the potency ratio 11R:11S of compounds so obtained was similar to that from log K, salivation and mydriasis measurements (Table 6). The low 11R:11S ratio in the tremor test is probably unreliable because compound 11S had to be given in a dose which was close to the intravenous LD50 for that drug [80 μmol kg^{-1} (95% confidence limits, 35–111 μmol kg^{-1})]. The above results therefore provide evidence that the receptors with which anti-acetylcholine drugs interact are essentially the same in the cat cns, mouse cns, iris and salivary gland and the guinea-pig ileum and are consistent with the results of recent *in vitro* studies of Barlow, Franks & Pearson (1972). The claim (Biggs, Casy & Jeffery, 1972) that the muscarinic receptors in the guinea-pig ileum differ from those in the circular muscles of the eye is almost certainly in error because no attempts were made to measure mydriasis effects with time and the first observations were made (at 30 min) long after the peak of activity for the drugs examined.

In a previous paper (Brimblecombe & others, 1971b), the observation that the enantiomeric potency ratios *in vivo* of anti-acetylcholine drugs decreased as *in vitro* potency increased, was interpreted as indicating the anti-acetylcholine drugs only elicit maximal effects if administered above a certain minimal dose. This interpretation explained adequately why in certain instances enantiomeric potency ratios were smaller *in vivo*, for example in the tests for measuring antagonism of oxotremorine-induced tremors and salivation, than *in vitro*, but neglect of time–activity profiles in some of the *in vivo* tests could have been responsible for the reduced enantiomeric potency ratios observed. The results now reported—that the enantiomeric potency ratios were not seriously in error because of this omission (see Table 6), strengthens the argument that maximal anti-acetylcholine effects cannot be observed unless the anti-acetylcholine drug is administered above a minimal dose. The results of the mydriasis experiments in Table 2 also substantiate this finding. There is a highly significant ($P < 0{\cdot}001$) linear regression of log (dose to produce 20 arbitrary mydriatic units) on log K for compounds with log K $< 9{\cdot}49$ ($b = -0{\cdot}76 \pm$ s.e. 0·08); there was no significant regression or correlation between the two variables for compounds with log K $\geqslant 9{\cdot}49$. The relation between log (dose to produce mydriasis) and log K is illustrated in Fig. 2. From Fig. 2 it can be seen that below a dose of 0·026 μmol kg^{-1} no anti-acetylcholine compound produces maximal mydriatic effects and that all anti-acetylcholine compounds having affinity constants log K $\geqslant 9{\cdot}49$ are active at approximately this dose.

If it is accepted that the receptors for anti-acetylcholine drugs are similar both in the cns and pns and in different species it is clear that dissociation of anti-acetylcholine effects can only be expected if there are gross differences in the distribution of anti-acetylcholine drugs. Thus no large differences are to be expected, nor are they observed for the various peripheral activities of any one anti-acetylcholine drug (Rama Sastry, 1970). The ability or otherwise of drugs to penetrate the blood–brain barrier (Brodie, Kurz & Schanker, 1960) can account for the fact that quaternary and highly lipophilic tertiary anti-acetylcholine drugs are much less potent in the cns than in the pns. However, there is no obvious mechanism whereby anti-acetylcholine drugs can preponderate in the cns compared with the pns and there is also no evidence

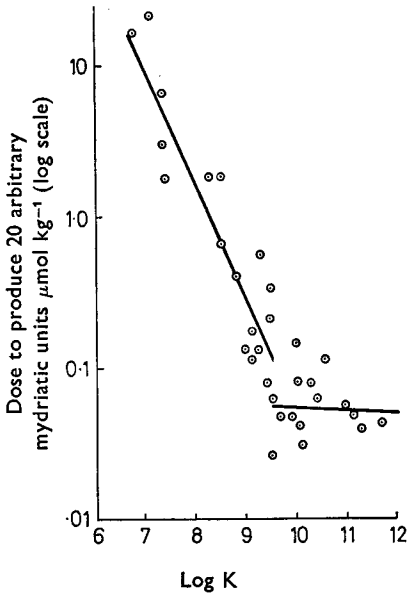

FIG. 2. Graph showing relation between Log K and log dose required to produce mydriasis for 33 anti-acetylcholine compounds.

from distributional studies with radiochemically-labelled drugs (Upshall, personal communication; Karlen & Jenden, 1970) for such a preponderance. Nevertheless there have been apparent claims that compounds which are more potent in the cns than pns have been prepared (Parkes, 1965; Lindqvist, Lingdren & others, 1970; Brown, Hughes & Mehta, 1969; Herz, Teschemacher & others, 1965). We believe that such claims have been based on misleading experimental evidence.

Methods for comparing the potency of anti-acetylcholine drugs in the cns and pns should ideally be measures of the efficiency of these drugs in antagonizing the effects of endogenous acetylcholine. However, although measurements of mydriasis are convenient for estimating anti-acetylcholine activity in the pns, there is no correspondingly simple test for central anti-acetylcholine activity, since positive central anti-acetylcholine effects such as behavioural disturbances are particularly difficult to quantify. Thus it is a logical choice to seek to compare anti-acetylcholine drugs on the basis of their ability to antagonize the effects of exogenous acetylcholine-like drugs in the cns and pns. Unfortunately the choice of exogenous acetylcholine-like drugs is limited because only tertiary muscarinic drugs such as pilocarpine, arecoline and oxotremorine can penetrate into the cns, and of these only oxotremorine (Cho, Haslett & Jenden, 1962) fulfils all the requirements necessary for estimating anti-acetylcholine activity in the cns and pns. Pilocarpine suffers from the disadvantage of being a partial agonist with only weak cns activity (Barlow, 1964) and arecoline from the disadvantage that relatively high doses are required to produce central effects which are of very short duration (Zejmal & Votava, 1961). Thus we have chosen to determine pns : cns ratos for anti-acetylcholine drugs by measuring the

antagonism of salivation and tremors* induced by equipotent doses of oxotremorine in the two tests. (Equipotent doses rather than equimolar doses of oxotremorine were used because it was assumed that anti-acetylcholine drugs competitively antagonize the effects of oxotremorine in the cns and pns.)

Using this procedure a pns:cns ratio of 66 (Table 3) was obtained for atropine. This value is close to that which may be calculated from results of experiments with atropine in man. In man the dose of atropine required to produce peripheral effects is 0·25–2 mg, whereas to produce central effects which relieve symptoms of Parkinson's disease, 54 mg day^{-1} has been given (Martindale, 1968); further, single doses of 30–50 mg of atropine are required to produce central (psychotomimetic) effects in man (Ostfeld & Aruguette, 1962). From these figures the pns:cns ratio in man of atropine appears to be *ca* 50.

In contrast much lower pns:cns ratios for atropine were found (Parkes, 1965; Lindqvist & others, 1970; Brown & others, 1969; Herz & others, 1965; Leslie & Conway, 1970) from experiments in which antagonism of peripheral endogenous acetylcholine was compared with antagonism of central exogenous acetylcholine-like compounds or in which cns potency was assessed by other methods (Parkes, 1965) and in which time–activity profiles were not determined. It is not surprising therefore that results from procedures that apparently give erroneously high cns:pns potency ratios for atropine have also indicated that some anti-acetylcholine drugs are more potent in the cns than pns. The results in Table 3 show that no drugs, including those which have been claimed to be more potent in the cns than pns [compound 15, benapyrzine (Brown & others, 1969), compound 16 (Parkes, 1965)] are appreciably more potent in the cns than pns.

The limitations of published experimental procedures for comparing cns and pns potencies are clear and to our knowledge there have been no reported examples where unequivocal evidence has been provided to show that anti-acetylcholine drugs may be appreciably more active in the cns than pns. On the basis of the results presented in this paper it is unlikely that any such drugs will be prepared.

The results in Table 3 permit a number of observations about the rate of action of anti-acetylcholine drugs and about the effect of distributional factors on observed pharmacological effects. For any of the anti-acetylcholine drugs examined, the time to peak effect (when the drug is administered at the ED50) is essentially the same for antagonism of oxotremorine-induced salivation (a pns effect) as it is for tremors (a cns effect). This relation holds for relatively lipophobic drugs† such as atropine and

* Instead of measuring antagonism of oxotremorine-induced tremors, antagonism of oxotremorine-induced hypothermia or analgesia (Leslie, 1969) could have been studied and indeed the latter procedure has some practical advantages. It should be noted that various sympathetic blocking drugs (phenoxybenzamine, propranolol and reserpine) are effective in inhibiting oxotremorine-induced tremor in rats (Cox & Potkonjak, 1970) and that it is essential to show that a drug is a competitive antagonist of acetylcholine (e.g. by measuring affinity constants) before anti-oxotremorine tests can be used as measures of anti-acetylcholine activity.

† At pH 7·4 all the drugs used in this work with the exception of atropine and hyoscine, partitioned >90% into n-butanol from aqueous buffer. Correlations between central anti-acetylcholine activity and partition coefficients have been obtained (Herz & others, 1965) but are not necessarily valid. Even if it is justified to use aqueous buffer–organic solvent systems as models for the *in vivo* situation, the partition coefficients obtained by such procedures are subject to thermodynamic control whereas *in vivo*, kinetic factors will assume greater importance. Thus the rate of penetration from the pns to cns may possibly be correlated more realistically with the partition coefficient of the unionized drug than with the partition constant at any set pH. Unfortunately, physical chemical procedures have not yet been adapted to permit accurate measurement of the former parameter for relatively insoluble, alkali labile, esters of aminoalcohols. For these reasons no partition coefficients have been listed in this paper.

hyoscine as well as for highly lipophilic compounds† with pns:cns ratios that are close to unity. It is also the case for enantiomers such as 10R and 10S which must have similar partition properties and have similar pns:cns ratios but quite different time–activity profiles (10R requires 40–80 min to give maximal effects whereas 10S produces maximal effects in 2–8 min).

At first sight it might appear conflicting that a slow-acting drug with a pns:cns ratio close to unity should have a similar time–activity profile in the cns as it has in the pns and that a fast acting drug with a high pns:cns potency ratio should also have a similar time—activity profile in the cns as it has in the pns. The latter situation is apparently consistent with a situation where at the high dose necessary for maximal cns effects, metabolic and general non-specific absorption procedures are so fast that they prevent the pns acting as a reservoir from which slow diffusion of a drug such as atropine can occur. (In a situation where metabolism and other general absorption factors are absent or much slower than the rate of diffusion into the cns, it might be expected that any anti-acetylcholine drug would be equipotent in the cns and pns but that the rate at which cns effects were produced would be related to the partition properties of the drug. The observed ED50 values (Table 3) in the cns did not decrease with time until the pns ED50 value was reached and did not exhibit a prolonged value as might be expected if metabolic and absorption factors were slow).

In contrast, the results for slow-acting drugs such as 10R may be considered as evidence that metabolic and general absorption factors are slow and have little to do with the time–activity profile of anti-acetylcholine drugs either in the pns or cns. Direct evidence that metabolic factors have little to do with the variations in time–activity profiles or enantiomeric potency ratios of enantiomers has been provided by Upshall (personal communication) who has shown that for a series of anti-acetylcholine esters the metabolic rates of enantiomers vary only slightly although in some species there are quantitative differences in the products formed. Also Janssen and his co-workers (1971) have shown with benzetimide that potency differences bear no relation to metabolic differences.

To explain these results it is convenient first to summarise those time–activity data that are available from isolated tissue studies and *in vivo* experiments on the pns which are not complicated by the need to consider rates of diffusion through the blood–brain barrier. It has been shown previously (Thron & Waud, 1968; Brimblecombe & others, 1971b) that drugs with high affinity constants take longer to reach equilibrium with isolated tissue such as guinea-pig ileum than drugs with lower affinity constants, even where the drugs were compared in equal concentrations. Since in addition, *in vivo* rates of onset of peripheral anti-acetylcholine activity correlated with affinity constants and did not seem to vary with test procedure (mydriasis, Table 2; salivation, Table 3), it is reasonable to conclude that differences in rates of onset of effects depend primarily on events that occur in the immediate vicinity of the receptor. If additionally, the essential distribution of anti-acetylcholine drugs leading to anti-acetylcholine effects in both the pns and cns occurs very rapidly and is in competition with other rapid processes (non-specific absorption and to a lesser extent metabolism) which remove the anti-acetylcholine drugs from general circulation, there is no inconsistency in the results that all anti-acetylcholine drugs have similar time–activity profiles in the cns and pns irrespective of pns: cns potency ratios or whether the drug is fast-acting with a short duration or slow-acting with a long duration. The *in vivo* results appear to support

† See p. 368.

the *in vitro* studies of Thron & Waud (1968) who have suggested an attractive mechanism to account for the fact that times to onset of effects increase as affinity constants increase. In the context of the present discussion, the finding that times to onset of anti-acetylcholine effects are similar in the pns and cns and are related to the affinity constants of the drugs, provides additional evidence to support the conclusion that the anti-acetylcholine receptors in the cns and pns are similar.

Two further important points follow from the above discussion. The rapid non-specific removal of anti-acetylcholine drugs is probably one of the major reasons why maximum anti-acetylcholine effects are observed only above a certain minimum dose. This conclusion, coupled with the fact that times to onset and duration of action of anti-acetylcholine drugs are little affected by rates of distribution calls into question the relevance of most reported (Werner & Schmidt, 1968; Albanus, Hammarstrom & others, 1968; Kalser, Wills & others, 1958) distribution and metabolism studies of atropine and other anti-acetylcholine drugs. In many of these experiments measurements of drug distribution were made using unrealistically high doses of anti-acetylcholine drugs and at times completely unrelated to the time–activity profile of anti-acetylcholine effects.

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