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Research Papers

Absolute configuration and parasympathetic action: pharmacodynamics of enantiomorphic and diastereoisomeric esters of β-methylcholine

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The parasympathomimetic and atropine-like actions of enantiomorphic esters of β-methylcholine and various esters of choline or β-methylcholine containing an asymmetric centre in the acid moiety have been studied. The potent parasympathomimetic drugs have a configuration identical with that of muscarine, suggesting a close affinity with the receptor. There is a gradual change from agonist to antagonist with increasing molecular size of the acid moiety, antagonism beginning with the butyric ester. Optimal affinity as a mimetic in the lower homologues is obtained with the acetic ester. Maximum affinity as an antagonist is obtained with the ester of the bulky hexahydrobenzilic acid. The configuration of the choline part is irrelevant for high atropine-like potency in the compounds derived from β-methylcholine. Clear differences in affinity are found, however, between stereoisomers of potent antagonists containing an asymmetric centre in the acid moiety, provided the asymmetric carbon atom does not bear isosteric groups. It is concluded that the atropine-like agents, although being competitive antagonists of the parasympathomimetics at best cover the agonistic receptor area only partially.

THE optical isomers of muscarine and acetyl-β-methylcholine differ widely in potency (Major & Bonnet, 1935; Glick, 1938; Waser, 1961), and the configurations of the active isomers of both substances are identical at the asymmetric centre (Ellenbroek & van Rossum, 1960; Beckett, Harper, Clitherow & Lesser, 1961). Slight alterations in the structure of potent parasympathomimetic drugs lead to a loss in affinity (van Rossum & Ariëns, 1959; Waser, 1961). These results indicate that potent parasympathomimetic agents are highly complementary to their receptors.

For atropine-like drugs there is similarly a great difference in potency between optical isomers (Long, Luduena, Tullar & Lands, 1956); for instance, natural (−)-hyoscyamine is 50–100 times more potent than its (+)-isomer (Maffii, 1960). As the atropine-like drugs are always larger molecules than the parasympathomimetic drugs, it seems likely that the receptors for both are not identical (Ariëns & Simonis, 1960). It is therefore important to investigate whether the same configurational requirements hold for both parasympathomimetic and atropine-like drugs. To this end a series of esters of choline and β-methylcholine have been synthesised, several of which, notably those with atropine-like action, contain an asymmetric centre in the acid moiety. Data about their biological activity indicate that parasympathomimetics and their antagonists interact only partially with common receptors.

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Experimental

METHODS AND MATERIALS

The biological activity of parasympathomimetic compounds was assessed on the isolated rat jejunum by making cumulative dose-response curves (van Rossum, 1963). A typical record is given in Fig. 1. A

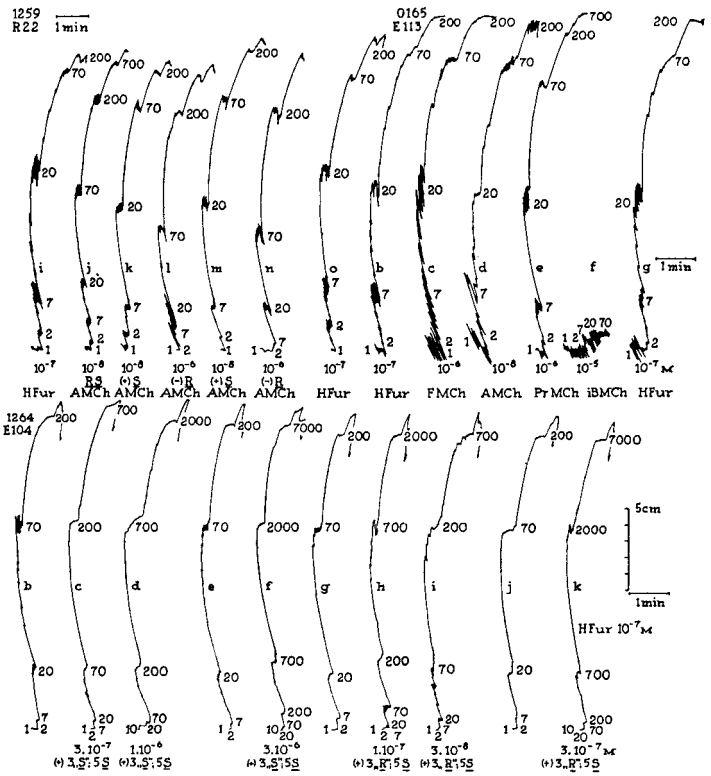


FIG. 1. Records of cumulative dose-response curves on the isolated rat jejunum. Experiment 1259 R 22: curves of the optical isomers [(+)*S* and (−)*R*] of acetyl-β-methylcholine (AMCh) and the racemic mixture (*RS*) compared with curves of the reference compound furtrethonium (HFur). Experiment 0165 E 113: curves of an homologous series of esters of racemic β-methylcholine. The esters of formic acid (FMCh), acetic acid (AMCh) and propionic acid (PrMCh) are full agonists whereas the ester of isobutyric acid (iBMCh) is practically inactive. Experiment 1264 E 104: dose-response curves of furtrethonium in the presence or absence of diastereoisomeric esters of hexahydrobenzilic acid. These diastereoisomers antagonize the action of furtrethonium but differ in potency. (The notation used is explained in the text.)

Tyrode solution of the following composition (g/litre distilled water) was used as bathing fluid: NaCl 8·0; KCl 0·2; $CaCl_2$ 0·2; $MgCl_2$ 0·1; $NaHCO_3$ 1; NaH_2PO_4 0·05; dextrose 1.

The potency of the parasympathomimetic agents has been expressed in terms of intrinsic activity and affinity (van Rossum, 1963; Ariëns, 1964). The intrinsic activity is the ratio of the maximum effect obtained with a

drug under study and the maximum effect of the reference compound furtrethonium. The pD_2-value was used as a measure of the affinity; this is the negative logarithm of the molar concentration that causes 50% of the maximum effect of the compound in question, and can be calculated from the dose-response curves of the parasympathomimetic compounds (see Fig. 2).

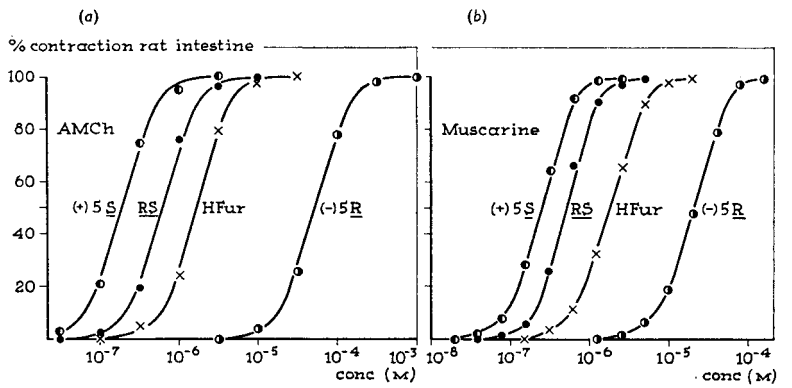


FIG. 2. Dose-response curves of optical isomers and racemic mixtures. (*a*) Curves of acetyl-β-methylcholine (AMCh) and the reference compound furtrethonium (HFur). (*b*) Curves of natural muscarine (5*S*) and the optical isomer (5*R*) compared with the reference compound furtrethonium. Note a large difference in the potency of the optical isomers as parasympathomimetic agents. The configuration of the potent isomers in position 5 is identical (see Table 5).

The atropine-like drugs which are antagonists of the mimetic drugs have an intrinsic activity equal to zero. They were therefore studied in combination with an agonist (see Fig. 1). The affinities of the antagonists are expressed as pA_2-values, this parameter being the negative logarithm of that molar concentration causing a shift by a factor of 2 in the dose-response curve of an agonist (see Figs. 4 and 5). A difference in the pD_2- or pA_2-value of 0·3 is significant at the 5% level.

The various isomers and racemic substances were synthesised in the department of organic chemistry (Ellenbroek, 1964). The notation for absolute configuration (*R* and *S*) is used according to the proposals of Cahn, Ingold & Prelog (1956).

Results

OPTICAL ISOMERS OF PARASYMPATHOMIMETIC AGENTS

(±)-Acetyl-β-methylcholine (methacholine, Mecholin, AMCh) is slightly less potent than acetylcholine as a parasympathomimetic drug in the isolated rat jejunum. Both drugs are agonists having intrinsic activities equal to that of the reference drug furtrethonium (HFur). The (+)-isomer (+)*S*-AMCh is about twice as potent as the racemate, but its potency exceeds that of the (−)-isomer (−)*R*-AMCh by about a factor of 300. Dose-response curves for these compounds calculated from similar experiments to those recorded in Fig. 1 are given in Fig. 2a. The curves

are identical in shape, but different in their position on the abscissa. This indicates that the differences in potency are due only to differences in affinity, while the intrinsic activities of the compounds are equal.

Similar results are obtained in an analogous manner with the optical isomers of natural muscarine and its racemic form (Fig. 2b). The average pD_2-values and intrinsic activities calculated from the dose-response curves are in Table 1. Here the affinity ratio S/R is about 320 in both cases.

TABLE 1. INTRINSIC ACTIVITIES (i.a.) AND AFFINITIES (pD_2 OR pA_2) OF ENANTIOMORPHS OF MUSCARINE AND ACETYL-β-METHYLCHOLINE AND SOME REFERENCE COMPOUNDS

Drug	Configuration*	i.a.	pD_2 (pA_2)	Relative affinity (acetylcholine = 100)	Affinity ratios
(+)-Muscarine	2*S*; 3*R*; 5*S*	1	7·1	160	5*S*/5*R* = 320
(−)-Muscarine	2*R*; 3*S*; 5*R*	1	4·6	0·5	
(+)-Acetyl-β-methylcholine	5*S*	1	6·9	80	5*S*/5*R* = 320
(−)-Acetyl-β-methylcholine	5*R*	1	4·4	0·25	
Acetylcholine	—	1	7·0	100	
Furtrethonium	—	1	5·9	8	
Atropine	racemate	0	(8·8)	6,400	
(−)-Hyoscyamine	3*S*	0	(9·1)	12,500	

* The numbering of the atoms has been explained in Table 5.

However, if only 0·3% of the *S*-compound were present as an impurity of the *R*-compound, the effect could be attributed to the impurity. Thus it is uncertain if the compounds with the *R*-configuration have any agonistic activity. Those with the *S*-configuration approach the potency of acetylcholine, suggesting that the β-methyl group in the *R*-configuration is an interfering factor in receptor occupation.

FROM PARASYMPATHOMIMETIC AGENTS TO ATROPINE-LIKE DRUGS

Slight alterations in the molecular structure of potent mimetic drugs cause a decrease in both intrinsic activity and affinity. Introduction of substituents with increasing molecular weight often results in a transformation of agonists into competitive antagonists, sometimes via intermediates or partial agonists (van Rossum & Ariëns, 1959; Ariëns, 1964).

A similar study was made beginning with (±)-acetyl-β-methylcholine. Dose-response curves of various esters from formic to butyric are given in Fig. 3*a*. It can be seen that the formic ester (FMCh), the acetic ester (AMCh) and the propionic ester (PrMCh) are pure agonists having the same intrinsic activity as the reference drug. The cyclopropanecarboxylic ester (CpMCh), which has a partially agonistic character, is noteworthy. There is an affinity optimum in acetyl-β-methylcholine in this series of agonists (see Table 2). This result agrees with that of the analogous series of choline esters.

The higher homologues are inactive. All these compounds, however, behave as antagonists of furtrethonium. They are atropine-like drugs, as can be seen for the isobutyric ester of β-methylcholine (iBMCh) in Fig. 3*b*. The dose-response curve of the standard of comparison is

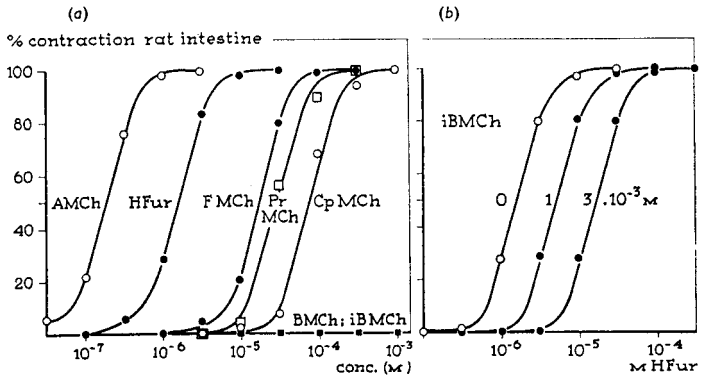
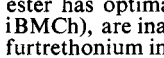
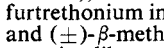
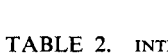


FIG. 3. (*a*) Dose-response curves of an homologous series of esters of aliphatic carboxylic acids and racemic β-methylcholine. The lower homologues FMCh, AMCh, PrMCh and in this particular experiment, the ester of cyclopropane carboxylic acid (CpMCh) are full agonists although they differ greatly in potency. The acetic ester has optimal affinity. The higher homologues, the butyric esters (BMCh and iBMCh), are inactive as parasympathomimetic agents. (*b*) Dose-response curves of furtrethonium in the presence of constant concentrations of the ester of isobutyric acid and (±)-β-methylcholine which shows that the inactive homologue of (*a*) acts as an atropine-like agent although having low affinity.

TABLE 2. INTRINSIC ACTIVITIES (i.a.) AND AFFINITIES (pD_2 OR pA_2) OF ESTERS OF (±)-β-METHYLCHOLINE AND VARIOUS CARBOXYLIC ACIDS

R in R—C(=O)—O—C(—C)—C—$\overset{+}{N}Me_3$	i.a.	pD_2	pA_2	Relative affinity (acetylcholine = 100)
H—	1	4·9	—	0·8
C—	1	6·7	—	50
C—C—	1	4·8	—	0·6
	0·6*	4·2	—	0·16
C—C—C—	0·1	—	3·8	0·06
	0	—	3·8	0·06
	0	—	4·6	0·4
	0	—	7·4	400
	0	—	8·3	2,000
	0	—	8·5	2,500

* Mean of values between 1 and 0·3.

shifted to higher concentrations by isobutyryl-β-methylcholine. Similar families of curves were obtained with the other antagonists. In Table 2 average pD_2- and pA_2-values, obtained from dose-response curves of various esters of β-methylcholine are given. There is a sharp rise in affinity of the antagonists if the acid moiety contains planar rings and polar groups.

ATROPINE-LIKE ESTERS FROM SYMMETRICAL BULKY ACIDS AND *R*- OR *S*-β-METHYLCHOLINE

Esters were synthesised from the optical isomers of β-methylcholine with diphenylacetic acid as well as with benzilic acid. In Fig. 4 various dose-response curves of furtrethonium in the presence of the two isomers of the benzilic esters are given. The average pA_2-values and relative affinities, calculated from these curves, are presented in Table 3.

In clear contrast to the results mentioned for the agonistic parent compound ($S/R = 320$), the difference in affinity of the enantiomorphs now appears to be small in both cases, the *R*-compounds having slightly greater affinities.

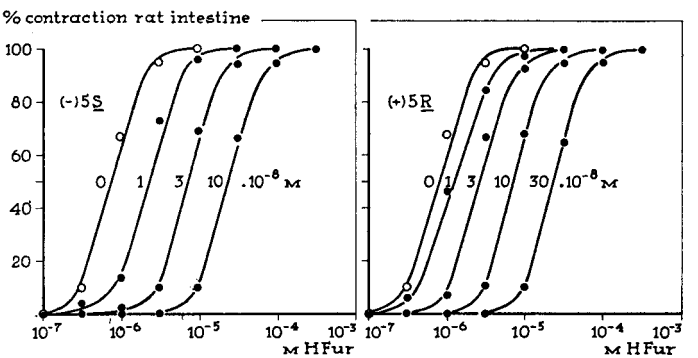


FIG. 4. Dose-response curves of furtrethonium in the presence of optical isomers of the esters of benzilic acid and β-methylcholine. Both esters are atropine-like drugs since they cause a shift in the dose-response curves and have affinities of similar magnitude.

TABLE 3. INTRINSIC ACTIVITIES (i.a.) AND AFFINITIES (pA_2) OF VARIOUS STEREOISOMERIC ESTERS

Configuration	[α]D in methanol	i.a.	pA_2	Relative affinity (atropine = 100)	Affinity ratios
Enantiomorphic esters of diphenylacetic acid and β-methylcholine					
5S	−12·4	0	5·0	0·016	*5S*/*5R* = 0·2
5R	+12·5	0	5·7	0·08	
Enantiomorphic esters of benzilic acid and β-methylcholine					
5S	+48·3	0	8·0	16	*5S*/*5R* = 0·8
5R	−48·1	0	8·1	20	
Diastereoisomeric esters of *O*-acetylmandelic acid and β-methylcholine					
Acid moiety / Alcohol moiety					
3R / *5R*	−93·9	0	3·7	0·0008	*3R*: *5S*/*3R*: *5R* = 2
3R / *5S*	−38·5	0	4·0	0·0016	*3S*: *5S*/*3S*: *5R* = 3
3S / *5R*	+39·9	0	3·7	0·0008	*3S*: *5R*/*3R*: *5R* = 1
3S / *5S*	+90·2	0	4·2	0·0025	*3S*: *5S*/*3R*: *5S* = 1·6
Enantiomorphic esters of phenyl-2-thienylglycollic acid* and choline					
(−)	−8·9	0	8·6	60	(−)/(+) = 5
(+)	+7·2	0	7·9	12·5	

* Configuration not known.

ATROPINE-LIKE ESTERS OF OPTICALLY ACTIVE BULKY ACIDS AND *R*- OR *S*-β-METHYLCHOLINE

A further step in the analysis of the stereochemical requirements for potent atropine-like action was the investigation of esters having an asymmetric centre in the acid moiety. For this purpose the four esters of *R*- and *S*-β-methylcholine and (+)- and (−)-α-methyltropic acid were prepared (Ellenbroek, 1964). The absolute configuration of the α-methyltropic acids is not known with certainty, but in view of the known configurations of the tropic acids (Fodor & Csepreghy, 1961) it seems likely that the (−)-isomer is the *S*-compound, the (+)-isomer having the *R*-configuration. In Fig. 5, dose-response curves of the four diastereoisomeric esters are presented.

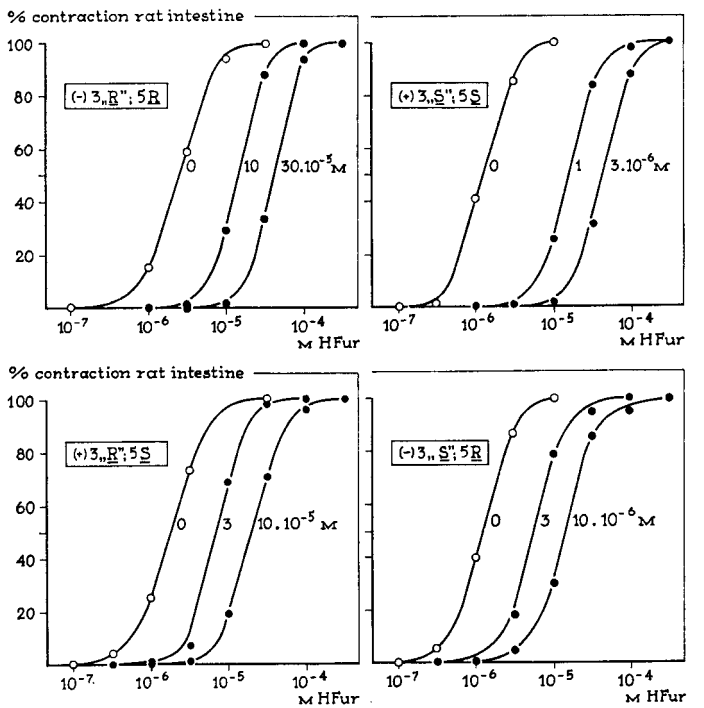


FIG. 5. Dose-response curves of furtrethonium in the presence of constant concentrations of the 4 possible isomeric esters of α-methyltropic acid and β-methylcholine. The optical isomers differ in potency. The configuration in the acid part (position 3) is of much greater importance than that in the choline moiety (position 5).

Both esters derived from the (−)-α-methyltropic acid are seen from the Figures to have much larger affinities than those from the isomeric acid. In Table 4, average pA_2-values and relative affinities are given. Diastereoisomeric esters, which are derived from the same acid isomer and differ only in the configuration of their β-methylcholine component exhibit only

a small difference in affinity. Their affinity ratios are about 1, as was found also with the β-methylcholine esters of diphenylacetic acid and benzilic acid. However, large ratios, of about 100, are found for the esters in which the configuration of the acid moiety differs. Similar measurements with the enantiomorphic esters of choline and α-methyltropic acid (see Table 4) reveal also that the ester of *S*-α-methyltropic acid is a much more potent atropine-like agent than is the corresponding *R*-acid ester.

TABLE 4. INTRINSIC ACTIVITIES (i.a.) AND AFFINITIES (pA_2) OF VARIOUS STEREOISOMERIC ESTERS

Configuration*						
Acid moiety	Alcohol moiety	$[\alpha]_D$ in methanol	i.a.	pA_2	Relative affinity (atropine = 100)	Affinity ratios
Diastereoisomeric esters of α-methyltropic acid and β-methylcholine						
3"*S*"	5*R*	−37·7	0	6·2	0·25	3*R*:5*S*/3*R*:5*R* = 1·5
3"*S*"	5*S*	+48·4	0	6·9	1·3	3*S*:5*S*/3*S*:5*R* = 5
3"*R*"	5*R*	−47·3	0	4·7	0·008	3*S*:5*R*/3*R*:5*R* = 30
3"*R*"	5*S*	+43·3	0	4·9	0·013	3*S*:5*S*/3*R*:5*S* = 100
Enantiomorphic esters of α-methyltropic acid and choline						
3"*S*"		−2·2	0	8·1	20	3*S*/3*R* = 300
3"*R*"		+1·8	0	5·6	0·06	
Diastereoisomeric esters of hexahydrobenzilic acid and β-methylcholine						
3"*R*"	5*R*	−39·7	0	8·9	125	3*R*:5*S*/3*R*:5*R* = 0·24
3"*R*"	5*S*	+32·0	0	8·3	30	3*S*:5*S*/3*S*:5*R* = 0·6
3"*S*"	5*R*	−35·1	0	6·9	1·3	3*R*:5*R*/3*S*:5*R* = 100
3"*S*"	5*S*	+37·9	0	6·7	0·8	3*R*:5*S*/3*S*:5*S* = 40
Enantiomorphic esters of hexahydrobenzilic acid and choline						
3"*R*"		−5·3	0	10·4	4,000	3*R*/3*S* = 100
3"*S*"		+6·1	0	8·4	40	

* The notation "*S*" and "*R*" for the configuration of the acids is only tentative. The numbering of the atoms has been explained in Table 5.

Results obtained with the four diastereoisomeric esters of β-methylcholine and hexahydrobenzilic acid substantiate these findings. Affinities calculated from dose-response curves are given in Table 4. As with the previously mentioned compounds the absolute configuration of the acids is unknown. However, the most potent antagonists in this series are the esters derived from the (−)-acid and it seems probable that this enantiomorph will have the same configuration as "*S*"-α-methyltropic acid. In the notation of Cahn & others (1956) (−)-hexahydrobenzilic acid would be the "*R*"-acid, a designation we use tentatively. Also the optical isomeric esters of choline and hexahydrobenzilic acid which have an asymmetric centre only in the acid moiety, differ widely in potency (see Table 4).

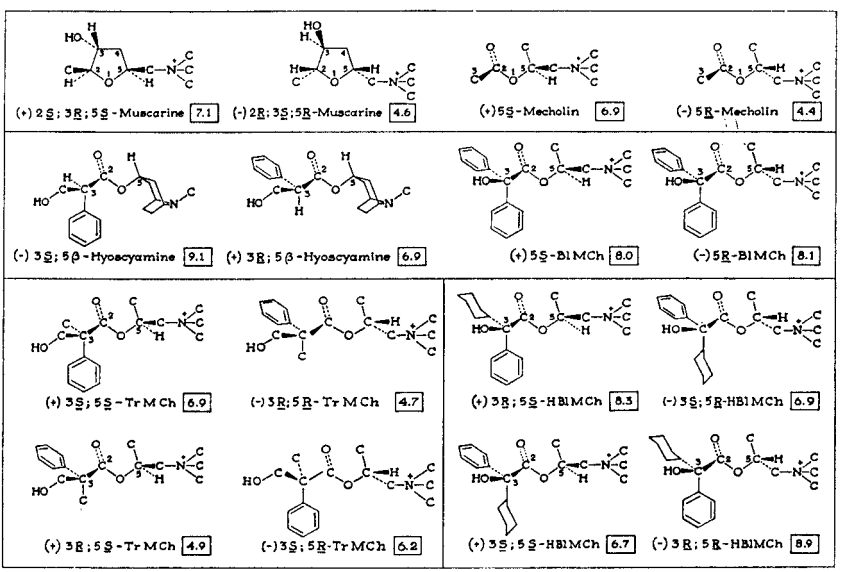
As the absolute configuration of the *O*-acetylmandelic acids is known, an attempt was made to obtain more precision about the required absolute configuration of the acid component in the asymmetric atropine-like esters by examination of the esters of *O*-acetylmandelic acid and β-methylcholine. However, the various isomers all appeared to be weak atropine-like drugs, which apparently do not fit the receptors very well. As a consequence their stereospecificity is small and the affinity ratios (3*S*/3*R* as well as 5*S*/5*R*) are both about 1 (Table 3).

Finally the isomers of the choline ester of phenyl-2-thienylglycollic acid have been investigated. Both are potent antagonist drugs and differ only slightly in affinity (Table 3). Apparently the receptor does not differentiate between the phenyl and thienyl groups.

Discussion

Pharmacologically active stereoisomers of the parasympathomimetic drugs muscarine and methacholine have identical absolute configurations at the asymmetric carbon, β to the ammonium group (position 5 in Table 5). In the notation of Cahn & others, this configuration must be indicated as *S* in both compounds. Recently it was shown that the most potent of the four diastereoisomers of (2-methyldioxolan-4-ylmethyl)trimethylammonium iodide (Methyldilvasène, F 2268) had the 2*S*:4*R*-configuration (Belleau & Puranen, 1963). The 4*R*-carbon in Methyldilvasène corresponds to the 5*S*-carbon of muscarine and acetyl-β-methylcholine. Thus,

TABLE 5. ABSOLUTE CONFIGURATION OF RELATED PARASYMPATHOMIMETIC AND ATROPINE-LIKE AGENTS (THE POTENCY AS pD_3- OR pA_2-VALUES IS GIVEN IN A BOX AFTER THE NAME OR THE ABBREVIATION OF THE VARIOUS COMPOUNDS)



all these compounds have an identical spatial arrangement around the asymmetrical carbon atom bearing the trimethylammonium-methyl group. These results indicate that all these compounds interact with a common cholinergic receptor and are highly complementary to it.

Exchange of the acetyl group in acetyl-β-methylcholine for other acyl residues causes a decrease in the intrinsic activity (see Table 2). The formic and propionic esters are still complete agonists like the parent compound, but with the esters of the four-carbon acids (cyclopropanecarboxylic acid, butyric acid and isobutyric acid) a lowering of the intrinsic

activity is observed; the cyclopropanecarboxylic ester is only a partial agonist, and the isobutyric ester is completely inactive as such. On introduction of still heavier groups the character of the compounds do not alter further; they remain "inactive". The inactive compounds are, however, inactive as agonists while still having affinity for the receptors. They therefore behave as competitive antagonists. The higher homologues are potent atropine-like agents approaching the affinity of atropine compounds.

The affinity of the β-methylcholine esters presents a more complex picture. The optimum in affinity is reached with the acetic ester. With increasing molecular size there is a gradual decrease in affinity. However, the esters of β-methylcholine and bulky acidic components, especially those containing large ring systems and polar groups, exhibit large affinities, which often exceed that of the parent mimetic compound. Similar findings have been reported for several series of related com-, pounds, for example, acetylcholine or Dilvasène (Schneider & Timms 1957; van Rossum & Ariëns, 1959; Koopman, 1960).

To explain the sharp increase in affinity as a consequence of the introduction of large acyl moieties, the presence of additional receptor areas located in the surroundings of the parasympathetic receptor has been postulated (Ariëns & Simonis, 1960). They would provide the corresponding drug-receptor complexes with a gain in binding energy. It even seems possible that the receptors for agonist and antagonist compounds, although neighbours, are completely different in location, but nevertheless identical in a functional sense.

In accordance with this hypothesis it appears that the asymmetric centre in the choline part, which is in a key position in parasympathomimetic esters of β-methylcholine, does not occupy this critical position in the homologous esters with atropine-like character; the enantiomorphic esters of diphenylacetic acid and benzilic acid (Table 3) showed only small differences in affinity.

The concept that atropine-like agents adhere to slightly different positions on the receptor surface in comparison with mimetics is corroborated by the data given in Table 4. From the results with choline esters of α-methyltropic acid and hexahydrobenzilic acid, it appears that the receptor strongly differentiates between isomeric compounds with an asymmetric centre in the acid component. Unfortunately, the absolute configurations of the stereoisomeric forms of the acids are not known with certainty. Considering the pharmacological measurements, it seems attractive to suppose that the most potent compounds of both pairs of isomers contain acids with identical configurations, tentatively referred to as "*S*"-α-methyltropic acid and "*R*"-hexahydrobenzilic acid.

A similar picture is obtained with the esters of the same acids with β-methylcholine as alcoholic component. Now four diastereoisomeric forms are possible. Higher affinities are found with the esters of "*S*"-α-methyltropic acid and "*R*"-hexahydrobenzilic acid. The configuration of the β-methylcholine is irrelevant. Clearly the stereochemical specificity

of the receptor is mainly directed to the asymmetric centre in the acid moiety of the atropine-like esters.

At first sight the measurements with the esters of *O*-acetylmandelic acid and phenyl-2-thienylglycollic acid, summarised in Table 3, seem to be in contradiction with this proposition, but it appears that the affinities of all diastereoisomeric esters of *O*-acetylmandelic acid and β-methylcholine are very low. Apparently none of the compounds fit very well to the receptor and therefore the stereospecificity is small. For the enantiomorphic esters of phenyl-2-thienylglycollic acid and choline, which are both very potent atropine-like agents, it might be suggested that the receptor does not differentiate between the isosteric thienyl and phenyl groups. Comparable biological activity of compounds which differ only by substitution of an ethylene group (—CH=CH—) in a ring system for a sulphur atom has also been found in other instances (Martin-Smith & Reid, 1959). The high affinity of the "*S*"-hexahydrobenzilic ester of choline, which is the less potent enantiomorph, may be ascribed also to the fact that phenyl and cyclohexyl rings can interchange their roles on the receptor surface to some extent. The extremely high affinity of the ester of choline and "*R*"-hexahydrobenzilic acid cannot be explained.

Pfeiffer (1956) has plotted potency ratios of many pairs of optical isomers against the effective doses used in clinical or animal experiments. He found the ratio to be larger if the effective dose was lower. This means that large affinity ratios between isomers can be anticipated when the affinity of a racemate or the most potent enantiomorph is high, whereas small ratios are to be expected with compounds of low affinity. From the experiments described in this paper, Pfeiffer's statement should be extended with two comments. Firstly, the rule holds good only if the asymmetric centre has a key position in the drug-receptor complex. Secondly, the rule fails when two groups at the asymmetric centre of potent enantiomorphs are bio-isosteric.

The results presented in the paper suggest the general conclusion that parasympathomimetics and their antagonists react with different or partially different receptors, although they are competitive antagonists of each other. It is therefore not justified to follow the procedure of Barlow, Scott & Stephenson (1963) who calculate relative efficacies by assuming that ethylation in a series of agonists results in the same affinity change as in a series of antagonists.

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