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SHORT COMMUNICATIONS

Inversion of Stereospecificity by Methylation of Compounds Acting at Acetylcholine Receptors

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SUMMARY

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On guinea pig ileum *R*-(−)-3-acetoxyquinuclidine is less than one-tenth as active as the *S*-(+) enantiomer, but the methiodide of the *R* compound is about 40 times as active as its *S* enantiomer. Inversion of stereospecificity occurs because methylation reduces the activity of the stronger (*S*) base about 1000-fold whereas it reduces the activity of the weaker (*R*) base only about 2-fold. The affinity of the *S* base is reduced about 100-fold, and this marked effect of methylation may be associated with the rigidity of the quinuclidine ring and indicate a particularly close fit between *S*-(+)-3-acetoxyquinuclidine and the "muscarinic" receptor. On the frog rectus there is no detectable stereospecificity and activity is not reduced by methylation, but the compounds are not very active.

INTRODUCTION

Ever since the work of Waser (1) and Gyermek and Unna (2) it has been known that relatively small changes in structure may produce an inversion of stereospecificity. In this instance the change, from —CHOH— in muscarine to —CO— in muscarone, is chemically fairly drastic. Recently it has been shown that marked differences in the degree of stereospecificity may be produced by simply methylating a tertiary nitrogen atom (3), and we now wish to report an example in which this change actually leads to an inversion of stereospecificity.

The compounds studied were the *S*-(+) and *R*-(−) enantiomers of 3-acetoxyquinuclidine and the *R*-(−) and *RS* forms of 3-acetoxyquinuclidine methiodide. The enantiomeric forms of the methiodide have already been described by Robinson, Belleau, and Cox (4). Their absolute configurations were incorrectly assigned but are now known from X-ray diffraction studies (5). Relative activities have been measured on the isolated guinea pig ileum and frog rectus preparations.

EXPERIMENTAL PROCEDURE

Compounds. *R*-(−)-Quinuclidin-3-ol was obtained by the method of Sternbach and Kaiser (6) and had $[\alpha]_D^{20}$ −44.3° (*C*, 2.5, in 1 N HCl) compared with $[\alpha]_D^{25}$ −43.8° (*C*, 3.0, in 1 N HCl). Partially resolved *S*-(+)-quinuclidin-3-ol was obtained by the method of Kalir, Sali, and Shirin (7). The hydrochlorides of the acetate esters were crystallized from ethanol–ether. *R*-(−)-3-Acetoxyquinuclidine HCl had m.p. 208–209° [Pyttel and

Robinson (8) reported 200–201°].

Calculated: C 52.54, H 7.78, N 6.81
Found: C 52.02, H 7.74, N 6.81

The samples of the *S*-(+) and *RS* compounds both contained water of crystallization detectable from the infrared absorption spectrum (9). The *RS* compound had m.p. 180°.

$C_9H_{16}NO_2Cl \cdot 0.5H_2O$

Calculated: C 50.35, H 7.93, N 6.53
Found: C 50.40, H 8.05, N 6.52

The *S*-(+) compound had m.p. 173–175°. The rotations (0.1 M in water) were $[\alpha]_D^{20}$ −11.5°, +7.7°; $[M]^{20}$ −24°, +16° (589 nm); −143°, +118° (300 nm); −181°, +141° (280 nm). If the *R* compound is stereochemically pure, the rotations indicate that the stereochemical purity of the *S* compound is 83%.

R-(−)-3-Acetoxyquinuclidine methiodide had m.p. 204–206°; $[\alpha]_D^{20}$ −9.3°; $[M]^{20}$ −29° (589 nm); −175° (300 nm); −202° (280 nm) (0.1 M in water). Robinson, Belleau, and Cox (4) reported m.p. 203–204°; $[\alpha]_D^{25}$ −11° (c, 2.03, in water). The methiodide of the *RS* compound had m.p. 164–164.5°; Robinson, Belleau and Cox (4) reported m.p. 164–165.5°.

Biological preparations. The guinea pig ileum was set up in aerated Tyrode's solution containing hexamethonium (276 μM) at 37°; the frog rectus (*Rana temporaria*) was set up in aerated frog Ringer's solution at room temperature (10). Drugs were applied to the ileum with automated apparatus, allowed to act for 30 sec, and given once every 90 sec. They were applied to the rectus by hand, allowed to act for 3 min, and given once every 15 min. Only small responses were obtained with the quinuclidine derivatives on the rectus, and their activity relative to carbachol could only be assessed very roughly. In the experiments on the ileum several pairs of alternate large and small responses were obtained with each compound and used to calculate concentrations which should produce matching responses. Provided the concentrations of the two drugs being compared are selected so that the observed responses are closely matched and the correction for an exact match is small, this procedure has been shown to give accurate results (11).

RESULTS AND DISCUSSION

On the guinea pig ileum the sample of *S*-(+)-acetoxyquinuclidine was 12.7 times as active as the *R*-(−) compound (Table 1). This agrees well with the results obtained by Weinstein, Maayani, Srebrenik, Cohen, and Sokolovsky (12). The methiodide of the *R* compound was 1.60 times as active as the methiodide of the *RS* compound, indicating a stereospecific index of 40:1. Robinson, Belleau, and Cox (4) obtained a ratio of about 60:1 for these compounds, although the weaker (+) enantiomer was only a partial agonist, which makes it impossible to assign an exact ratio (their results indicate a range from 23 to 69). Methylation has accordingly led to an inversion of stereospecificity.

Methylation reduces the activity of the *R*-(−) base about 2-fold, but that of the *S*-(+) base, over 1000-fold. As the compounds are agonists, the change may reduce efficacy (ability to activate receptors) as well as affinity, but there is evidence that the main effect is on affinity. It is possible to estimate the affinity constant of the partial agonist, *S*-(+)-acetoxyquinuclidine methiodide, from matching concentrations of partial and full agonists calculated from the log dose-response curves published by Robinson, Belleau, and Cox (4), fitted to a hyperbolic expression (13); the value of log *K* was estimated to be 2.95. This is 2 log units less than estimates for acetylcholine (log *K*, 5.04) (14) and less even than for nonesterified substances such as tropine methiodide (log *K*, 3.14) (15). It seems likely, therefore, that the 1000-fold reduction in the activity of *S*-(+)-acetoxyquinuclidine by methylation is due mainly to a reduction in affinity by about 100-fold, although there must also be some reduction in efficacy because the methiodide is a partial agonist.

There is evidence (16) that the part of the receptor with which the onium group interacts is of limited size, and the results may therefore be explained by supposing that the fit of the quinuclidine portion in the *S*-(+) base is very close and that

TABLE 1

3-Acetoxyquinuclidines and their methiodides

The equipotent molar ratios (EPMR) relative to carbachol are only approximate; direct comparisons were made between the *S* and *R* bases and the methiodides of the *R* and *RS* compounds, and the mean values are shown with the standard error and number of estimates. As the stronger (*S*) base is incompletely resolved (see EXPERIMENTAL PROCEDURE), the stereospecific index will be slightly greater than the ratio shown. The values for the methiodide of the *S* compound relative to carbachol and to the methiodide of the *R* compound are calculated from the result for the racemate relative to the methiodide of the *R* compound. Note that on the ileum *S*-(+)-acetoxyquinuclidine is much more active than its *R* enantiomer but the *S* methiodide is much less active than its *R* enantiomer. Although the compounds had some activity relative to carbachol on the frog rectus, comparisons of the enantiomers were made on only two preparations. In other experiments the racemate of the methiodide was found to be slightly less active than methylquinuclidinium iodide. Racemic quinuclidin-3-ol and its methiodide had negligible activity.

Compound	Ileum		Rectus	
	EPMR	Ratio	EPMR	Ratio
S-(+) base (HCl)	6	12.7 ± 0.5 (6)	125–250	~1
R-(−) base (HCl)	80		125–250	
R-(−) methiodide	160	1.60 ± 0.05 (6)	—[a]	
RS methiodide	250		135–170	
S-(+) methiodide calculated	6400	40		

[a] Hydrolyzed.

because the ring is rigid an extra methylene group cannot be accommodated and the main bulk of the quinuclidine ring does not contribute to the binding of the methiodide. At these receptors the maximum increase in log *K* produced by a methylene group appears to be just below 1 log unit (17), which could account for a decrease in affinity of the size indicated. This decrease is not out of keeping with results with other esters of quinuclidine which are antagonists, in which it was found that *N*-methylation can reduce affinity 25-fold (17). If the quinuclidine ring in the weaker *R*-(−) base fits the receptor less closely, *N*-methylation may have a less adverse effect on affinity and hence the inversion of stereospecificity. The *S*-(+) enantiomer of 3-acetoxyquinuclidine seems therefore to be a good model for discussing fit to the "muscarinic" receptor, but not necessarily for discussing ability to activate receptors. It is interesting that the N—C—C—O torsion angle in both enantiomers is anticlinal, as in the proposed active conformation of acetylcholine and other flexible compounds (for review, see ref. 18).

The situation is quite different at the nicotine-sensitive receptors in the frog rectus. There is no detectable stereospecificity and activity is not reduced by methylation, although the hydrolysis of the methiodide of the *R* compound complicates estimates of activity because it affects the time course of the response. The compounds are only feebly active, being about as potent as methylquinuclidinium iodide. As the enantiomeric forms of nicotine differ about 10-fold on this preparation (19), the absence of stereospecificity with 3-acetoxyquinuclidines and their methiodides, together with their low activity, suggests that their action is associated with a less intimate fit to these receptors as opposed to the muscarine-sensitive receptors and the active site of acetylcholinesterases.

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