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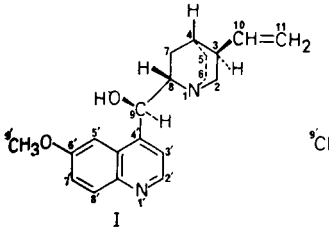
COMMUNICATIONS

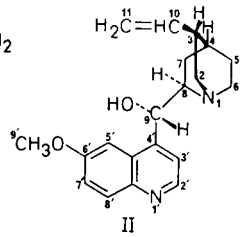
Low stereospecificity of quinine taste receptors

P. C. SCHOBER, P. W. BOWERS, S. E. SMITH*, *Department of Pharmacology, St. Thomas's Hospital Medical School London SE1 7EH, U.K.*

A wide variety of chemical compounds excites a bitter taste in man, but characterization of the receptors involved is limited by the paucity of studies of structure-acitivity relations among closely-related compounds (Kubota & Kubo, 1969; Belitz, 1973). This paper describes an experiment in which taste thresholds of quinine (I) and 10 structurally-related compounds were measured. An attempt is made to reconcile taste activity to structure.



I



II

Twelve healthy volunteers of either sex aged 18–32 years took part in the experiment. None smoked nor took drugs of any sort during the study period. Taste thresholds were measured by the forced choice method of Harris & Kalmus (1949), using the solution number system of Fischer (1967). This scale is a $\log_2$ series of ascending concentration in which solution no. 12 is $1{\cdot}5 \times 10^{-3}$M. Intervening solutions were prepared by diluting the whole number solutions by a factor of $\sqrt{2}$ to provide half number solutions. All compounds were dissolved in deionized distilled water and pH adjusted to neutrality. Two compounds, quininone and quinicine, gave slightly coloured solutions; for testing these the subjects were blindfolded. Measurements were performed twice weekly in a quiet air-conditioned room of ambient temperature 20°, each subject being tested at the same time of day on every occasion. Solutions were equilibrated to ambient temperature overnight. Each volunteer was given a trial test with quinine for familiarization with the method; the results of this test were discarded. Each substance was tested once except quinine itself which was tested at the beginning, middle and end of the experiment. Comparison of the first and third determinations showed satisfactory repeatability (Fisher's (1970) intrapair correlation of 0·92).

* Correspondence.

Structural modifications to the quinine molecule and the mean taste thresholds, threshold differences from quinine and relative potencies of all the compounds studied are given in Table 1. Only hydroquinine was more potent than quinine itself, all other derivatives being less potent by a factor of 3 to 10 fold. The overall potency range was 25·5-fold.

Studies with Dreiding models of the four stereoisomers quinine, quinidine, epiquinine and epiquinidine showed that interactions between (i) the quinoline nucleus, (ii) the hydroxyl group on C9 and (iii) C6, C7 and the nitrogen atom of the quinuclidine ring severely restrict conformational freedom. Each isomer has only one stable spatial conformation, with limited torsional movement about the C8–C9 and C9–C4′ bonds. In all cases the C9 hydroxyl group would be expected to lie out of the plane of the quinoline ring system. The four stereoisomers differ in respect of their combinations of two structural characteristics:

(i) the separation of the quinuclidine N from the C9 hydroxyl group (3·6 Å in quinine and quinidine, which can adopt an extended *trans* conformation, and 2·6 Å in epiquinine and epiquinidine, which adopt a *gauche* conformation); and

(ii) the folding in or extension of the vinyl group at C3 in relation to the quinoline moiety (extended in quinine and epiquinine, folded in across the quinuclidine ring in quinidine and epiquinidine; see II).

Hydroquinine, quinine ethyl carbonate and cinchonidine have the conformation of quinine, cinchonine that of quinidine. Compounds which are symmetrical at C9 and have no large substituent groups at this position, namely desoxyquinine, quininone and especially quinicine, possess wide rotational mobility and therefore do not adopt single fixed conformations.

Interpretation of the structure-activity relations in this series of compounds is complex. Certain features are, however, clear. First, all the compounds were bitter and their potencies fell within a remarkably small range, considering their structural and conformational differences. This indicates a very low stereospecificity of the taste receptors involved, a conclusion which is in accord with observations on the great range of substances which have a bitter taste. Secondly, the findings are at variance with the observations of Kubota & Kubo (1969) with diterpenes which suggest that bitter tasting compounds react with the bitter receptor protein (Dastoli, Lopiekes & Doig, 1968) by paired hydrogen

Table 1. *Taste thresholds of quinine and related compounds.*

Compound	Structural difference from quinine	Mean† threshold	Difference from† quinine (Mean ± s.e.m.)	Relative potency (quinine = 100)
Quinine	—	4·62*	—	100
Hydroquinine	Saturation of vinyl group on C3	3·29	−1·33 ± 0·30	251
Quinicine (Quinotoxine)	Carbonyl substitution at C9 and opening of N1–C8 bond	6·08	1·46 ± 0·37	36·3
Epiquinidine	Stereoisomer, opposite configuration at C8	6·08	1·46 ± 0·29	36·3
Desoxyquinine	Reduction to CH_2 at C9	6·25	1·63 ± 0·19	32·3
Cinchonidine	No OCH_3 group at C6′	6·33	1·71 ± 0·18	30·6
Quininone	Carbonyl substitution at C9	7·04	2·42 ± 0·23	18·7
Epiquinine	Stereoisomer, opposite configuration at C9	7·08	2·46 ± 0·29	18·2
Quinidine	Stereoisomer, opposite configuration at C8 and C9	7·25	2·63 ± 0·27	16·2
Quinine ethyl carbonate (Tasteless quinine)	Esterification at C9	7·92	3·30 ± 0·23	10·2
Cinchonine	No OCH_3 group at C6′ and stereoisomer, opposite configuration at C8 and C9	7·96	3·34 ± 0·38	9·9

* Quinine threshold = 4·62 s.d. 0·43 (Mean of 3 determinations in 12 subjects with s.d., n = 36). This mean concentration = $9{\cdot}04 \times 10^{-6}$M.

† Solution number ($\log_2$ scale).

bonding with a pair separation of 1·5 Å. The proposed hydrogen donor and acceptor groups are present in the quinine molecule (C9 hydroxyl and quinuclidine N) but they are too far separated. Furthermore, the degree of separation does not correlate with the individual taste thresholds among the four stereoisomers. It must be concluded that in these compounds the reactive parts of the molecule are different from those of diterpenes. Thirdly, further comparison of the stereoisomers indicates that the most important determinant of taste potency is the configuration of C9, which in its best position in quinine and epiquinidine places its hydroxyl group above the plane of the quinoline nucleus and the quinuclidine nucleus behind it. Folding in or extension of the C3 vinyl group, determined by the spatial orientation of the quinuclidine nucleus, is of only slight influence though its saturation (as in hydroquinine) increases potency to some extent. Fourthly, reduction or oxidation of C9 (to desoxyquinine and quininone respectively), which allows greater freedom of rotation about its bonds with C8 and C4′, in each case reduces potency, perhaps because the compounds are then less likely to take up the best (quinine) formation. Esterification of the C9 hydroxyl group (to quinine ethyl carbonate) reduces potency about 10-fold. Finally, the methoxy group at C6′ appears to exert some influence, quinine being more potent than cinchonidine and quinidine more potent than cinchonine. In the former its influence is, however, much greater indicating that in the quinine conformation this methoxy group can approach more closely to a reactive site.

These observations are consistent with the hypothesis that the quinuclidine group is unimportant for receptor attachment and there exists a flat hydrophobic receptor area (for the quinoline nucleus) with three reactive groups on the periphery, two electropositive for the C6′ methoxy and the C9 hydroxy groups and one electronegative for the quinoline N which is largely charged at physiological pH (pKa 9·7). Further experiments are, however, needed to elucidate receptor attachments more particularly by study of other quinoline derivatives.

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