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Relations between structure and nicotine-like activity: X-ray crystal structure analysis of (−)-cytisine and (−)-lobeline hydrochloride and a comparison with (−)-nicotine and other nicotine-like compounds

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1 Although (−)-cytisine is a rigid structure, it occurs in the crystal in two distinct but very similar conformations in which the pyridone ring is tilted relative to the charged nitrogen atom at much the same angle as the pyridine ring is in (−)-nicotine hydrogen iodide. The carbonyl group in the pyridone ring of (−)-cytisine, however, is on the side of the ring opposite to the pyridine nitrogen in (−)-nicotine.

2 The pK_a of (−)-lobeline HCl at 25°C is 8.6 (approx), indicating that (−)-lobeline is at least 90% in the protonated form at physiological pH (7.6). It is probably the phenyl 2-keto-ethyl part of (−)-lobeline, rather than the phenyl 2-hydroxy-ethyl part, which interacts with the receptor.

3 The combination within one molecule of a charged ('onium') nitrogen atom lying out of the plane of, and some distance (4.5–6.5 Å) from, an aromatic ring is common to many compounds with nicotine-like activity (e.g. nicotine, cytisine, choline phenyl ether bromide, dimethyl-phenyl-piperazinium (DMPP) iodide, coryneine iodide and *m*-hydroxyphenylpropyl trimethyl ammonium iodide). In some molecules the aromatic ring can be replaced by an unsaturated group, such as carbonyl (e.g. acetylcholine) or double-bonds (e.g. anatoxin).

4 Activity at nicotinic receptors appears to involve interactions between the positively charged nitrogen atom and a negatively charged group, probably close to cysteine residues 192 and 193 in the receptor. It is suggested that rather than specific groups in the molecule also being involved, activity at nicotinic receptors depends on interactions between a flat part of the drug containing double-bonds, or systems of double bonds, and a planar area in the receptor, possibly tyrosine or phenylalanine residues.

Introduction

The nicotine-like properties of the alkaloid (−)-cytisine have long been known (Dale & Laidlaw, 1912; Zachowski, 1938; Barlow & McLeod, 1969) and are of particular interest in the discussion of molecular interactions between agonists and nicotinic receptors, because (−)-cytisine should be a relatively rigid structure. This has been important in the ideas of Beers & Reich (1970) which led them to suggest that, as well as involving a charged 'onium' nitrogen, activity also involved a group, such as carbonyl, capable of forming a hydrogen bond with a donor group in the receptor. From modelling studies on the structures of nicotinic agonists, including cytisine, Sheridan *et al.* (1986) have claimed to identify a pharmacophore 'triangle', comprising 'the cationic center (A), an electronegative atom (B) and an atom (C) which forms a dipole with B'. In view of the significance which has been attached to the activity and structure of (−)-cytisine, it is important to make an X-ray structure analysis to determine its precise molecular parameters in the crystal.

An X-ray structure determination of the alkaloid (−)-lobeline has also been made. This is likewise classified as a nicotine-like agonist (Dixon, 1924). It is listed as a ganglion-stimulant (Dale & Rang, 1987) but interest in it has mainly been concerned with the marked reflex stimulation in respiration which it produced by an action on chemoreceptors in the

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aortic and carotid bodies (Dallemagne & Heymans, 1955). Reflex respiratory stimulation is also seen with nicotine and, to a lesser extent, with cytisine (Barlow & McLeod, 1969), but the effects of lobeline appear to have been so marked as to have justified its extensive use clinically (it has been included in the pharmacopoeias of many countries).

(−)-Lobeline is potentially a flexible molecule with no obvious structural resemblance to nicotine. Although the unit cell dimensions of lobeline HCl H_2O were described by Eeles (1953), a full X-ray structure analysis was not made. The pK_a of lobeline has also been measured as it could not be found in the literature. Both cytisine and lobeline are currently of interest because of their affinity for receptors in rat brain (Wonnacott, 1987) and ideas of how agonists interact with nicotinic receptors have been greatly helped by progress which has been made on the structure and amino acid sequence of the nicotinic receptor mostly from *Torpedo californica* (Noda *et al.* 1982) and *Torpedo marmorata* (Changeux *et al.*, 1984). It is now possible to try to identify and model putative binding sites using, for example, antibodies and peptide fragments from the receptor (Watters & Maeliecke, 1983; McCormick & Atassi, 1984; Smart *et al.*, 1984; Criado *et al.*, 1986; Kao & Karlin, 1986; Oblas *et al.*, 1986; Mulac-Jerevic & Atassi, 1986; Abramson *et al.*, 1988; Dennis *et al.*, 1988; Gotti *et al.*, 1988; Moskovitz & Gershoni, 1988).

Methods

Measurement of pK_a

Electrometric titrations were made as described by Armstrong & Barlow (1976), with a Metrohm E500 digital pH meter and an EA121 combination glass electrode, with 0.1 M sodium hydroxide added by means of an E412 Dosimat. The temperature was 25.0 ± 0.2°C. The experiments involved the addition of known amounts of alkali to a known amount of lobeline hydrochloride and measuring the pH. For each value of added alkali and pH the thermodynamic pK_a was calculated as described by Armstrong & Barlow (1976: the computer program used is listed by Barlow, 1983).

Lobeline base is not very soluble in water. Some titrations were made with 0.015 mmoles and a volume of 25 ml (giving an initial concentration of 0.06 mM) and at this dilution the base stayed in solution, but the results may not be accurate because of the need to weigh small amounts of lobeline and to use small volumes of alkali. Experiments were also made with larger quantities in 20 ml aqueous ethanol (50% by volume): with 0.2 mmoles in this medium the titration could proceed well beyond half-neutralization and with 0.1 mmoles the base remained in solution even when fully neutralized.

Crystal structure determinations

Crystal data for (−)-*cytisine* (I) $C_{11}H_{14}N_2O$, M_r = 190.3, orthorhombic, $P2_12_12_1$ (No:19), a = 7.175(2), b = 9.973(3), c = 26.639(9) Å, U = 1906(1) Å^3, Z = 8, D_x = 1.33 g cm^{-3}, Mo K_α X-radiation (λ = 0.71073 Å), μ = 0.81 cm^{-1}, $F(000)$ = 816, T = 298 K, $R(R_w)$ = 0.0325 (0.0303) for 1782 reflections with $I > 3\sigma(I)$.

Crystal data for lobeline hydrochloride (II) $C_{22}H_{28}O_2N^+ \cdot Cl^- \cdot H_2O$, M_r = 373.9, orthorhombic, $P2_12_12_1$ (No:19), a = 8.152(2), b = 14.370(4), c = 17.915(5) Å, U = 2099(1) Å^3, Z = 4, D_x = 1.24 g cm^{-3}, Mo K_α X-radiation (λ = 0.71073 Å), μ = 2.00 cm^{-1}, $F(000)$ = 840, T = 298 K, $R(R_w)$ = 0.0498 (0.0540) for 1515 reflections with $I > 3\sigma(I)$.

For both structure determinations diffracted intensity data were collected on Nicolet four-circle automated diffractometers by $\theta/2\theta$ variable speed scans. Table 1 gives the data collection parameters and details of data reduction, structure solution and refinement. Structure solution was by direct methods and Fourier difference synthesis. No corrections were applied for absorption or extinction. Structure I was refined by blocked-cascade least-squares on a Data General Desktop minicomputer with the *SHELXTL* package (Sheldrick, 1981), and structure II by full-matrix least-squares on a Digital MicroVAX II computer with the *SHELXTLplus* package (Nicolet Instrument Corp., 1988). All non-hydrogen atoms were refined with anisotropic thermal parameters. The phenyl rings in II were refined as regular hexagons (C—C 1.395 Å). Non-hydroxyl hydrogen atoms were incorporated at geometrically idealised positions (C—H 0.96Å, fixed U_{iso} of 1.2xU_{eq} of C) and refined by a riding model. Hydroxyl hydrogens in II were located from diffrence Fourier maps (low-angle, $\sin\theta/\lambda < 0.25$) and their position and isotropic thermal parameter were fixed during refinement. In compound I the piperidine N—H could not be located from a Fourier difference synthesis and so both *endo-* and *exo-* N—H positions were generated geometrically. Refinement of *endo-/exo-* site occupancy resulted in 90% of the *exo-* hydrogen occupancy. Residual peaks and troughs in the final ΔF maps were <0.26 e Å^{-3} for both structures. The function minimized during refinement was $\Sigma w(F_0 - |F_c|)^2$, where $w^{-1} = [\sigma^2(F) + gF^2]$ and values of g are given in Table 1. Atomic scattering factors and corrections for anomalous dispersion were taken from *International Tables for X-ray Crystallography, Vol. IV.*

Table 1 Data collection parameters, structure solution and refinement details for (−)-cytisine and (−)-lobeline hydrochloride monohydrate

Compound	*(−)-Cytisine*	*Lobeline hydrochloride monohydrate*
Crystal colour	Yellow	Colourless
Habit	Needle	Needle
Size (mm)	0.2 × 0.3 × 0.8	0.3 × 0.25 × 0.1
Diffractometer	R3m/V	$P2_1$
Unit cell determn.		
No. of reflns.	18	15
2θ range (°)	20–25	14–18
Scan range (°2θ)	2.9–50.0	2.9–52.5
Range of *hkl*	−2/8, −2/11, −2/31	0/10, 0/17, 0/22
Scan type	$\theta/2\theta$	$\theta/2\theta$
Scan speeds ($\cdot 2\theta\,\text{min}^{-1}$)		
Min.	2.00	1.50
Max.	29.30	29.30
No. of standard reflns.	3	3
Frequency of stds.	100	50
Variation/decay (%)	3	3
Data collected	3107	2512
Independent data	2479	2477
R_{int}	0.013	0.008
Solution method	Direct/ΔF	Direct/ΔF
Program system	*SHELXTL*	*SHELXTL*plus
Refinement method*	bcls	fmls
Refined data	1782	1515
$I > n\sigma(I)$: n	3	3
R	0.0325	0.0498
R_w	0.0303	0.0540
$w^{-1} = [\sigma^2(F) + gF^2]$;		
g-value	0.0005	0.002
Goodness-of-fit	1.135	1.080
Data:parameter ratio	7	6
Max. Δ/σ (final)	0.035	0.001
Max. $\Delta\rho$ final ($e\text{Å}^{-3}$)		
positive	0.19	0.26
negative	0.24	0.18
Hydrogen atoms:		
Location	Geometric	OH by ΔF, others geometric
Refinement	Riding (90% piperidine N–H *exo*-)	OH fixed, others riding

* bcls = blocked-cascade least-squares, fmls = full-matrix least-squares.

Materials

(−)-Cytisine (Sigma) was crystallized from acetone (Ing, 1931). (−)-Lobeline hydrochloride (Aldrich) was crystallized from aqueous ethanol (approx 20% water by volume).

Results

Final fractional atomic coordinates are given in Tables 2 and 3. The analysis shows two distinct conformers of cytisine in the asymmetric unit but these are very similar (Figure 1). The piperidine ring is fixed at right angles to a pyridone ring by a bond which forms another saturated six-membered ring.

In lobeline hydrochloride the nitrogen atom is again part of a piperidine ring but, like nicotine, it is a tertiary base and has an attached methyl group as well as a proton. The piperidine ring has two flexible chains linking a benzene ring to the carbon atom adjacent ot the nitrogen. In one the hydroxyl group and the benzene ring are almost coplanar (the C—C—C—OH torsion angle is −9°): in the other the keto group and the benzene ring are not coplanar (the C—C—C=O torsion angle is 38°). The two

Table 2 Atomic coordinates ($\times 10^4$) for (−)-cytisine

Atom	*x*	*y*	*z*
N(1)	5212(3)	3669(2)	624(1)
C(2)	3364(3)	3341(2)	569(1)
C(3)	2705(3)	2217(2)	803(1)
C(4)	3875(4)	1410(3)	1090(1)
C(5)	5692(4)	1748(3)	1146(1)
C(6)	6451(3)	2911(3)	918(1)
O(6)	8106(2)	3262(2)	947(1)
C(7)	6084(3)	4819(3)	359(1)
C(8)	4794(3)	5631(3)	24(1)
C(9)	3283(3)	4742(3)	−197(1)
C(10)	2159(3)	4211(3)	244(1)
C(11)	3881(4)	6789(2)	301(1)
N(12)	2745(3)	6291(2)	717(1)
C(13)	1297(3)	5384(3)	534(1)
N(1′)	8925(3)	9395(2)	1927(1)
C(2′)	7219(3)	9357(2)	2156(1)
C(3′)	5851(4)	8582(3)	1959(1)
C(4′)	6157(4)	7841(3)	1522(1)
C(5′)	7818(4)	7890(3)	1294(1)
C(6′)	9314(4)	8672(3)	1487(1)
O(6′)	10891(3)	8736(2)	1299(1)
C(7′)	10479(3)	10224(3)	2109(1)
C(8′)	10138(3)	10924(3)	2607(1)
C(9′)	8150(3)	11404(3)	2631(1)
C(10′)	6941(4)	10160(3)	2631(1)
C(11′)	10573(4)	10052(3)	3058(1)
N(12′)	9330(3)	8893(2)	3075(1)
C(13′)	7399(4)	9303(3)	3091(1)
H(3)	1414	1980	769
H(4)	3396	616	1247
H(5)	6485	1186	1346
H(7A)	6590	5413	608
H(7B)	7075	4476	153
H(8)	5557	5996	−238
H(9A)	2497	5254	−417
H(9B)	3832	4013	−380
H(10)	1153	3661	126
H(11A)	3102	7279	73
H(11B)	4830	7372	431
H(12A)	3529	5818	949
H(12B)	2169	7036	884
H(13A)	606	5041	814
H(13B)	473	5868	315
H(3′)	4659	8542	2122
H(4′)	5176	7298	1384
H(5′)	8007	7383	992
H(7′A)	11550	9656	2146
H(7′B)	10731	10898	1861
H(8′)	10980	11671	2621
H(9′A)	7950	11911	2933
H(9′B)	7868	11953	2345
H(10′)	5657	10430	2645
H(11C)	10415	10571	3359
H(11D)	11839	9747	3036
H(12C)	9606	8375	3370
H(12D)	9531	8354	2782
H(13C)	6616	8523	3094
H(13D)	7182	9818	3390

Table 3 Atomic coordinates ($\times 10^4$) for lobeline hydrochloride monohydrate

Atom	*x*	*y*	*z*
Cl(1)	974(2)	6193(1)	2386(1)
C(1)	1058(6)	3261(3)	1855(3)
O(1)	1676(5)	2325(3)	1820(2)
C(12)	−1022(5)	4316(2)	1262(2)
C(13)	−2123	4535	693
C(14)	−2408	3900	118
C(15)	−1591	3047	112
C(16)	−490	2828	682
C(11)	−205	3463	1257
C(2)	2531(6)	3903(3)	1787(3)
C(3)	3632(6)	3984(3)	2474(3)
C(4)	4936(7)	4725(4)	2380(3)
C(5)	5961(8)	4860(4)	3084(3)
C(6)	6630(7)	3923(4)	3359(3)
C(7)	5313(7)	3209(3)	3444(3)
N(1)	4400(5)	3060(3)	2714(2)
C(10)	5436(8)	2628(4)	2110(3)
C(8)	5901(7)	2277(3)	3756(3)
C(9)	6048(7)	2273(4)	4595(3)
O(9)	5835(7)	2976(3)	4955(2)
C(22)	5535(4)	1191(2)	5631(2)
C(23)	5802	357	6011
C(24)	6908	−294	5727
C(25)	7746	−111	5065
C(26)	7478	723	4685
C(21)	6373	1374	4969
O(1′)	7107(6)	3138(3)	6502(2)
H(1)	493	3354	2319
H(1′)	802	2021	1908
H(12)	−826	4752	1658
H(13)	−2685	5122	697
H(14)	−3165	4051	−274
H(15)	−1787	2611	−283
H(16)	72	2241	678
H(2A)	3195	3682	1382
H(2B)	2129	4514	1672
H(3)	2913	4172	2871
H(4A)	5649	4549	1978
H(4B)	4408	5304	2261
H(5A)	6863	5267	2975
H(5B)	5291	5131	3467
H(6A)	7146	4016	3835
H(6B)	7426	3700	3007
H(7)	4574	3461	3809
H(1A)	3548	2610	2797
H(10A)	6037	2118	2322
H(10B)	6190	3069	1900
H(10C)	4721	2400	1725
H(8A)	6959	2141	3546
H(8B)	5136	1802	3610
H(22)	4774	1639	5827
H(23)	5225	231	6467
H(24)	7091	−868	5988
H(25)	8506	−560	4869
H(26)	8055	848	4229
H(11′)	6898	2587	6363
H(12′)	7034	3574	6184

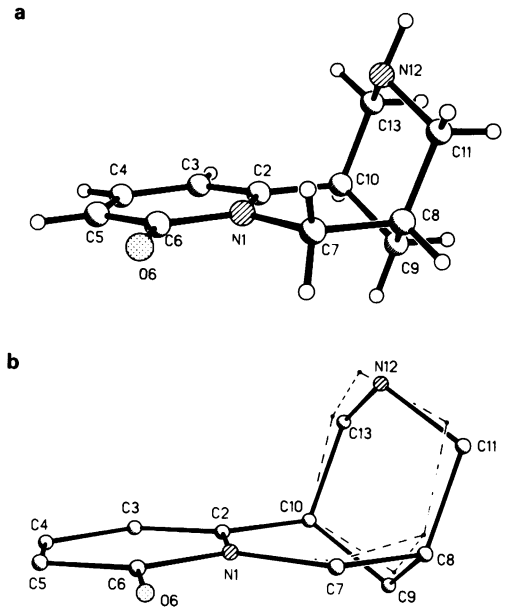


Figure 1 (a) The structure of (−)-cytisine showing the numbering: the basic nitrogen is at the top right and the pyridone ring on the left. (b) The two conformations of cytisine in the crystal superimposed.

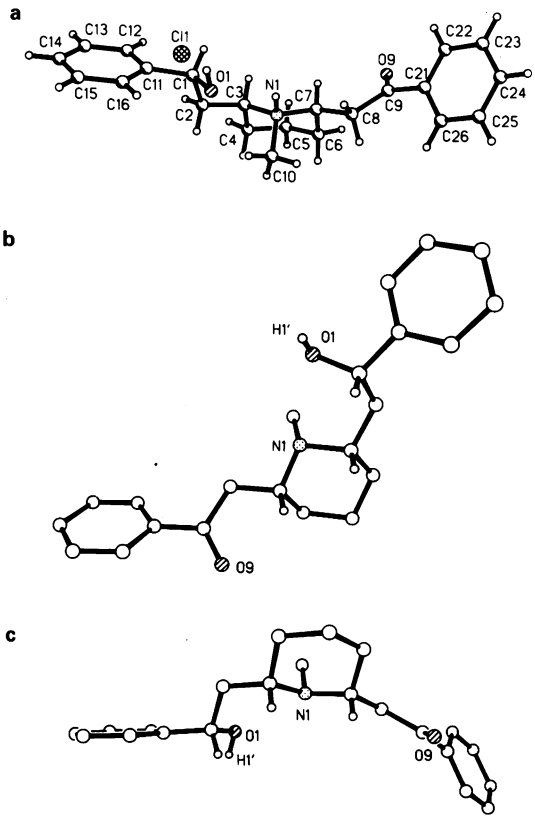


Figure 2 The structure of (−)-lobeline showing: (a) the numbering, (b) the keto-ethyl part and (c) the 2-hydroxyethyl part. Note the different positions of the nitrogen atom relative to the keto and hydroxyl groups.

links differ in that the keto group is orientated away from the nitrogen atom whereas the hydroxyl group is directed more towards the nitrogen (Figure 2).

Estimates (duplicates) of the pK_a of lobeline HCl in 50% aqueous ethanol were 8.53, 8.52 (2.5 mM), 8.60, 8.62 (5 mM) and 8.64, 8.75, (10 mM); in water, 8.62, 8.77 (0.6 mM). At physiological pH lobeline is likely to be at least 90% protonated. (−)-Lobeline is a stronger base at 25°C than nicotine (pK_as, 3.10 and 8.01; Barlow & Hamilton, 1962a) or cytisine, (pK_a 7.92; Barlow & McLeod, 1969). In part, at least, the greater basicity of lobeline can be ascribed to its nitrogen atom being less sterically hindered than the corresponding atom in cytisine or nicotine. From the effects of pH on the activity of nicotine it is known that it is the monoprotonated form which is biologically active (Barlow & Hamilton, 1962a). Similar experiments have shown that it is the protonated form of cytisine (and its mono-methyl analogue, caulophylline) which is active (Barlow & McLeod, 1969).

Although this study has been made with cytisine base, rather than with the protonated form, it seems highly unlikely that with such a locked cyclic molecule there can be much difference between the structures of the charged and uncharged forms.

Discussion

A comparison of the structures of (−)-cytisine and (−)-nicotine HI (Barlow *et al.*, 1986) is shown in Figure 3a, where arrowed atoms lie in the same plane. In cytisine and nicotine the aromatic ring is tilted to a similar extent. In Figure 3b the nicotine molecule has been moved so that the onium nitrogen atoms (point A in Sheridan's triangle) are superimposed. A horizontal rotation of −30° shows both rings edge-on (Figure 3c), but the pyridine nitrogen of nicotine and the amide carbonyl group of cytisine (point B in Sheridan's triangle) are on opposite sides of the ring. In theory the pyridine ring in nicotine could rotate and point B could be in a similar position in the two molecules but rotation is likely to be hindered. In the crystal structure of nicotine mono hydrogen iodide (the form which predominates at

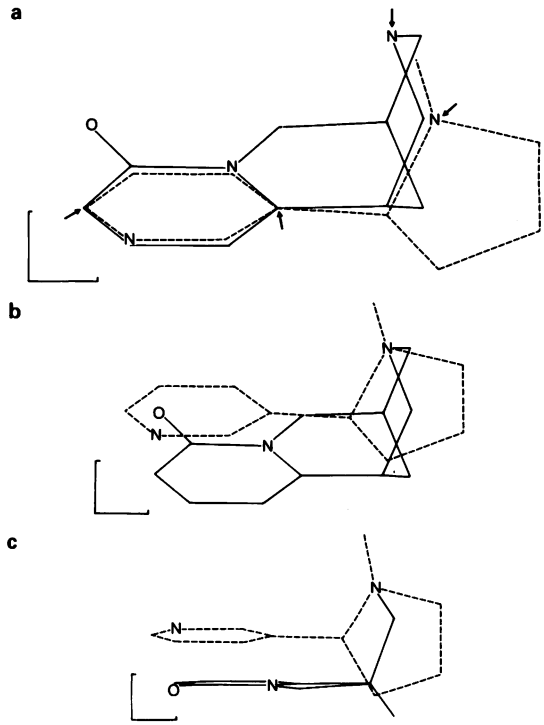


Figure 3 A comparison of cytisine and (−)-nicotine hydrogen iodide. The scale shows 1 Å (100 pm). In (a) the molecules have been aligned so that the ends of the aromatic rings (indicated by the arrows) form the X-axis and have a common origin at the righthand end of the ring. The basic nitrogen atoms (also indicated by the arrows) are set in the vertical plane and are some distance apart (1.35 Å). In (b) the nicotine molecule has been moved so that the basic nitrogen atoms are superimposed. In (c) a rotation of −30° about the X-axis has been made: this shows how closely parallel the rings are, relative to the basic nitrogen atom. In cytisine the pyridone ring is viewed edge-on and in nicotine it is only slightly different.

(−)-Cytisine is about 1.6 times as active as (−)-nicotine on the frog rectus and about 4 times as active as (−)-nicotine on guinea-pig ileum (ganglionic effects; Barlow & McLeod, 1969). It is from 2 to 6 times as active as (−)-nicotine in binding experiments with rat brain (Wonnacott, 1987).

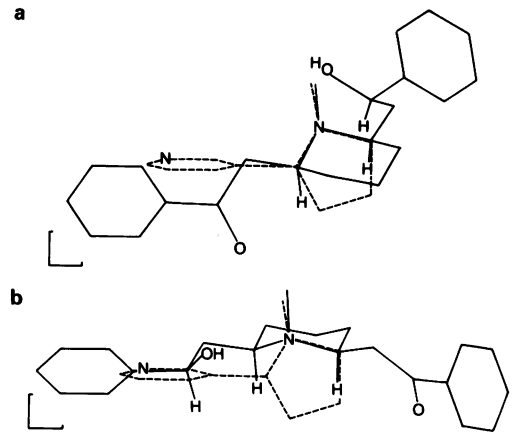
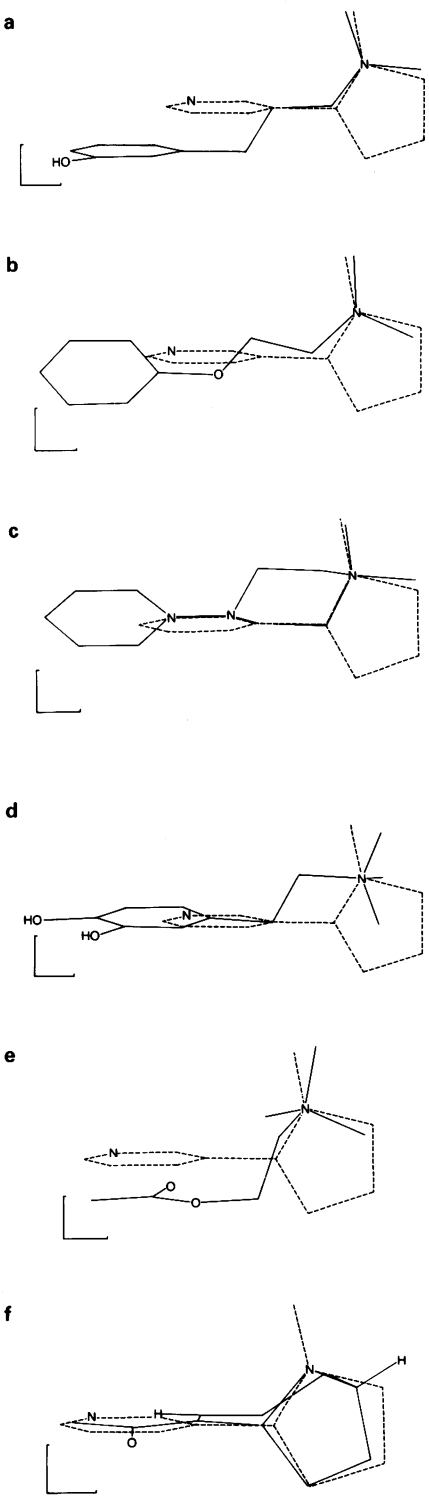


Figure 4 A comparison of lobeline with (−)-nicotine hydrogen iodide. The scale shows 1 Å (100 pm). The same procedure is followed as in Figure 3 but in (a) the phenyl ring of the 2-keto-ethyl part was superimposed on the pyridine ring of nicotine, with a common origin at the righthand end of the aromatic ring and the basic nitrogen atoms set in the plane of the paper. The origin was then moved so that these atoms are superimposed (cf. Figure 3b) and then a rotation of −30° about the X-axis has been made. In (b) the 2-hydroxy-ethyl part was superimposed on the pyridine ring, the origin moved and the rotation made.

Lobeline appeared to have less than 1% of the activity of nicotine on the frog rectus (Barlow & Veale, unpublished), on guinea-pig ileum it appeared to be about 10 times as active as nicotine but also blocked the preparation (possibly by blocking muscarinic receptors on the smooth muscle; Barlow & Franks, 1971). It is from 0.06 to 0.2 times as active as nicotine in binding experiments with rat brain (Wonnacott, 1987).

physiological pH) the two nitrogen atoms are orientated away from each other: the torsion angle about the bond linking the pyridine and pyrrolidine rings ((N)C—C—C—N(Me)) is 119°.

With lobeline either the 2-phenyl-2-ketoethyl part (Figure 4a) or the 2-phenyl-2-hydroxyethyl part (Figure 4b) can be compared with nicotine. In Figure 4a the phenyl ring of lobeline is almost at right angles to the pyridine ring of nicotine but in solution rotation may be possible in the lobeline molecule. The same applies to the phenyl and pyridine rings in Figure 4b but the onium groups in the two molecules are very differently placed. As the molecules are arranged, access to the pyrrolidine nitrogen in nicotine must be from the top whereas access to the nitrogen in lobeline must be from behind. There are also pharmacological reasons for believing that the phenyl 2-hydroxyethyl part is not likely to be involved. A 2-hydroxy group greatly reduces nicotine-like activity: the trimethylammonium derivative of adrenaline has feeble nicotine-like activity compared with coryneine (the trimethylammonium derivative of dopamine: Cuthbert, 1964) and 2-hydroxy phenethyl trimethyl ammonium bromide has negligible activity compared with phenethyl trimethylammonium bromide (Barlow & Gonzalez, 1986). In lobeline the hydroxyl group is actually 3



carbon atoms from the nitrogen but the groups are arranged less than 3 Å apart.

The X-ray crystal structures of many nicotine-like compounds have been obtained and Figure 5 shows a number of active compounds with their onium atoms superimposed on that of nicotine and presented as in Figure 3c and Figure 4. Their potency relative to nicotine is also indicated in the legend. Although many of these compounds are flexible and the conformation in the crystal may not indicate possible conformations in solution, there are structural similarities. As drawn, the compounds have the charged nitrogen atoms superimposed and the unsaturated ring lying to the left. The position and rotation of the ring varies from compound to compound but it is possible that they could all interact with some planar group in the receptor. With compounds such as *m*-hydroxyphenyl-propyl trimethylammonium iodide (Figure 5a) and coryneine (Figure 5d) the benzene rings are twisted at the same angle as in nicotine mono HI (and in cytisine). In choline phenyl ether bromide (Figure 5b) and dimethylphenylpiperazinium (DMPP) iodide (Figure 5c) the benzene ring is tilted differently, but rotation should be possible about the bond linking the benzene ring to the next atom (oxygen or nitrogen). With compounds lacking the aromatic ring, such as acetylcholine (Figure 5e) or anatoxin (Figure 5f), the comparison with nicotine can be made in many ways but it is still possible to observe a flat unsaturated part of the molecule in a position comparable to the aromatic ring.

It is possible to fit many of the structures (but not the hydroxyethyl part of lobeline) to the pharmacophore triangle described by Sheridan *et al.* (1986), but it is necessary to assume that in some molecules there is a conformational change (Table 4), e.g. with

Figure 5 A comparison of (−)-nicotine hydrogen iodide with: (a) *m*-hydroxyphenylpropyl trimethylammonium bromide (Barlow *et al.*, 1989). Activity F = 50, G = 1.25. (b) Choline phenyl ether (Celikel *et al.*, 1980). Activity F = 1.3, G = 1.25. (c) 1,1-Dimethyl-4-phenylpiperazinium iodide (DMPP: Chothia & Pauling, 1978). Activity G = 1 (but blocks muscarinic receptors), B = 0.02–0.2. (d) Coryneine iodide (Barlow *et al.*, 1989). Activity F = 12, G = 0.8. (e) Acetylcholine bromide (Svinning & Sorum, 1975). (f) Anatoxin (Huber, 1972; Koskinen & Rapoport, 1985): the structure shown is N-acetyl-anatoxin with the acetyl group removed.

The scale shows 1 Å (100 pm). The approximate potency relative to (−)-nicotine on the frog rectus is indicated by F (Barlow *et al.*, 1969), on guinea-pig ileum for effects at parasympathetic ganglia by G (Barlow & Franks, 1971; Barlow *et al.*, 1974) and in binding to rat brain by B (Wonnacott, 1987, with activities calculated from IC_{50} values given in Table 2).

Table 4 Comparison of the pharmacophore triangle with crystal structures: ± 0.3 represents the 'superposition tolerance'

	Atoms			*Distance* (Å)		
Compound	*A*	*B*	*C*	*AB*	*AC*	*BC*
Sheridan *et al.* (1986)				4.8 ± 0.3	4.0 ± 0.3	1.2
Nicotine HI	N	N	X*	4.74	3.78	1.4
Cytisine	N	=O	C(=O)	4.93	4.33	1.24
				4.87	4.24	1.24
Lobeline HCl						
Keto	N	=O	C(=O)	4.18	3.80	1.21
(CHOH−)	N	O(H)	C(OH)	2.93	3.14	1.44
Coryneine	N	*m*−O	*m*−C	6.83	5.94	1.36
(Rotated)				4.86	4.33	1.36
m-Hydroxyphenylpropyl trimethylammonium						
	N	*m*−O	*m*−C	8.39	7.35	1.34
(Rotated)				4.3	4.0	1.34

* Centroid of the pyridine ring (c.f. Sheridan *et al.*, 1986). The pharmacophore triangle used was that described by Sheridan *et al.* (1986).

coryneine iodide and *m*-hydroxyphenylpropyl trimethyl ammonium iodide, as with nicotine, the phenyl rings must be rotated. However, attempts to identifiy particular groups, other than the onium nitrogen atom, which are associated with activity have always been accompanied by argument (see, for instance, Hey, 1952; Barlow & Hamilton, 1962b; Barlow & Franks, 1973; Barlow *et al.*, 1969; 1974). While it is possible, for instance, that the carbonyl group in acetylcholine or 4-ketopentyl trimethylammonium can interact with a hydrogen donor group in the receptor, as postulated by Beers & Reich (1970), it is difficult to identify specific hydrogen acceptor groups in some of the other active compounds shown in Figure 5. Even if it is argued that *m*-hydroxyphenylpropyl trimethylammonium could act as a hydrogen acceptor as well as a hydrogen donor, the unsubstituted compound, phenylpropyl trimethylammonium has considerable activity on the frog rectus, being more active than choline phenyl ether (Barlow & Franks, 1973).

With the elucidation of the primary sequence of nicotinic receptor binding subunits it has been possible to try to identify particular groups which may be involved in the actions of agonists. The idea (Noda *et al.*, 1982) that the aspartate residue (138) interacts with the onium group of agonists now seems likely to be incorrect and some group in the region near the cysteine residues (192 and 193) seems much more probable. These groups are involved in the binding of α-bungarotoxin (Ralston *et al.*, 1987; Gotti *et al.*, 1988) and lophotoxin (Abramson *et al.*, 1988) and are labelled by a photo-affinity ligand (Dennis *et al.*, 1988). The adjacent tyrosine residue (190) is also labelled and there are aspartate residues nearby (195 and 200), though these are not among the major labelled products.

Although the precise geometry of the binding site is not yet known there is a fair indication of the groups likely to be involved (and the results obtained in this work at least provide coordinates for ligands which may be used in molecular modelling with the relevant part of the nicotinic receptor). These groups are consistent with the idea that agonist activity at nicotinic receptors may be associated with the charged nitrogen atom and an area of planarity such as an aromatic ring, rather than specific points such as in Sheridan's 'triangle'. This was suggested by Barlow *et al.* (1989) when describing the X-ray crystal structures of several phenolic quaternary ammonium salts with nicotine-like activity, some of which are included in Figure 5. The planar groups in the binding site could be tyrosine or phenylalanine and the idea that agonist activity involves a point and an area (rather than distinct points) may also explain why the enantiomeric potency ratio of nicotine is very variable.

Although natural (−)-nicotine is more active than (+)-nicotine in many tests, the potency ratio varies from 3:1 for neuromuscular block of the cat tibialis anterior muscle to 42:1 at parasympathetic ganglia in guinea-pig ileum (Barlow & Hamilton, 1965). Higher ratios have been obtained in binding experiments for sites in brain (88:1, Wonnacott, 1986; 177:1, Wonnacott, 1987). Indeed, from the existence of distinct groups of neuromuscular blocking and ganglion-blocking drugs it has long been known that the nicotinic receptors at these sites must be different.

Interactions involving a point and a flat area

would have no asymmetry if the point lies vertically above the area, but if it lies to either side of the vertical (as does the pyrrolidine nitrogen relative to the pyridine ring in nicotine in Figure 3a) there is asymmetry. The enantiomeric potency ratio of nicotine would, therefore, depend on the position of the onium binding group relative to the flat binding area (possibly tyrosine or phenylalanine), which could well differ from one type of receptor to another. The results obtained with cytisine and, to a lesser extent, with lobeline support the idea that the activity of agonists at nicotinic receptors, as well as involving the onium group, may also involve interactions between flat areas in drug and receptor. In addition to hydrophobic effects, binding could involve pi electrons in aromatic systems in compounds like nicotine, cytisine, *m*-hydroxyphenylpropyl trimethylammonium, coryneine and leptodactyline (not included in Figure 5), or double bonds or systems of double bonds in compounds such as acetylcholine, anatoxin, and 4-ketopentyl trimethyl ammonium iodide and unsaturated esters of choline such as murexine (not included in Figure 5: review, Barlow, 1964) and *iso*-arecolone methiodide (Spivak *et al.*, 1986; Waters *et al.*, 1988).

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