Stereoselective LSD-like Activity in d-Lysergic Acid Amides of (R)- and (S)-2-Aminobutane

Robert Oberlender,1 Robert C. Pfaff,1 Michael P. Johnson, Xuemei Huang, and David E. Nichols*

Departments of Medicinal Chemistry and Pharmacognosy and Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907. Received May 15, 1991

The (R)- and (S)-2-butylamides of d-lysergic acid were prepared and evaluated in behavioral and biochemical assays of 5-HT₂ agonist activity. In rats trained to discriminate 0.08 mg/kg LSD tartrate from saline, both isomers completely substituted for the training stimulus. Similarly, both isomers were found to possess very high affinity in displacing [125I]-(R)-DOI ([125I]-(R)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) from rat cortical homogenate 5-HT₂ receptors and in displacing [3H]-8-hydroxy-2-(di-n-propylamino)tetralin) from rat hippocampal 5-HT₁A receptors. The difference in activity between the two isomeric amides was significant in both the behavioral and binding assays, with the R isomer possessing greater potency. Molecular mechanics were used to predict the active geometries of the subject compounds. It was found that the (R)-2-butylamide has a conformation quite similar to LSD, while the (S)-2-butylamide does not. These results suggest that stereochemical properties of the amide substituent of hallucinogenic lysergamides may exert a critical influence on activity. It is concluded that the conformation of the amide function may directly affect binding through stereoselective interactions with a hydrophobic region on the receptor, indirectly by inducing conformational changes elsewhere in the molecule, or by a combination of these two mechanisms.

From studies relating structure and activity, it was noted more than 30 years ago that minor molecular modifications in ergot alkaloids produce surprisingly great changes in pharmacological effect.1 Included among these compounds are various amides of lysergic acid. In the years following Hofmann's synthesis and discovery of the hallucinogenic properties of the N,N-diethylamide of d-lysergic acid (LSD, 1),2 a large number of chemical modifications were investigated in structure-activity relationship (SAR) studies. As discussed previously,3 the results of these early studies generally confirmed that hallucinogenic potency in the lysergamide series seemed to be optimized in 1. Although very few new compounds have been prepared in SAR studies of hallucinogenic lysergamides in recent years, evidence that 1 may not possess uniquely high potency has been reported. Derivatives in which various alkyl groups replaced the N(6)-methyl of 1 were identified that retained or even surpassed the potency of 1.4-7 Of these compounds, the N(6)-n-propyl derivative was found to be equipotent to 1, while the N(6)-ethyl and -allyl derivatives were more potent than 1 in a rat behavioral model.5

In view of a renewed interest in hallucinogenic agents, related to the recent emphasis in the neurosciences on the role of serotonin in behavior,8 further studies of 1 SAR using more up-to-date techniques were thought to be quite valuable. Previous work regarding stereochemical aspects of lysergamide pharmacology has demonstrated that inversion of the configurations of either of the two chiral centers (5R,8R) of 1 results in loss of activity. That is, the 5S,8S, 5S,8R, and 5R,8S isomers were inactive in man as...
This demonstrates the high degree of stereoselectivity in the ergoline ring. Very little is known, however, about possible stereochemical influences on the interaction between the amide moiety of hallucinogenic lysergic acids and the biological target sites of these drug molecules.

The focus of this study was directed toward evaluating potential stereoselective effects of 1-like lysergamides containing a chiral N-alkyl substituent. The simplest of these are the N-2-butylamides of d-lysergic acid (2 and 3). These stereoisomers are novel compounds with a molecular weight identical to, and size very similar to the diethylamide (1). Although the mixture of 2 and 3 has previously been reported for analytical purposes, the isolomers had not been separated, nor had any biological data been reported for the individual isomers. Therefore, the DD paradigm was employed as a means of evaluating these chiral analogues in rats. In addition, receptor binding studies were employed as an in vitro measure of the 5-HT2 and the 5-HT1A receptor interactions for the two chiral lysergamides synthesized in this experiment, relative to 1. As in previous studies, this was measured as affinity for [3H]-DOPI-(1H]-DOPI-(R)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane)-labeled 5-HT2 receptors in rat cortical homogenates and as affinity for [3H]-8-OH-DPAT (1H]-8-hydroxy-2-(dipropylamino)tetralin)-labeled 5-HT1A receptors in rat hippocampal homogenates. Since [3H]DOPI-(R)-DOPI also binds with high affinity to 5-HT1C receptors (see e.g. ref 8) it is possible that this receptor subtype is involved in hallucinogenesis. However, our focus has been on the more prevalent view that the 5-HT2 subtype is more critical.

Chemistry

The syntheses involved the amide-forming condensation of the appropriate amines with d-lysergic acid. The classical methods for preparing amides by acylation of amines with esters or acid chlorides were known to fail when applied to lysergic acid. The condensation was accomplished using the practical and efficient method of Johnson et al., in which phosphorus oxychloride and the amine were added simultaneously to a chloroform suspension of lysergic acid at reflux, was employed for the more bulky 2-butylamines. After workup, the normal (8S) isolomers were purified by centrifugal chromatography (chromatotron) as described previously. The diastereomeric 2 and 3 could also be separated using the chromatotron. Data from analyses utilizing 1H NMR, IR, and high-resolution mass spectrometry were all consistent with this structural assignment and agreed with relevant data previously reported for the unseparated mixture of 2 and 3.

Pharmacology

Using methods described previously, compounds 1, 2, 3, and 4 were evaluated in the two-lever drug discrimination assay in a group of rats trained to discriminate the effects of ip injections of saline from those of 1 (LSD) tartrate (0.08 mg/kg, ip). Potencies were measured using ED90 values with 95% confidence intervals for those compounds that completely substituted for 1. The methods used in binding experiments have also been described in earlier reports. Briefly, the ability of 1, 2, and 3 to displace 0.25 nM [3H]-DOPI from rat frontal cortex homogenate and 1.0 nM [3H]-8-OH-DPAT from rat hippocampus homogenate was measured.

Molecular Modeling

Knowledge of the biologically active conformations of lysergic acid derivatives would clarify understanding of their hallucinogenic potencies and of the nature of the
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5-HT2 receptor. Although lysergamides are relatively rigid, they do have conformational flexibility in two places, the amide substituent and the D ring. In particular, the D ring of 1, and of most related ergolines, has been found to exist in the C(7) "flap-up" conformation in CDCl3 solution24-28 as well as in the crystal structure of 1-p-iodobenzoate monohydrate.27,28 However, Pierri et al.29 have reported that ergotamine exists in the C(7) "flap-down" conformation. The position of the amide substituent is variable and considered fluxional within the bounds of hydrogen bonding and steric influences.

Classical molecular mechanics were performed on 1, 2, and 3. The molecular flexibility of the lysergamides is principally described by two dihedral angles, identified in structure 1. In addition, the 2-butyllysergamides, 2 and 3, exhibit a third important torsion angle, identified in structure 2.

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\begin{align*}
\tau_1 &= C(5)-N(6)-C(7)-C(8) \\
\tau_2 &= C(7)-C(8)-C(18)-O(19) \\
\tau_3 &= C(18)-N(20)-C(21)-C(22)
\end{align*}
\]

As such, \(\tau_1\) describes the envelope conformation of the D ring, with positive values of \(\tau_1\) corresponding to flap-down conformations and negative values of \(\tau_1\) corresponding to flap-up conformations. The angle \(\tau_2\) describes the position of the amide carbonyl group relative to the D ring, with values of \(\tau_2\) in the approximate range -90° to 90° corresponding to the amide carbonyl pointing above the D ring (\(\beta\)) and values of \(\tau_2\) in the approximate range -90° to -180° and 90° to 180° corresponding to the carbonyl pointing below the D ring (\(\alpha\)). The angle \(\tau_3\) measures the position of the amide alkyl group relative to the carbonyl. All three dihedral angles were investigated by performing geometry searches, or grid scans, with minimization at each step. The structures as drawn illustrate the compounds with \(\tau_1 \approx 65°\), \(\tau_2 \approx -90°\), and \(\tau_3 \approx 0°\).

**Results and Discussion**

The results of the drug discrimination testing are given in Table I. The two 2-butylamides, 2 and 3, and the pyrrolidine, 4, completely substituted for 1. All eight rats tested with saline selected the saline lever. Based on the 95% confidence intervals, 1 and 2 were of comparable potency, which was significantly greater than the potencies of 3 and 4. The latter two compounds did not have significantly different potencies in substituting for 1. In terms of disruptive effects, none of the test compounds produced large numbers of disruptions at stimulus-generalization doses. The compound which produced the highest degree of disruptive effects was 4.

The results suggest that stereochemical differences in the amide substituent of lysergamides may have a significant influence on hallucinogenic activity, as reflected in the DD model. The relative potency of 4 (16% of 1) is in agreement with clinical evaluations (5–10% of 1),24,30 thereby lending support to the validity of this model. It is particularly noteworthy that the (R)-2-butyl group of 2 represents the first amide substituent to give a lyseramide with potency comparable to that of 1. This is the first indication that the diethylamide may not in fact be "unique." In addition, the sensitivity of activity to the amide substituent is not merely related to the size or physicochemical properties of this portion of the molecule. Since the two 2-butylamide isomers differ only in the spatial arrangement of identical atoms, amide conformational properties are likely of significance in optimizing interactive forces at the receptor(s) involved in 1-like actions.

This is partially supported by the binding affinities for the test compounds using [3H]-[(R)-DOI] and [3H]-8-OH-DPAT as the radioligands (Tables II and III). Both 2 and 3 have high affinity for the 5-HT2 and 5-HT1A sites. It is noteworthy that the difference observed in the potency of the 1-like DS properties of these compounds is mirrored by their significantly different affinities for [3H]-[(R)-DOI]-labeled 5-HT2 binding sites and their affinities for [3H]-8-OH-DPAT-labeled sites. In both cases the (R)-2-butyllysergamide (2) had higher affinity than the (S)-2-butyllysergamide (3). However, the higher affinity of 2 compared to 1 at both sites was not reflected in the behavioral potency, where 2 and 1 were approximately equipotent, while 3 was significantly less potent than either drug. The reasons for this are not clear, although this may indicate that factors other than 5-HT2 and 5-HT1A interactions are involved. For instance, it is possible that, compared to the hallucinogenic phenethylamines, lysergamides may produce DS effects that involve important contributions from activity at receptors other than the 5-HT1A and/or 5-HT2 subtypes. Alternatively, if I-like drugs are acting as partial agonists at 5-HT receptors (e.g.,...
As anticipated, two minima were identified, corresponding to the C(7) flap-up (–65°) and the C(7) flap-down (+65°) conformations. Conformational energy increases toward the strain of flattening of the D ring. The slight discontinuity observed at 0–10° is an artifact of N(6) inversion, moving the methyl from β-pseudoequatorial to α-pseudoequatorial.

of the difference in potency between 2 and 3 is consistent with the idea that the amide substituent interacts with a sterically sensitive region of the receptor. The existence of such a hydrophobic region which can accommodate, in a predictable way, the substituents of the amide nitrogen has been suggested previously by Dunn and Bederka.33 These workers also speculated that the conformational rigidity of cyclic amides may impair receptor contact. The results of the present study suggest that if a hydrophobic bonding site is involved, stereochemical aspects of the amide alkyl groups that interact with it exert an important influence on the drug–receptor interaction.

Alternatively, the alkyl group(s) of the amide substituent may indirectly affect binding by influencing the conformation of another portion of the molecule. For example, the orientation of the carbonyl oxygen of 1 is probably influenced by factors including the relative stability of the possible amide N-alkyl conformations. The various optimally stable positions adopted by the different alkyl groups in the lysergamide series may force the carbonyl oxygen to reside in slightly different orientations in each derivative. It is conceivable, therefore, that the diethylamide of 1 may help to orient the carbonyl oxygen in such a way as to optimize hydrogen-bond formation between its unshared electron pairs and a proton of one of the amino acids which comprise the receptor. This possibility has been discussed previously by Kumbars24 in an empirically derived conformational analysis of 1 and several other lysergamides. It was found in that study that the directionality of the carbonyl oxygen, observed in 1, changed when the diethyl groups were substituted with other alkyl substituents. The angle of deviation from the preferred carbonyl conformation of 1 correlated with hallucinogenic potency, thus providing support for the importance of a hydrogen-bonding interaction involving the oxygen.34 It is clear from our study, for example (Figure 3), that the carbonyl will resist orientations where $\tau_2$ is near $45^\circ$ or $180^\circ$.

However, from our modeling data it appears that the differences in potency and binding shown by these compounds are not directly due to conformational energy considerations. Stereoviews of the fully minimized structures of the free bases of 1, 2, and 3 (shown in Figure 5) indicate that all three possess virtually identical con-

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Figure 3. Energy of carbonyl torsion ($\tau_2$) for LSD (1) and 2-butyllysergamide free bases 2 and 3.

Figure 4. Energy of amide torsion ($\tau_3$) for 2-butyllysergamide free bases 2 and 3 in the flap-up conformation.
formations, differing most in the positions of the amide alkyl groups. Figure 6 illustrates the most detailed comparison of the D ring and amide functionalities.

In view of the conformational similarities identified for 1, 2, and 3, it seems most probable that the differences in measured affinity are rooted in subtle steric effects. In terms of the portions of the 2-butyl group directed toward the β-face of the molecule (up in Figure 6), 2 has an ethyl extended up, similar to 1. By contrast, 3 has a propyl extended up. Therefore, the β-face of 2 seems similar to that of 1, while the β-face of 3 is considerably different. This may suggest that neither the 5-HT₂ nor the 5-HT₁A receptor can optimally accommodate a group larger than ethyl on the β-side of the molecule.

In addition, the role of the lone pair electrons of the tertiary nitrogen atom in the ergoline D ring has been discussed extensively with respect to binding forces of the drug–receptor interaction. Variations of the amide

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**Figure 5.** Stereoviews of fully minimized structures: (a) LSD (1), (b) (R)-2-butyllysergamide (2), and (c) (S)-2-butyllysergamide (3) as the free bases in the flap-up conformation.

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alkyl substituent may also affect the conformation of the
D ring and consequently the directionality of the nitrogen
lone pair electrons. In fact, the rigidity of 1 tends to
increase the importance of conformational properties of
the amide as influential in the determination of the shape
of the molecule as a whole.

In conclusion, the present study has demonstrated that
stereochemical properties of the amide substituent of
lysergamides exert a significant influence on potency in
producing 1-like discriminative stimulus effects in rats.
Since this behavioral paradigm seems to provide a rea-
sonably good model for human hallucinogenic activity, a
similar influence on the latter may also exist. Receptor
affinities are also sensitive to stereochemical effects of
the amide substituent. The conformation of the amide func-
tion may directly affect binding through stereoselective
interactions with a hydrophobic region on the receptor, or
indirectly by inducing conformational changes elsewhere
in the molecule.

Experimental Section

Melting points were determined with a Thomas-Hoover ap-
paratus and are uncorrected. 1H NMR (200 MHz) were obtained
in CDCl3 with a Chemagnetics A-200 spectrometer. Chemical
shifts are reported in 6 values (parts per million) relative to an
internal reference of tetramethylsilane (δ 0). Abbreviations used
in NMR analysis are as follows: br s = broad singlet, s = singlet,
d = doublet, t = triplet, m = multiplet. Elemental analyses were
performed by Galbraith Laboratories, Knoxville, TN, and
were within ±0.4% of the calculated values.

N-((R)-1-Methylpropyl)-9,10-didehydro-6-methyl-
ergoline-82-carboxamide (2). (+)-Lysergic acid monohydrate
(150 mg, 0.52 mmol) and 25
ml of dry, ethanol-free CH2Cl2
were placed in a flame-dried 50 mL, three-necked, round-bottom flask
equipped with a condenser, N2 line, and septa inlets. The stirred
slurry was brought to reflux in a preheated 90 °C oil bath after
which 384 mg (5.2 mmol) of (R)-(-)-2-butylamine (Aldrich) in 1.0
mL of CH2Cl2 and 160 mg (1.04 mmol) of POCl3 were added
simultaneously, via syringes, over 3 min. The mixture was allowed
to stir at reflux for an additional 5 min and was then cooled to
room temperature. The clear amber CH2Cl2 solution was then
washed with 1 M NH4OH (3 × 30 mL) and brine (1 × 10 mL)
and dried (Na2SO4). The drying agent was removed by filtration,
and the solution was concentrated in the dark by rotary evap-
oration at 30 °C.

The residue was purified and fractionated by radial centrifugal
chromatography (Chromatotron, Harrison Research) using a silica
gel rotator and eluting with ethyl acetate in an H2O-ammonia at-
mosphere. TLC (silica gel, EtOAc-NH3) showed a large blue
fluorescent product spot at 1R, 0.21 corresponding to the (R)-
butyllysergamide and a much smaller spot at 1R, 0.15 corre-
sponding to the S isomer. The faster moving component was col-
clected and concentrated by rotary evaporation. The residue was
then taken up in CH2Cl2, washed with H2O, and dried (MgSO4),
and the CH2Cl2 was removed by rotary evaporation followed by pumping
under high vacuum. The free base (158 mg, 94% yield) was taken
up into 2 mL of methanol, and 67 mg of maleic acid in 0.75 mL
of methanol was added. The maleate salt (1:1 stoichiometry
spontaneously crystallized as a white crystalline solid: mp 210
°C dec; 1H NMR (free base, CDCl3) δ 0.91 (t, J = 7.5 Hz, 3 H),
1.13 (d, J = 6.7 Hz, 3 H), 1.36–1.56 (m, 2 H), 2.68–2.82
(m, 2 H), 3.05–3.13 (m, 1 H), 3.31–3.56 (m, 3 H), 3.89–3.98
(m, 1 H), 6.45 (s, 1 H), 6.60 (d, J = 8.0 Hz, 1 H), 6.91 (s, 1 H),
7.12–7.22 (m, 3 H), 8.15 (br s, 1 H); [α]D = +48° (c = 0.1, H2O); IR (free
base, neat) 1650 cm–1 (C=O). Anal. (C26H28N2O5) C, H, N.
N-((S)-1-Methylpropyl)-9,10-didehydro-6-methyl-
ergoline-82-carboxamide (3). An exact replication of the above
procedure using 150 mg of (+)-lysergic acid monohydrate and 384
mg of (S)-(-)-2-butylamine (Aldrich) gave 154 mg (91.7% yield)
of the free base. The free base in methanol was combined with
55 mg of maleic acid in methanol to again yield a white crystalline
solid with 1:1 stoichiometry: mp 213 °C dec; 1H NMR (free base, CDCl3) δ 0.93 (t, J = 7.5 Hz, 3 H), 1.13 (d, J = 6.7 Hz, 3 H),
1.37–1.58 (m, 2 H), 2.60 (s, 3 H), 2.66–2.82
(m, 2 H), 3.05–3.13
(m, 1 H), 3.31–3.56 (m, 3 H), 3.89–3.98
(m, 1 H), 6.45 (s, 1 H),
6.80 (d, J = 8.0 Hz, 1 H), 6.92 (s, 1 H), 7.12–7.22 (m, 3 H), 8.02
(br s, 1 H); [α]D = +59° (c = 0.1, H2O); IR (free base, neat) 1650
cm–1 (C=O). Anal. (C26H28N2O5) C, H, N.

Twenty male Sprague–Dawley rats (Harlan Laboratories, Indi-
anapolis, IN) weighing 200–240 g at the beginning of their use
as experimental subjects were employed for this study. These
rats had previously received drugs and behavioral training as part
of our ongoing investigations.36 Water was freely available in

(38) Weinstein, H.; Green, J.; Osman, R.; Edwards, W. Recognition
and activation mechanisms on the LSD/serotonin receptor:
The molecular basis of structure-activity relationships. In
Quantitative Research Monograph 22; Barnett, G., Trsic, M.,
Willette, R., Eds.; U.S. Government Printing Office: Wash-
their individual home cages, and a rationed amount of supplemental feed (Purina Lab Blox) was made available after experimental sessions so as to maintain approximately 85% of the free-choice weight gain. The temperature of the animal facility remained within the range of 22–24 °C. The humidity was maintained at 40–50% and the lights were on between 6 am and 8 pm.

Apparatus. Six standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA) consisted of modular test cages enclosed within sound-attenuated cubicles with fans for ventilation and background white noise. A white house light was centered near the top of the front panel of the test cage, which was also equipped with two response levers, separated by a food hopper, all positioned 2.5 cm above the floor. Solid-state logic, in an adjacent room, interfaced through a Coulbourn Instruments Dynaport to an IBM PC, controlled reinforcement and data acquisition with locally written software.

Drug Administration. The training drug, 1-(d-LSD) tartrate (NIDA, 156 nmol/kg, 0.06 mg/kg), or saline was administered ip, 30 min prior to sessions. All drugs were administered dissolved in saline such that a volume of 1 mL/kg of body weight was used. Solutions were sterilized prior to use by filtration through a sterile 0.2 μm filter (Millipore) into an autoclaved vial.

Discrimination Training. A fixed ratio (FR) 50 schedule of food reinforcement (Bioserv 45 mg dustless pellets) in a two lever paradigm was used. Initially, rats were taught to lever press on an FT 1 schedule so that one food pellet was dispensed for each press. Half the rats were trained on drug-L, saline-R, and the other half R, saline-L, to counterbalance preference. Training sessions lasted 15 min and were conducted at the same time each day, Monday through Friday. Levers were cleaned with a 10% ethanol solution in order to avoid olfactory cues. Both levers were present during all phases of training, but reinforcements were delivered only after responses on the stimulus-appropriate lever. Presses on the incorrect lever were recorded but had no programmed consequence. After initially learning to lever-press for food, saline and drug sessions were randomly ordered, with neither treatment given more than three consecutive sessions. As responding rates stabilized, the schedule of reinforcement was gradually increased from FR 1 to FR 50. Once at FR 50, training continued until an accuracy of at least 85% (number of correct presses × 100/number of total presses) was attained for 8 of 10 consecutive sessions.

Once criterion performance was attained, test sessions were interspersed between training sessions either one or two times per week. At least one drug and one saline session separated each test session. Rats were required to maintain the 85% correct responding criterion on training days in order to be tested. In addition, test data were discarded when the accuracy criterion of 85% was not achieved on the training sessions following a test session. Test sessions were run under conditions of extinction, with rats removed from the operant box when 50 presses were emitted on one lever. If 50 presses on one lever were not completed within 5 min, the session was ended and scored as a disruption due to a specific activity of 2000 Ci/mmol. [3H]-8-OH-DPAT was purchased from New England Nuclear (Boston, MA) at a specific activity of 185.9 Ci/mmol. The procedure of Johnson et al.37 was employed with minor modifications. Briefly, the frontal cortex and hippocampal brain regions from 10 to 20 male Sprague–Dawley rats (175–190 g, Harlan Laboratories, Indianapolis, IN) were pooled and homogenized (Brinkman Polytron, setting 6 for 2 × 20 s) in 4 or 8 volumes of 0.32 M sucrose for frontal cortex or hippocampus, respectively. The homogenate was centrifuged at 3600g for 10 min, and the resulting pellets were resuspended in the same volume of sucrose. Separate aliquots of tissue were then frozen at −70 °C until assay.

For each separate experiment, a tissue aliquot was thawed slowly and diluted 1 to 25 with 50 mM Tris HCl (pH 7.4). The homogenate was then incubated at 37 °C for 10 min and centrifuged twice at 3600g for 10 min, with an intermittent wash. The resulting pellet was resuspended in 50 mM Tris HCl with 0.5 mM Na2EDTA, 0.1% Na ascorbate, and 10 mM pargyline HCl (pH 7.4). In experiments with [3H]-DOI and DOI, 10 mM MgCl2 was also included. A second preincubuation for 10 min at 37 °C was conducted and the tissues were then cooled in an ice bath.

All experiments were performed with triplicate determinations using the appropriate buffer, to which 200–400 μg of protein was added, giving a final volume of 1 mL. Displacement and saturation experiments were conducted with [3H]-DOI as described in Nichols et al.37 Tubes were allowed to equilibrate for 15 min at 37 °C before filtering through GF/C filters. Specific binding was defined as that displaced with 1 μM DOI, that displaced more than 95% of [3H]-DOI was found to bind to a single site (Hill coefficient of 0.99 ± 0.03) with a Bmax of 46 ± 3 fmol/mg protein and a KD of 1.34 ± 0.12 nM. The ability of the test drugs to displace 0.25 nM [3H]-DOI was determined. Filters were allowed to air dry before counting with a gamma counter.

Displacement and saturation experiments were conducted with [3H]-8-OH-DPAT according to the methods of Gozlan et al.35 Tubes were allowed to equilibrate for 10 min at 37 °C before filtering through GF/C filters. Specific binding was defined as that displacable with 10 μM serotonin. Under these conditions [3H]-8-OH-DPAT was found to bind to a single site (Hill coefficient of 1.00 ± 0.01) with a Bmax of 119 ± 8 fmol/mg protein and a KD of 2.49 ± 0.23 nM. The ability of the test drugs to displace 1.0 nM [3H]-8-OH-DPAT was determined. Filters were air-dried and placed in vials, 10 mL of scintillation fluid was added (Budget Solv, Research Products International, Mount Prospect, IL) and left to set overnight before counting. At least seven samples for 125I and 34% for 3H, binding parameters were determined using the computer programs ERBA and LIGAND as described elsewhere. The values from three to four separate experiments were combined. Protein determinations were made using the procedure of Bradford.44 Free energy of binding at 37 °C (310 K) was estimated from ΔG° = −RT ln (1/KD).

Molecular Modeling. Preliminary computations were performed on a Silicon Graphics Personal Iris workstation running Polygen Quanta and CHARMM molecular mechanics software. Further calculations were done using the programs CNINDO (Quantum Chemistry Program Exchange program 141) and AMPAC

The Three Binding Domain Model of Adenosine Receptors: Molecular Modeling Aspects

Michael J. Dooley and Ronald J. Quinn*

Division of Science and Technology, Griffith University, Brisbane, Queensland 4111, Australia. Received April 15, 1991

Using molecular modeling, adenosine receptor ligands were fitted together to maximize correlations between the three most important factors controlling binding to the receptor, namely steric, hydrophobic, and electrostatic complementarity. Structure-activity relationships can be explained by three binding domains on the receptors. These are hydrophobic, aromatic, and ribose binding domains. We propose that the N6, C2, and C8 hydrophobic binding domains are not discreet but occupy the same region of the receptor.

Adenosine (1) is a naturally occurring endogenous nucleoside which has generated much interest due to its biological activity. Much of this activity is mediated via membrane-bound extracellular receptors which bind adenosine, its analogues, alkylxanthines, and various miscellaneous heterocycles.1-3 Pharmacologically distinct receptors which inhibit (A1) or stimulate (A2) adenylate cyclase activity have been identified on the basis of differing structure-activity profiles. Interpretations of structure-activity relationships, based on the structure of (R)-(phenylisopropyl)adenosine and other N6-substituted analogues, have resulted in details of the N6 binding domain. The four atoms of the alkylamino moiety of (R)-(phenylisopropyl)adenosine lie in a Y-shaped groove, there is a site to fit the phenyl ring and in close proximity, capacious binding domains called S1 and S1-A based on evidence for little stereoselectivity and the ability to bind three carbons in this area.4 There is a potential hydrogen-bond acceptor next to this N6-subregion.4 Novel N6-bicyclo[2.2.1]alkyldenosines with unusually high potency allowed the receptor-excluded volume to be probed in considerable detail.5 The sophistication of the model was increased using computer molecular modeling.6 A molecular mechanics calculates energies relative to a hypothetical "perfect" geometry, rather than an absolute energy, and uses a temperature of 0 K. The structures cited here are those obtained from molecular mechanics, and the structure of LSD is in good agreement with the reported X-ray results.27 Calculations were performed on the free bases as well as on the N(6)-protonated cations, in vacuo, and no attempt was made to consider the effect of solvation. In addition, conformations where the N(6)-methyl was equatorial were of lower energy than those in which the methyl was axial. Therefore, only the equatorial conformations were used in the analyses. As stated above, the molecular flexibility of 1, 2, and 3, is described primarily by the torsion angles \( \tau_1, \tau_2 \), and \( \tau_3 \). All three dihedral angles were investigated on the CAChe system by performing geometry searches or grid scans, with minimization at each step. In these searches, the molecular mechanics settings were as stated above. The angle \( \tau_1 \) was searched over the range \(-70^\circ\) to \(0^\circ\) and \(+70^\circ\) to \(0^\circ\), in 5-deg increments. This angle was scanned from both directions in order to better control the inversion of N(6) and to maintain the equatorial position of the N(6)-methyl. Similarly, \( \tau_2 \) and \( \tau_3 \) were searched over the range \(-180^\circ\) to \(+180^\circ\), in 15-deg increments.

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Registry No. 2, 137765-82-3; 2-maleate, 137765-83-4; 3, 137765-84-5; (+)-lysergic acid, 82-58-6; (R)-(−)-2-butylamine, 13250-12-9; (S)-(−)-2-butylamine, 513-49-5.

v1.21 (Quantum Chemistry Program Exchange program 506). Final computations were performed on a Tektronix CAChe worksystem running Tektronix proprietary software6 including their implementations of ZINDO v2.2 and MOPAC v5.10.24 CAChe molecular mechanics uses Allinger's MM2 force field40 as augmented by Tektronix. With both AM1 and MOPAC, the AM1 Hamiltonian40 was used.

The molecular mechanics of 1, 2, and 3 were investigated on the CAChe system41 by minimizing the total molecular energy according to the molecular mechanics expression

\[
E_{\text{total}} = E_{\text{bonding}} + E_f + E_{\text{improp}} + E_{\text{dihedral}} + E_{\text{water}} + E_{\text{Ha}}
\]

where \( E_{\text{bonding}} \) describes bond lengths, \( E_f \) describes bond angles, \( E_{\text{improp}} \) describes improper torsions, \( E_{\text{dihedral}} \) describes dihedral angles, \( E_{\text{water}} \) describes the van der Waals interactions, and \( E_{\text{Ha}} \) describes hydrogen bonding. Further, molecular mechanics uses Allinger's MM2 force field40 as augmented by Tektronix. With both AM1 and MOPAC, the AM1 Hamiltonian40 was used.

As stated above, the molecular flexibility of 1, 2, and 3, is described primarily by the torsion angles \( \tau_1, \tau_2 \), and \( \tau_3 \). All three dihedral angles were investigated on the CAChe system by performing geometry searches or grid scans, with minimization at each step. In these searches, the molecular mechanics settings were as stated above. The angle \( \tau_1 \) was searched over the range \(-70^\circ\) to \(0^\circ\) and \(+70^\circ\) to \(0^\circ\), in 5-deg increments. This angle was scanned from both directions in order to better control the inversion of N(6) and to maintain the equatorial position of the N(6)-methyl. Similarly, \( \tau_2 \) and \( \tau_3 \) were searched over the range \(-180^\circ\) to \(+180^\circ\), in 15-deg increments.

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Registry No. 2, 137765-82-3; 2-maleate, 137765-83-4; 3, 137765-84-5; (+)-lysergic acid, 82-58-6; (R)-(−)-2-butylamine, 13250-12-9; (S)-(−)-2-butylamine, 513-49-5.

1 CAChe Version 2.5, Tektronix, Inc., 1990.
2 MOPAC v5.10 settings: optimization by the AM1 Hamiltonian; singlet multiplicity; C1 level = default; maximum SCF cycles = 200; BFGS converger, XYZ coordinates. ZINDO v2.2 settings: energy only; singlet multiplicity; maximum SCF cycles = 200; INDO/1; SCF type = RHF.
5 CAChe Molecular Mechanics v2.5 settings: optimization by the block-diagonal Newton-Raphson method; relaxation factor = 1.00; energy value tolerance = 0.001 kcal/mol. Included terms: bond stretch, bond angle, dihedral angle, improper torsion, van der Waals, electrostatic, and hydrogen bonding.

Using molecular modeling, adenosine receptor ligands were fitted together to maximize correlations between the three most important factors controlling binding to the receptor, namely steric, hydrophobic, and electrostatic complementarity. Structure-activity relationships can be explained by three binding domains on the receptors. These are hydrophobic, aromatic, and ribose binding domains. We propose that the N6, C2, and C8 hydrophobic binding domains are not discreet but occupy the same region of the receptor.