Table I—Distribution of Ampicillin (Micrograms per Milliliter or Micrograms per Gram) after Oral Administration in the Rabbit of Anhydrous Ampicillin, Trihydrate Ampicillin, and Metampicillin at 50 mg./kg. as 6-[D(−)-α-Aminophenylacetamido]penicillanic Acid

Hours after Antibiotics Administration	Antibiotics[a]	Plasma	Organs							
			Kidney	Stomach	Duodenum	Liver	Lung	Brain	Muscle	Spleen	Heart
0.5	AA	1.31 ± 0.27	9.32 ± 2.52	20.09 ± 2.37	68.21 ± 9.01	0.42 ± 0.10	0.74 ± 0.17	0.22 ± 0.03	0.65 ± 0.11	0.64 ± 0.09	0.88 ± 0.17
	TA	1.06 ± 0.16	7.57 ± 1.52	19.97 ± 2.72	58.20 ± 6.51	0.58 ± 0.09	0.73 ± 0.06	0.39 ± 0.04	0.46 ± 0.03	0.72 ± 0.12	0.92 ± 0.12
	MA	1.20 ± 0.22	8.12 ± 1.13	25.46 ± 3.78	57.42 ± 5.56	0.34 ± 0.08	0.88 ± 0.10	0.29 ± 0.07	0.59 ± 0.11	0.66 ± 0.10	0.96 ± 0.07
1	AA	2.25 ± 0.29	15.89 ± 2.32	8.69 ± 1.91	18.54 ± 2.02	0.44 ± 0.09	1.94 ± 0.37	0.47 ± 0.11	0.94 ± 0.09	1.26 ± 0.23	1.52 ± 0.20
	TA	2.36 ± 0.34	19.91 ± 3.64	10.39 ± 1.24	22.29 ± 2.41	0.51 ± 0.09	1.86 ± 0.44	0.44 ± 0.04	1.00 ± 0.18	1.79 ± 0.11	1.88 ± 0.31
	MA	2.19 ± 0.38	17.27 ± 2.06	9.70 ± 1.66	21.24 ± 2.62	0.55 ± 0.04	1.67 ± 0.23	0.57 ± 0.12	1.00 ± 0.21	1.33 ± 0.25	1.63 ± 0.29
2	AA	1.16 ± 0.21	10.77 ± 1.62	1.59 ± 0.15	6.82 ± 1.05	0.40 ± 0.12	0.59 ± 0.11	0.26 ± 0.05	0.58 ± 0.06	0.55 ± 0.06	0.59 ± 0.08
	TA	0.95 ± 0.26	10.88 ± 1.33	1.49 ± 0.34	5.30 ± 0.57	0.40 ± 0.11	0.67 ± 0.16	0.32 ± 0.08	0.79 ± 0.25	0.64 ± 0.11	0.53 ± 0.11
	MA	1.11 ± 0.17	8.18 ± 1.10	1.87 ± 0.28	6.29 ± 0.50	0.48 ± 0.09	0.84 ± 0.13	0.22 ± 0.06	0.65 ± 0.08	0.61 ± 0.08	0.68 ± 0.07
4	AA	0.45 ± 0.06	3.66 ± 0.47	0.56 ± 0.10	1.97 ± 0.30	0.06 ± 0.03	0.32 ± 0.07	0.13 ± 0.03	0.42 ± 0.08	0.24 ± 0.07	0.30 ± 0.05
	TA	0.54 ± 0.11	3.80 ± 0.78	0.50 ± 0.07	1.67 ± 0.13	0.12 ± 0.06	0.39 ± 0.13	0.13 ± 0.03	0.40 ± 0.11	0.20 ± 0.03	0.29 ± 0.04
	MA	0.38 ± 0.04	3.28 ± 0.67	0.68 ± 0.12	2.45 ± 0.34	0.11 ± 0.04	0.38 ± 0.07	0.16 ± 0.04	0.47 ± 0.08	0.31 ± 0.06	0.37 ± 0.05

[a] AA = anhydrous ampicillin; TA = trihydrate ampicillin; and MA = metampicillin. $n = 5$.

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Translocation of Ligand Conformational Free Energy in Receptor Activation: A Possible Functional Role of Conformational Isomerism in Drug Action

Keyphrases □ Ligand conformational free energy—receptor activation □ Conformation isomerism relationship—biological activity

Sir:

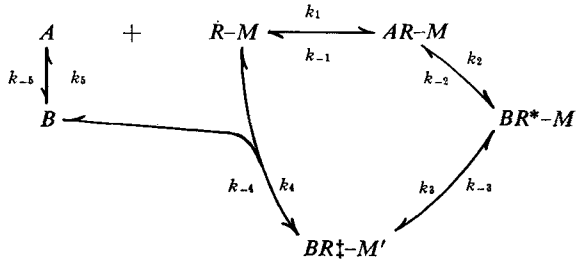
Numerous attempts have been made to define the pharmacophoric conformations of physiologically active molecules (1). Some have postulated that the preferred conformation is the pharmacophoric species (2, 3), while others have concluded that, at least in one case, an energetically unfavorable conformation is bound to the receptor (4–6). On the other hand, Gill (7) implied that more than one conformation may be involved, and Shefter (8) recently pointed out the inadequacies of associating a unique conformation of cholinergic ligands with biological activity. None of these speculations has ascribed a functional role to conformational equilibria in the ligand–receptor interaction.

We wish to propose a model in which conformational free energy of the ligand is utilized to perform work in assisting conformational reorganization of the receptor assembly. The concept described is based on the widely accepted hypothesis (9–12) that a specific conformational change of the receptor assembly gives rise to a biological effect.

The model envisages ligand–receptor association and events leading to depolarization and recovery as a cyclic process (Scheme I). The agonist exists as an equilibrium mixture, $A \rightleftarrows B$, of a preferred (B) and a higher energy (A) conformer. It is assumed that A combines with resting receptor component R faster then does B and that $k_1 \gg k_{-1}$. The receptor component is considered to be a regulatory site (11) which is coupled to one or more macromolecular membrane subunits (M). The activated receptor component (R^*) is capable of triggering a conformational reorganization of M to M', thus giving rise to depolarization.

The salient features associated with various phases of the cycle are as follows:

1. The *higher energy* conformer (A) has greater affinity for R when compared to B; the difference in



Scheme I

affinity resides primarily with association rate constant k_1.

2. Complex *AR–M* undergoes a conformational reorganization, resulting in the transfer of conformational free energy from the ligand to the receptor component. This process involves the decay of high energy conformer *A* to the lower energy conformer *B*, with a concomitant elevation of the receptor component to activated state *R**. It is conceivable that a *BRM* complex may also lead to an activated receptor component (*BR*–M*), although it would occur with greater difficulty because a higher activation energy would have to be surmounted for this process. The difference in activation energy would be equivalent to ΔG for the conformational equilibrium, $A \rightleftarrows B$.

3. Activated receptor assembly *BR*–M* undergoes conformational reorganization to *BR‡–M′*. The energy of *R** is considered to be dissipated in the triggering process. This is accompanied by a conformational change of the activated receptor component to *R‡*, which is in an energy state similar to that of *R*. Depolarization occurs in the *M′* state.

4. The drug–receptor assembly complex (*BR‡–M′*) dissociates to complete the cycle. This may involve several intermediates but, for the sake of simplicity, the model depicts this as a single step. The dissociation of *B* ($k_4 \gg k_{-4}$) is facilitated by the conformational state of the receptor component (*R‡*).

5. The low energy conformer, *B*, which is released from the complex, reestablishes equilibrium with *A* by the extraction of environmental thermal energy.

If this model is interpreted in terms of the rate theory of drug action (4, 6), then the effect of conformational isomerism on biological activity would depend on which step is rate limiting in the cycle. According to this concept, the affinity of the ligand for the receptor will reflect the ratio of k_1 and one of the rate-limiting kinetic constants (k_2, k_3, k_4, or k_5). A complete kinetic description of the model will be presented in a future publication.

The model, therefore, envisages the interaction of neurotransmitter agents with receptors as providing the necessary chemical information to allow this cycle to proceed in a clockwise fashion. The transformation of random thermal energy into a specific energy source (*i.e.*, conversion of the stimulant to a higher energy conformer) is viewed as a means of lowering the activation energy for receptor activation. While the model most probably is not applicable in all cases of conformational isomerism in drug action, it may be relevant to the naturally occurring neurotransmitters, particularly if evolutionary factors are of prime importance in shaping molecules possessing maximum efficiency as chemical messengers.

The model does not rule out the possibility that totally rigid ligands can act as stimulants, although it suggests that such molecules should be less active than the naturally occurring agonist. Unfortunately, there are no reported examples of totally rigid analogs of neurotransmitters. Elaboration and testing of totally rigid ligands might be an approach to testing this model.

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