The preferred conformation of noradrenaline

The structural theory of the adrenergic receptor and transmitters and their complexes is currently in a primitive, though active, state. A recent review (Korolkovas, 1970) summarizes various structural postulates. Rapid advances in computer and mathematical techniques have recently made it possible to consider fairly elaborate quantum mechanical studies on some of the "intermediate" sized biologically important molecules. For instance, the simple but powerful extended Hückel method (Hoffmann, 1963) and the more advanced CNDO/INDO methods (Pople, Beveridge & Dobash, 1967) have been used to study a variety of problems of interest in quantum chemistry in the past several years. Kier (1969) has recently systematically examined the structural properties of neural compounds including noradrenaline with the extended Hückel method. He found that the calculated lowest energy conformation was consistent with his postulates on the structure of the α-receptor site. By examining rotation about the $-HC(OH)CH_2$ bond he found the most stable conformation to be that with the catechol rotated 180° from the $-NH_3^+$. Because of the important question involved here it seemed reasonable to examine the same problem with more sophisticated, yet still tenable, INDO theory suggested by Pople & others. In addition, a study of the dependence of the conformation of the α-receptor portion of the molecule (the $-NH_3^+$) upon chelation of the β-receptor portion of the molecule (the catechol functional groups) was examined.

The INDO method is an approximate molecular orbital method which explicitly treats all valence electrons in the same framework; the σ–π separability approximation of earlier theories is not made, This theory (Pople & others, 1967) along with its predecessor, the CNDO method, have proved successful in predicting geometries, electron paramagnetic resonance hyperfine coupling constants, nuclear spin coupling constants and barriers to internal rotation. The essential approximation made is to assume zero all integrals for which the product $\phi_\nu(1)\phi_\mu(1)$ has ν and μ on different atoms. In particular, the electronic wave function is an antisymmetrized product of molecular orbitals $\psi_i = \Sigma C_{i\nu}\phi_\nu$, composed of Slater atomic orbitals. The self consistent field equations are iterated allowing the α-spin and β-spin molecular orbitals to vary independently until some external criterion on self-consistency (for instance non-changing total energy upon reiteration) is met. This method has proved to be successful in predicting barriers to internal rotation; the barriers of CH_3CH_3, CH_3NH_2 and CH_3OH are found to be in the correct 1:2:3 ratio.

The co-ordinates for the calculation were evaluated using the parameters suggested in the crystallographic study on the noradrenaline HCl derivative (Carlstrom & Bergin, 1967). For the chelated catechol study, a hydrogen was replaced by Li and the Li then placed 0·2 nm from one oxygen and 0·22 nm from the other. Two waters of hydration were then placed in a tetrahedral arrangement (one above, one below the plane of the catechol ring). The Li–H_2O distance was taken to be 0·23 nm (Li–O) and the two water oxygens were taken to be 0·29 nm apart. The total charge on the chelated molecule was then held at +1 as the protonated form.

Fig. 1 displays the dependence of the total energy on the rotation about the $-HC(OH)CH_2$ bond. For noradrenaline two conformations, the 60° and 180° structures, have the same energy with a small barrier of 6·276 kJ (1·5 kcal) between them at 120° rotation. The 180° degree structure agrees with Kier's extended Hückel calculation (Kier, 1968); however, he found this structure to be substantially lower in energy than two other minima which appeared in his results: one at 60° and one at 300°. The 300° minima (60° in the notation of Kier) does not occur in the INDO calculation. The largest barrier (29·288 kJ; 7 kcal) in our calculation occurs at the 240° conformation.

When the $-Li(H_2O)_2$ chelate is substituted for one of the hydrogens, the rotational

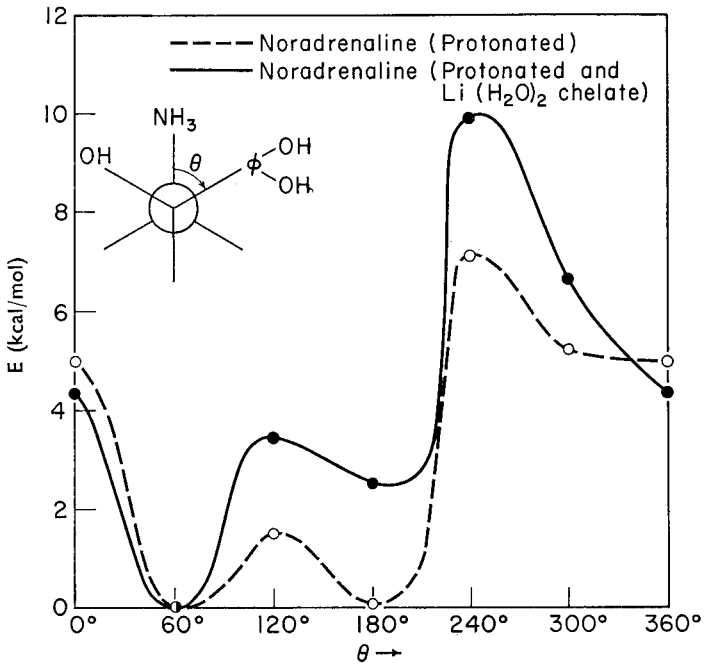

FIG. 1. Energy versus $-CH(OH)-CH_2-$ rotation angle.

minimum at 180° essentially disappears and is replaced by a slight minimum about 10·46 kJ (2·5 kcal) above the 60° minimum which remains. Thus, a change in environment at the β-receptor site can have an important effect on the α-receptor site conformation. The largest barrier for the chelated compound still occurs at the 240° rotation and is increased over the non-chelated case to 41·84 kJ (10 kcal).

The origin of the barrier seems to be a delicate balance of several factors. Examination of the nuclear repulsion term shows that the 180° conformation is most favoured. The attraction between the NH_3^+ group and the alkyl OH will be a maximum at 120° rotation but this is an "eclipsed" configuration. It is therefore not unreasonable that the minimum occurs at either 60° or 180° with a small hump between resulting from eclipsed repulsions. The 240° configuration is the least stable probably because it is an eclipsed configuration and the (NH_3^+)—OH distance (attractive) is quite large.

We conclude that the likely conformation in solution will be the 60° configuration. This assumes a strong interaction through a metal at the β-receptor site and assumes that the actual binding will not be substantially different from that of the "gaseous" molecule we have studied. The 180° well is only 10·46 kJ (2·5 kcal) above the 60° well; thus, a receptor favoring 180° over 60° could, by increased bonding opportunity for the 180° conformation, overcome this relatively small difference. It may be of some interest to point out that Li^+ and Mg^{2+} have almost the same ionic radii and that Mg is thought to be one of the metals binding noradrenaline to ATP. Li has a maximum co-ordination of four whereas Mg has a maximum co-ordination of six. Thus, it may be that Li^+, when given in the treatment of manias, may temporarily increase but eventually decrease the free noradrenaline in the brain by replacing Mg^{2+} and thereby weakening the bonding to the noradrenaline storage region.

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A simple method for the quantitative extraction of dye extravasated into the skin

Increased vascular permeability is usually demonstrated by the leakage of certain dyes injected intravenously. Though the intensity of this reaction has been measured in various ways (Jori, Bentivoglio & Garattini, 1961; Parratt & West, 1958; Frigeni, Gazzanica & Bonanomi, 1970), the best way is to extract and determine the extravasated dye quantitatively. Of several methods for achieving this some do not give satisfactory recovery (Jancsó-Gábor, Szolcsányi & Jancsó, 1967), while others are laborious (Nitta, Hayashi & Norimatsu, 1963; Judah & Willoughby, 1962). The dye can be determined quantitatively by relatively simple procedures (Ankier & Whiteside, 1969; Udaka, Takeuchi & Movat, 1970), but this takes a long time at relatively high temperatures. We previously proposed a method in which the chopped skin is violently agitated in a mixed solvent of acetone and a commercial detergent, Emal, in a homoblendor for 15 min (Harada, Takeuchi & Katagiri, 1966). This method, however, had two demerits; (1) requirement of volume adjustment after the homoblendor process and (2) the use of a detergent that is a mixture of various compounds. Both defects have since been improved as follows. (1) Volume change due to volatilization of acetone was easily prevented by replacing the homoblendor procedure by a 24 h incubation at room temperature with occasional shaking. (2) Each ingredient of the detergent was examined individually and the component effective in the extraction was identified as sodium sulphate. The following experiment demonstrates the usefulness of this improved method.

First, the recovery of dye injected intradermally was examined. Various amounts of azovan (Evans) blue were injected into the skin of rats. After 30 min, each blue area, which was about 10–15 mm in diameter, was erased, cut into about 10 pieces with scissors and mixed with a medium composed of 14 ml of acetone and 6 ml of a 0·5% aqueous solution of sodium sulphate in a test tube. The tube was closely firmly with parafilm and left to stand for 24 h at room temperature (20°) with occasional mild shaking. Each preparation was then centrifuged for 10 min at 300 rev/min and the supernatant separated. Percentage recovery of the dye was calculated by comparing the absorbance of the supernatant at 620 nm with that of a standard sample prepared by mixing the corresponding amount of azovan blue and normal skin pieces in the same medium *in vitro*. The method gave a recovery of over