tracture of the slow skeletal muscle fibres of the frog, in agreement with the previous report from our laboratory[7]. In contrast, even after 10^{-3} g/ml of ouabain, no contractile response could be induced in the slow skeletal muscle fibres of the toad, although the muscle responded to 10^{-6} g/ml of ACh with a definite contracture, just as the slow skeletal muscle fibres of the frog did, indicating that the slow muscle fibres of the toad are not insensitive to acetylcholine. It may, therefore, be concluded that the observed lack of response of rectus abdominis muscle of the toad to ouabain resulted, not from the insusceptibility of the muscle to acetylcholine, but from the insusceptibility of the small motor nerve towards cardiotonic steroids.

2. *Potassium contracture of the slow skeletal muscle fibres.* Potassium contracture (K-contracture) of the slow skeletal muscle fibres was induced in the rectus abdominis muscle by replacing an equivalent amount of NaCl by KCl. It may be seen from Figure 2 that 10^{-6} g/ml of digitoxigenin was without effect on the K-contracture of the slow skeletal muscle of the toad, while there was a clear-cut potentiation of the K-contracture of the slow skeletal muscle of the frog.

Zusammenfassung. Untersuchungen der Wirkung von g-Strophanthin auf das Tonusfasersystem des Krötenherzens ergab selbst bei einer Konzentration von 10^{-3} keine kontraktile Reaktion. Auch die Kalium-Kontraktur der Tonusfasern wurde durch Verabreichung von Digitalis-Verbindungen nicht potentiert.

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Molecular Orbital Studies on the Conformation of γ-Aminobutyric Acid and Muscimol[1]

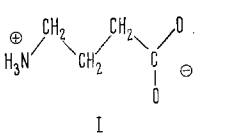
At the present time, a substantial amount of evidence is available which implicates γ-aminobutyric acid (GABA) (I) as a central inhibitory transmitter[2-6]. Evidence has also been presented which indicates that glycine may act as an inhibitory transmitter in the cat spinal cord[7-8] and on neurones in the caudate nucleus[9]. It was recently shown that GABA is considerably more active than glycine as an inhibitor of cortical neurones[10]. Other evidence suggests that glycine is probably not an inhibitory transmitter in the cortex of several species[11].

The role of neutral or neutrally charged endogenous amino acids, including GABA, in abnormal nervous conditions has been long sought[12-14]. These conditions include seizures associated with dietary deficiencies[15], epilepsy[16], and barbiturate abstinence convulsions[17].

It is conceivable that the design of substances with GABA-like activity capable of passing the blood-barrier may provide an approach to the supplementation of GABA deficiencies in these pathological conditions. In this regard, it is of interest that a substance with GABA-like central activity has been recently reported[18]. This compound, muscimol (II), is an isoxazole betaine found in the mushrooms of the genus *Aminata*[19].

The suggestion was made that, with the observation of the GABA-like action of muscimol, the examination of similar molecules with this type of conformational restriction might be rewarding in understanding the interaction of GABA and its receptor[18]. If GABA and muscimol act at the same receptor, and if their preferred conformations are pertinent to this interaction, then both GABA and muscimol should be able to readily exist in conformations in which comparably charged atoms or groups are presented to a receptor in a similar pattern[20].

In the present study, we have undertaken predictions of the preferred conformation of GABA and muscimol using molecular orbital theory. This same approach has been used in this laboratory to predict the conformational preference of numerous physiological mediating agents[20].



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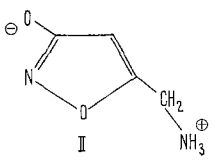

II

[1] Supported by National Institutes of Health (US) Grant No. GM-16312.

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The calculations were made using the molecular orbital procedure of HOFFMANN[21] known as extended HUCKEL theory. The method calculates the total energy of the molecule as a function of the coordinates of all of the atoms. Thus the geometry may be varied in a regular manner and the minimum energy sought in a series of conformations and corresponding total energies. The conformation associated with the lowest calculated energy is predicted to be the preferred conformation.

The calculations indicated that the GABA molecule prefers a conformation in which the onium and methylene groups are all trans or staggered, while the carboxylate is free to rotate 360°. A representative set of evergy vs. angle curves are shown in Figure 1. A range of distance between the nitrogen atom and one oxygen atom was calculated to be 5.0 to 6.1 Å.

The calculations indicated that the muscimol molecule prefers a conformation in which the sidechain is coplaner with the ring and directed toward the ring oxygen, as depicted in II (see Figure 2). In this calculated conformation, the charged exocyclic oxygen atom is 5.8 Å from the onium nitrogen atom.

The structural relationship between the two molecules, with respect to an onium group and an ionized oxygen atom in each molecule, is obvious from the calculations. The $0^{\ominus}$–$N^{\oplus}$ distance in muscimol, 5.8 Å, is well within the range calculated to prevail in GABA between comparable atoms, 5.0–6.0 Å.

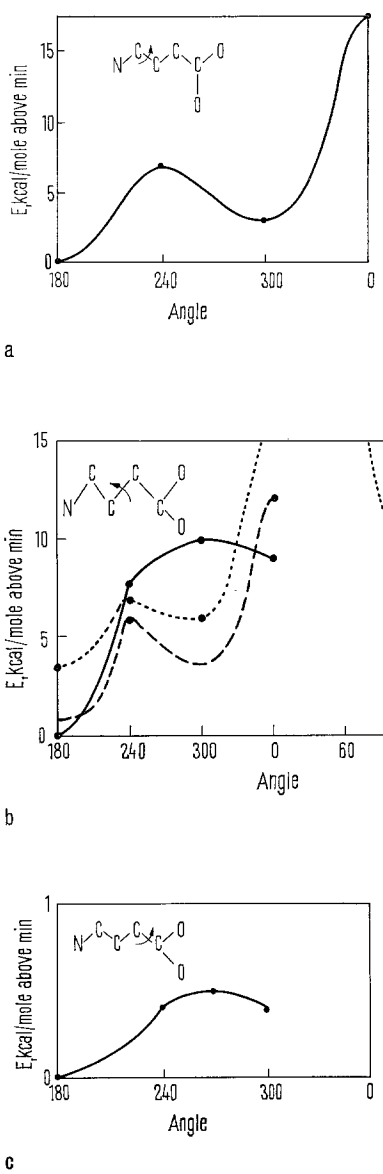

Fig. 1. Calculated energy vs. bond angles in GABA. a) Rotation of first methylene-methylene bond with other bonds in trans positions. b) Rotation of second methylene-methylene bond with other bonds in trans positions (solid line), first methylene-methylene bond trans and carboxylate group 90° from trans (dashed line), and first gauche and carboxylate group 90° from trans (dotted line). c) Rotation of carboxylate group with other bond trans.

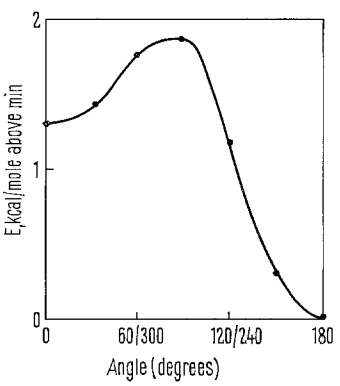

Fig. 2. Calculated energy vs. angle of side chain in muscimol. At 180° the side chain is coplaner with the ring and directed toward the ring oxygen.

The finding of a closely similar pattern of comparably charged atoms in 2 chemically different molecules with the same biological activity suggests that this pattern has a relevance to receptor interaction. We have made this same observation in studies of other potent agonists[20], in which more than one molecule in a pharmacological class was found to possess comparable patterns of charged atoms or groups.

Our calculations on GABA and muscimol lead us to the hypothesis that the central inhibitory receptor requires a molecule possessing at least 2 highly charged regions, of opposite sign, separated by at least 5 Å, but more likely approximately 6 Å.

Zusammenfassung. Mit Hilfe der Molecular Orbital Theorie werden die bevorzugten Konformationen für die γ-Aminobuttersäure und das strukturell verwandte Muscimol, die neurologisch bedeutsam sind, berechnet.

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