Volume 231, number 1, 5–10 FEB 05688 April 1988

Hypothesis

A partial structure for the γ-aminobutyric acid ($GABA_A$) receptor is derived from the model for the nicotinic acetylcholine receptor

The anion-exchange protein of cell membranes is related to the $GABA_A$ receptor

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Received 28 January 1988

Based on the nicotinic acetylcholine receptor model [(1987) Eur. J. Biochem. 168, 431–449], a partial model is constructed for the exobilayer portion of the $GABA_A$ receptor, an approach justified by the superfamily relationship of the two receptors [(1987) Nature 328, 221–227]. The model predicts succesfully the excess positive charge on interior strands which constitute the ligand-responsive portion of the receptor. Binding to GABA expands the exobilayer portion of the receptor, opening a pathway to a chloride channel. Separate binding sites for antianxiolytics (benzodiazepines) and hypnotics (barbiturates) are suggested, with prolongation of chloride entry projected as a consequence of stabilization of the open form. The anion-exchange protein (AEP) of membranes (band 3 of red blood cell membranes) is similar in some respects to the γ-aminobutyric acid ($GABA_A$) receptor. Both proteins are inhibited and labeled by diisocyanatostilbenedisulfonate (DIDS), both transport Cl^- and HCO_3^-, and both are membrane proteins. Starting with the lysines known to be labeled in band 3 protein, searches of the amino acid sequences of the $GABA_A$ receptor α- and β-subunits reveal at least 4 reasonably homologous sequences. The relationship between AEP and $GABA_A$ receptor leads to the idea that the chloride/bicarbonate channel may be the ancestor of all ligand-gated channels, with ligand gating by γ-aminobutyric acid and acetylcholine arising later in evolution.

GABA receptor; Nicotinic acetylcholine receptor; Anion-exchange protein; Amino acid sequence; Sequence homology; Structural model

1. INTRODUCTION

Superfamily relationships have been discovered among ligand (photon) activated G-protein receptors [1–6], voltage-gated ion channels [7–13] and ligand-activated ion channel receptors [14–16] on the basis of amino acid sequence homologies and domain distributions. These have stimulated the hope that conclusions on the structure of one member of a superfamily might be used to elucidate the structure of another member of the same superfamily [17,18]. It is accepted that, within families like those of the opsins [19] and nicotinic acetylcholine receptors [20], homologous structures occur.

The amino acid sequences for both subunits of the γ-aminobutyric acid ($GABA_A$) receptor and one subunit of the glycine receptor have been carefully examined. The structural features identified are four hydrophobic transmembrane α-helices and an exobilayer 'disulfide loop' [21]. It seemed worthwhile to test the arrangements deduced in the model for the nicotinic acetylcholine

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

(nACh) receptor on the $GABA_A$ receptor so that a plausible structural and dynamic model might be available for further analysis.

A recent report suggested that bicarbonate can exit through the $GABA_A$ channel which normally admits chloride into the cell [22,23]. It seemed desirable to make a comparison between the amino acid sequences of the $GABA_A$ and glycine receptors and those of the cell membrane anion-exchange proteins (AEPs) which exchange HCO_3^- for Cl^-. The prototypical AEP is the red blood cell AEP (band 3 in red blood cell membrane protein electrophoretograms) [24–26]. The red blood cell AEP was included among the group of chloride channels similar to the $GABA_A$ and glycine receptors [27].

We now report that a partial model for the $GABA_A$ receptor can be derived from the nACh receptor model on the basis of the superfamily relationship. In addition, a number of homologous amino acid sequences in the $GABA_A$ and glycine receptors and the AEP suggest a possible common origin. Given the likelihood that chloride was present in the primitive environment before $GABA_A$, it seems plausible that the $GABA_A$ receptor may have evolved from the chloride AEP. The relationship of the $GABA_A$ receptor to the nACh receptor then implies that ligand-gated channels for cations are derived from the same origin. The existence of an acetylcholine-activated receptor in *Aplysia* which controls the entry of Cl^- is consistent with this proposition [28]. The calcium channel has been suggested as precursor for the sodium channel [29].

2. RESULTS AND DISCUSSION

The superfamily relationship between the $GABA_A$ and nACh receptors implies that a structural similarity might exist. Cartoons for receptors are attractive but do not have sufficient physical and chemical detail to explain receptor behavior on the molecular level. We therefore modeled the $GABA_A$ receptor using the scheme developed for the nACh receptor [30]. The exobilayer portion of the $GABA_A$ receptor is divided into 20 amino acid sections to obtain 11 strands analogous to those proposed for the nACh receptor model. An overall view of the strand arrangement is shown in fig.1. The lower strands (5,6,9–11) are on the inner side

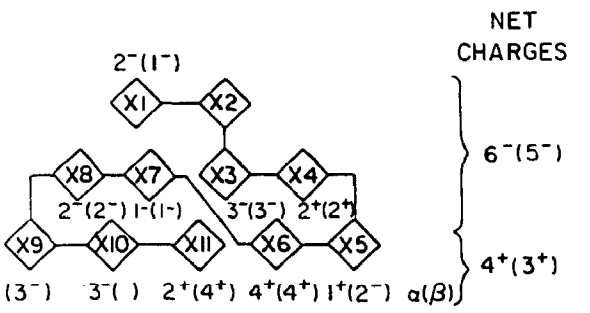

Fig.1. Schematic exobilayer strand arrangements for the α- and β-subunits of the γ-aminobutyric acid ($GABA_A$) receptor. Charges for each strand of the α-subunit are shown next to each strand. The net charges for the β-subunit are indicated in parentheses. The charges on the strands are asymmetrically distributed in two ways. First, the net charge of the outer strands is negative while that of the inner strands is positive. Second, the charges on the inner strands, those which face the pathway to the channel, are quite asymmetric, the right-hand and center strands being very positive, while the strands on the left are negative. The strand arrangements are those shown in [30] for the nACh receptor.

of the exobilayer portion of the receptor. Charged amino acids (Lys^+, Arg^+, Glu^-, Asp^-) are counted and the net charges shown next to each strand. No corrections are made for the possibility that adjacent amino acids might point in opposite directions on a β-strand. The surprising result is that the net charge is asymmetrically distributed, with the inner strands positive and the outer strands negative. The positively charged regions of the inner strands should favor the association and passage of Cl^- to the bilayer channel. Barnard et al. [14,21] pointed out that there were many positive charges near the bilayer, but could not define their relationships.

The 'flower' model for the nACh receptor is a reasonable choice for the $GABA_A$ receptor, as shown in fig.2, since the phenomena of activation and desensitization are parallel in the two cases [31]. The composition of the receptor, $\alpha_2\beta_2$, is different from that of the nACh receptor. Activation of the receptor opens up a pathway to Cl^-, which should involve the positively charged regions of the strands. Activation is achieved by 2 molecules of $GABA_A$ binding between two subunits of the receptor, either two α- or two β-subunits. Desensitization involves rotation of the $GABA_A$ molecule(s) to a position parallel to the strands, allowing the flower to close somewhat and cutting off the flow of Cl^-.

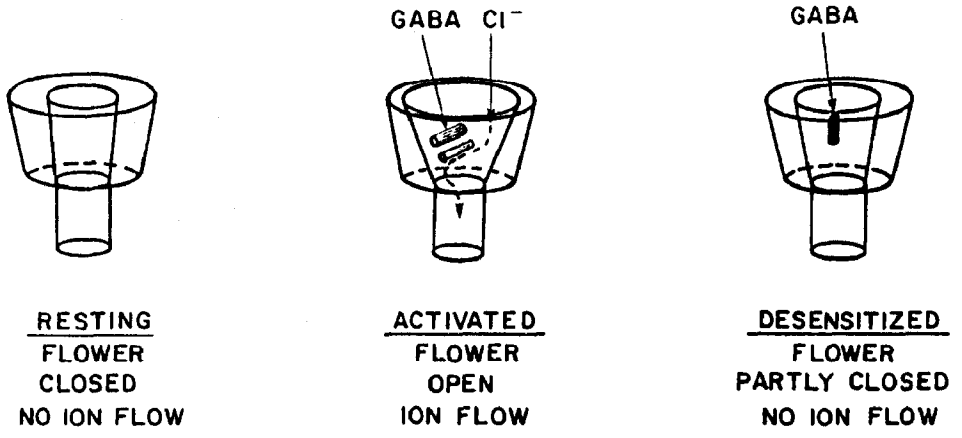

Fig.2. Activation and desensitization mechanism for the $GABA_A$ receptor. Two GABA molecules bind to the interior of the exobilayer portion of the receptor, opening a pathway for Cl^-. A reorientation of the GABA molecule or molecules leads to a partial closing of the pathway and blocks the flow of Cl^-. (Adapted from [30].)

Antianxiolytic and hypnotic drugs inhibit nervous activity by increasing the open time for the $GABA_A$ chloride channel. A schematic mechanism for the conversion of the resting (closed channel) to the activated state (open channel) is shown in fig.3. Single group rotation [30] changes the orientation of the binding groups, and combination of GABA with the receptor (an α-α site is arbitrarily chosen) expands the receptor from approx. 16 Å to 21 Å for the α-α dimension. The expansion opens up a pathway to the bilayer channel, which for the moment is unspecified (see below and [14,21]). Prolonging the channel open time might be achieved through binding of drugs to the $\alpha5/\beta8$ and $\beta4/\alpha9$ strand combinations. The drug complex stabilizes the activated, expanded state of the receptor, retarding the closure of the chloride pathway. The benzodiazepine-binding site must be accessible to the milieu since a benzodiazepine affinity column is used to purify the receptor. The model accounts for two different types of prolongations for channel opening, one due to antianxiolytics and the other due to depressants. Since the chloride pathway is close to the $\alpha5/\beta8$ corner, we suppose this to be the depressant-binding site. Channel blocking inhibitors like picrotoxin and *t*-butylbicyclophosphorothionate [32,33] bind on the interior to several positively charged groups. A detailed hydrogen-bonding scheme for the binding of barbiturate to two peptide strands shows quite clearly that the strands must be antiparallel (fig.3). More specific binding site groups for $GABA_A$ might have been chosen using the same theoretical approach as that used for ACh [30], but has not yet been tried. Experimental and genetic data for checking prospective binding sites will surely appear within a year or two.

2.1. *Relationship between $GABA_A$ receptor and AEP*

The AEP of membranes (band 3 of red blood cell membranes) is similar in some respects to the $GABA_A$ receptor. Both proteins are inhibited and labeled by diisocyanatostilbenedisulfonate (DIDS), both transport Cl^- and HCO_3^-, and both are membrane proteins.

The finding that HCO_3^- could exit from cells through the chloride channel of the $GABA_A$ receptor suggested that a search be made for a possible connection between the receptor and AEP. Amino acid sequences were chosen for examination on the basis of their importance to the activity of AEP. Starting with the lysines known to be labeled in band 3 protein, searches of the amino acid sequences of the $GABA_A$ receptor α- and β-subunits were begun with critical pairs. Pairs comprising the labeled lysines plus a neighboring amino acid were selected, all contained within the amino acid sequences compared by Demuth et al. (fig.5 in [24]) for the 'membrane domains' of HKB3 (human non-erythroid band 3 protein) [24], MEB3 (mouse erythrocyte band 3 [25]) and HEB3 human erythrocyte band 3, 72 peptide amino acid sequence [26]. The tyrosine included in the search is

labeled extracellularly by radioiodination. Sequences which contained the pairs were then examined for matches in neighboring segments. The search revealed at least 4 reasonably homologous sequences.

The matches found at critical segments of the $GABA_A$ receptor are not present in randomly selected portions of the receptor amino acid sequences. We can conclude that there is a relationship between the AEP and the $GABA_A$ receptor. The results are shown in the homology search scheme.

Given the high probability that chloride was present in the primitive environment, it seems reasonable to propose that a chloride channel (or AEP) preceded ligand-gated chloride channels in evolution. Hille [29] suggested that calcium channels were the earliest of the voltage-gated channels. Apparently, chloride and calcium channels are the elementary primitive channels. A link between these two 'primary' classes is that both are inhibited by pyrethroids [34].

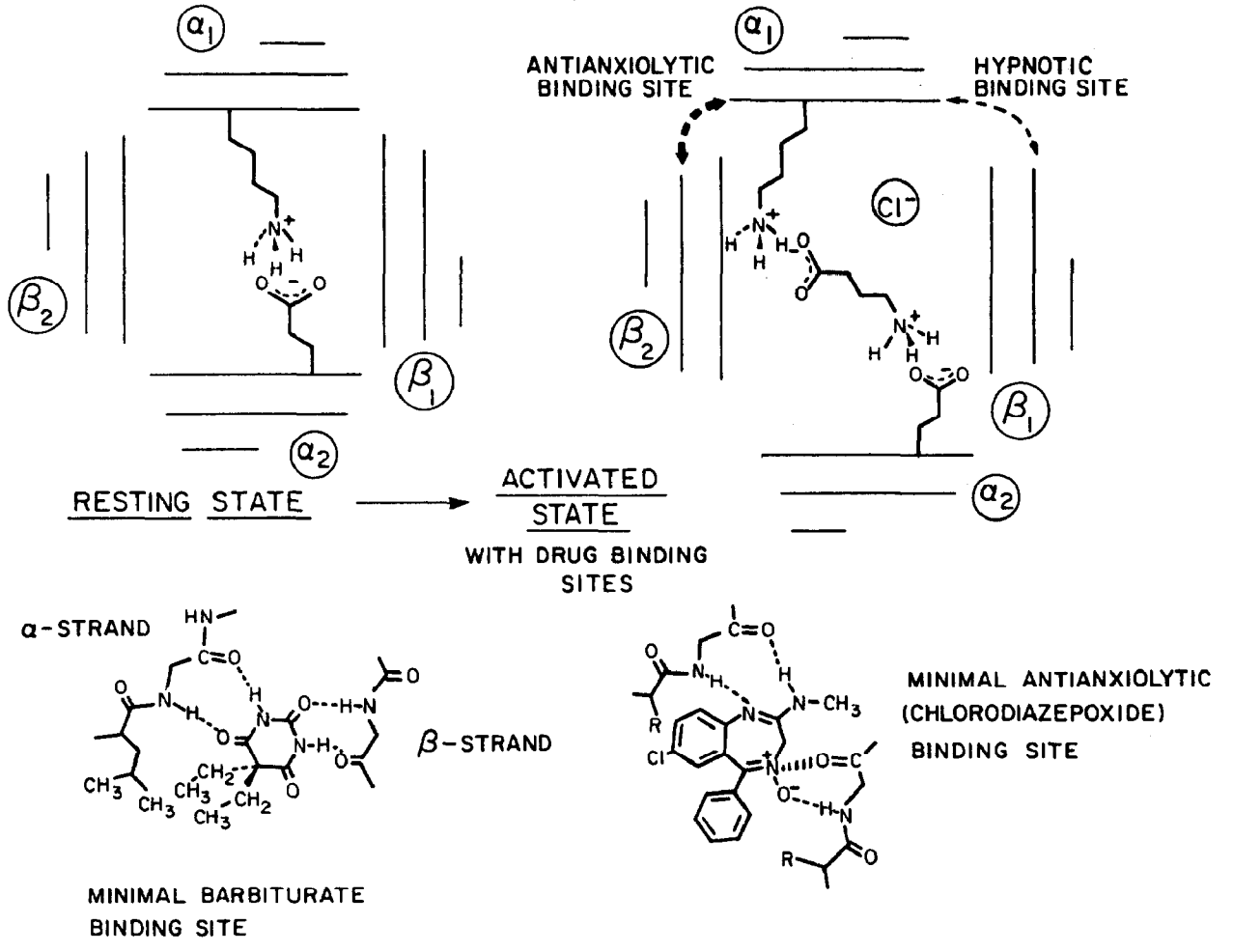

Fig.3. Schematic illustration of the mechanism of activation of the $GABA_A$ receptor by GABA, in which the resting (closed) form of the receptor is converted into the expanded (opened) form of the receptor. Lines represent the strands shown individually in fig.1. The closed form is estimated to have a separation of 16 Å between opposed subunits, whereas the opened form has an α-α separation of approx. 21 Å. The binding site groups for GABA are shown in a particular initial conformation in the resting form, but change orientation by single group rotation in the activated form. The binding sites for antianxiolytics and hypnotics between two antiparallel peptide strands are illustrated with detailed hydrogen-bonding schemes for a barbiturate and a benzodiazepine. The drug-binding sites must include hydrophobic groups shown as the side chain of leucine for barbiturate and as R for the benzodiazepine.

2.2. *Chloride channel in bilayer*

As pointed out by Barnard and co-workers [14], amphiphilic segments like those noted for the nACh receptor [30] cannot be located in either the $GABA_A$ or glycine receptors. The finding that 1,2-cyclohexanedione, an arginine-binding agent, is an AEP channel blocker without interfering with the binding of Cl^- [35] may be used to infer the presence of an arginine in the channel region of the red blood cell AEP. The mechanism proposed by Falke and Chan [36] for translocation of ions in AEP seems quite different from that envisaged here for the $GABA_A$ receptor. Nevertheless, it is similar in the sense that a physical barrier opens or

Scheme 1

Homology search

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Sequences containing pairs derived from MEB3 proteins:

MEB 448 EKTRNLMGVSELLISTA     Lys 449 [25]
MEB 556 FSKLIKIFQDYPLQQTY     Lys 558, 561 [25]
MEB 606 LRKFKNSTYFPGKLRRV     Lys 608, 610, 618 [24, 26]
MEB 645 TYTQKLSVPDGLKVSNS     Tyr 646  [24, 37]

FPS = functionally plausible substitutions

Matches:

117                                  Identical   FPS    Similar  TOTAL
  KLLRITEDGTL  GABA_A-α
  KLIKIFQDYPL  MEB3                    4/11      3/11     1/11    8/11
  KLVKIFQEHPL  HKB3                    4/11      3/11     1/11    8/11
557


380
  RKPMSSREGYGR GABA_A-β
  RKFKNSTYFPGK MEB3                    4/12      1/12             5/12
  RKFKNSRFFPGR HKB3                    6/12       -               6/12
  RKFKNSSYFPGK HEB3                    4/12      1/12             5/12
607


196
  KKVEFTTGAYPRL GABA_A-β
  RKFKNSTYFPGKL MEB3                   3/13      4/13      -      7/13
  RKFKNSRFFPGRI HKB3                   2/13      4/13      -      6/13
  RKFKNSSYFPGKL HEB3                   2/13      5/13      -      7/13
607


347
  SYTPNLARGD GABA_A-α
  TYTQKLSVPD MEB3                      4/10      1/10      -      5/10
  TYTQKLSVPS HKB3                      3/10      1/10      -      4/10
  TYTQ....   HEB3                      2/4       1/4       -      3/4
646
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The MEB numbering system [25] is used to denote the amino acid sequences [24]

closes the pathway for Cl^-. The question of the presence of another transmembrane segment between M3 and M4, as well as the related question of the orientation of M4 is best left for the future, pending further experimental data. As pointed out elsewhere [30], there is no really persuasive evidence concerning the orientation of M4 in the nACh receptor and whether or not it ends extra- or intracellularly.

3. CONCLUSIONS

The present partial model for the $GABA_A$ receptor provides a reasonable basis for further work on the structure of the receptor. Further studies using molecular graphics might identify the binding sites for GABA and channel-opening drugs. Drug design for two important classes of compounds might be carried in new directions by the present approach.

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