A MOLECULAR MODEL FOR AN ACETYLCHOLINE BINDING SITE

Ion Channel and the Bilayer Helices of the Acetylcholine Receptor Assigned Using Single Group Rotation Theory and Electrostatic Interactions

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We recently presented structural assignments (1, 2) for 24 transmembrane segments of the acetylcholine receptor (AChR), a membrane complex with five subunits, two α- and one each of β-, γ- and δ-subunits (3, 4). Derived from the complete amino acid sequences for the subunits (5–8), 17 of these segments had marked hydrophobic character, and the other seven segments were identified as channel elements on the basis of single group rotation (SGR) theory (9). We now show that these independently chosen channel elements form a molecular combination that is plausible for an acetylcholine binding site, an ion channel, and a "gate" for opening the ion channel. In addition, a ring of 17 hydrophobic helices surrounds the inner channel within the phospholipid bilayer.

THEORY AND DISCUSSION

The functional arrangement of the channel elements is not obvious from the amino acid sequences (7, 8). The channel elements (Table I) were chosen (1, 2) on the basis of a model for the channel amino acid sequence Glu (1), Lys (5), Glu (8), Lys (12) . In fact, a high proportion of the amino acids at the SGR-designated locations in the channel elements are Lys (21/21) and Glu (16/20) (1, 8). We approached the problem of function in steps. First, the α-helix is represented in a simple way. A binding site is then chosen for acetylcholine. Last, the α-helices are brought together in a way that maximizes electrostatic interactions on one side and hydrophobic "bonding" to hydrophobic helices and/or phospholipids on the other.

The acetylcholine binding site was sought on the basis of certain minimal expectations (1, 9): (*a*) a negative charge (Glu or Asp) for the positive trimethylammonio group; (*b*) a hydrogen bonding group (ϵ-ammonium ion of Lys) for the ester carbonyl; (*c*) a negative charge (i.e., a Glu near the Lys) to favor the association of the ACh carbonyl group with the Lys positive charge; (*d*) matched groups in the same relative positions on the helices.

The channel elements for the α-subunit could be readily fitted to an acetylcholine molecule in the expected manner. The binding site is near the opening of the channel according to its location among the channel elements.

Channel elements from the β-, γ- and δ-subunits were combined with the "nucleus" afforded by the ACh-binding site made by the α-subunits through matching groups with

TABLE I
CHANNEL ELEMENTS DERIVED FROM THE α, β, γ, AND δ SUBUNITS OF THE ACETYLCHOLINE RECEPTOR

	4α-2	5α-2	3β-	4α-1	5α-1	3γ-	3δ-
							—
							360*s*
							361*s*
	362	363*am*		362	363*am*		362*s*
	361	364*t*		361	364*t*		363
	360	365*P*	392*t*	360	365*P*		364*g*
	359–	366	393*am*	359–	366		365
	358g	367	394*P*	358g	367	—	366
	357*t*	**368+**	395	357*t*	**368+**	**360+**	367*s*
	356	**369*am***	396*t*	356	**369*am***	**361*a***	**368+**
	355*am*	**370*P***	397	355*am*	**370*P***	**362–**	**369*a***
	354+	**371–**	398*P*	**354+**	**371–**	**363–**	**370*am***
	353*g*	372	399*am*	**353*g***	372	**364**	**371–**
B	**352*s***	**373+**	400–	**352*s***	**373+**	**365**	372
I	**351**	**374*s***	401	**351**	**374*s***	**366**	373
L	**350–**	**375*a***	**402+**	**350–**	**375*a***	**367+**	374*am*
A	349*s*	**376**	**403–**	349*s*	**376**	→**368+**	375
Y	348	**377–**	**404*a***	348	**377–**	**369*P***	**376+**
E	347–	**378*g***	**405**	347–	**378*g***	**370*rg*+**	**377*s***
R	346	**379**	**406–**	346	**379**	**371*s***	**378*rg*+**
	345–	**380+**	**407*a***	345–	**380+**	**372–**	**379*s***
	344–	**381**	**408**	**344–**	**381**	**373**	**380–**
	343*a*	**382**	**409+**	**343*a***	**382**	**374**	381
	342	**383*a***	**410**	**342**	**383*a***	**375**	382
	341	**384–**	**411**	**341**	**384–**	*r***376–**	383
	340+	**385*h***	**412*a***	**340+**	**385*h***	**377–**	384–
	339*am*	**386**	**413–**	339*am*	**386**	**378*am***	**385+**
	338–	**387+**	414*am*	**338–**	**387+**	**379+**	**386*am***
	337*am*	**388*s***	415	**337*am***	**388*s***	380–	**387*s***
	336+	**389–**	416–	**336+**	**389–**	381*rg*+	**388–**
	335–	**390–**	417*s*	**335–**	**390–**	382*h*	389*rg*+
	334+	**391–**	418*a*	**334+**	**391–**	383*g*	390*h*
	333*s*	392*s*	419*s*	333*s*	392*s*	—	391*g*
	332*a*	393*s*	420–	332*a*	393*s*	—	392
	331+*rg*	394*am*	421	331+*rg*	394*am*		393
	330+		422–	330+			
	329		423–	329			
	—		424	—			
	—		425+	—			

Key: +, Lys; *rg*+, Arg; –, Glu or Asp; *S*, Cys; *s*, Ser; *t*, Thr; *h*, His; *g*, Gly; *P*, Pro; *a*, Ala; *am*, Gln or Asn. All hydrophobic residues [Leu, Ile, Val, Phe, Tyr, Trp, Met] unmarked. →, channel-type sequence restarts; *r*, reverse charge order. (α-Asn 217, 297, 339, 369; Asp 238, 344, 345, 347, 350, 371, 389, 407; β- Asp 400, 422, 423; γ- Asp 380; δ- Asn 374). Numbers above the channel elements indicate the place in the sequence of the bilayer helices for a given subunit (1, 2).

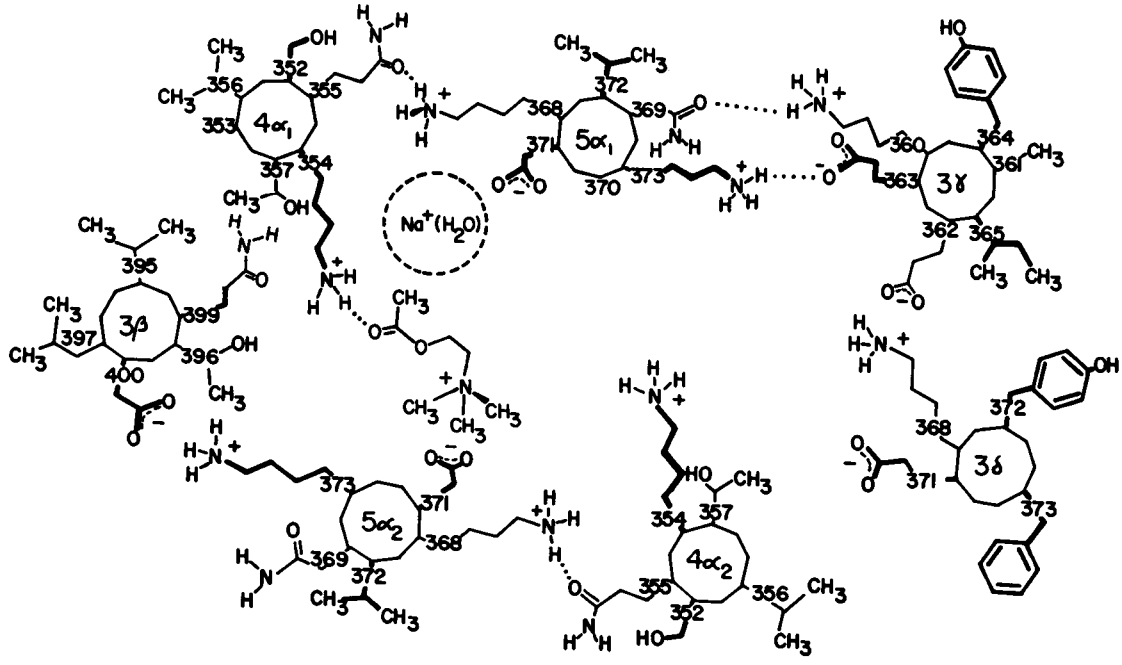

FIGURE 1 First level of the acetylcholine receptor ion channel and the binding site with acetylcholine in the open conformation. The channel elements (transmembrane helices of 24 amino acids, ca. 6.8 turns) are divided into four levels of six amino acids each. Each level is represented as a nonagon; the vertices numbered 1, 4, 7, 9, 3, and 6 are designated as the sites for the amino acids, a span corresponding to 1.56 turns of an α-helix. Continuing the vertex numbering through a second level nonagon with 8, 2, 5, 7, 1, and 4, we note that the 11th amino acid is correctly found in the same orientation as the first, but three turns down. Although this representation for the α-helix is approximate, it is simpler and faster to use than a "helical wheel" (10). Light lines to side chains are used for the first three amino acids ("first" turn), dark lines for side chains for the next three amino acids ("second" turn). Proximal groups in adjacent helices are thus identifiable.

electrostatic or hydrogen-bonding interactions. A single group rotation of the Lys-ACh combination in the binding site leads to a space large enough for a hydrated Na^+ ion. The "gate" for the ion channel is explained in this way. Note that one natural consequence of the model is that the Lys rotates to a position near a Gln (β-399). The overall result is shown in Fig. 1 for the first level (six amino acids from each subunit) of the AChR ion channel and the ACh-occupied binding site.

The details of construction of the AChR ion channel, including the ring of 17 hydrophobic helices and other molecular aspects of the AChR, will be described in subsequent communications.

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