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A. O. ŽUPANČIČ

A MODEL OF CHOLINORECEPTORS BASED ON CONFORMATIONAL TRANSITIONS OF MEMBRANE-BOUND ACETYL-CHOLINESTERASE PROTEIN

1. INTRODUCTION

According to modern views, receptors can be defined as allosteric proteins of effector membranes which, under the influence of activators. undergo a conformational transition triggering herewith the chain of effects. Such conformational transitions cannot be directly proven, but can be inferred from the kinetics of reactions between active substances and the receptor protein. The working hypothesis on the identity of anionic sites of membrane-bound acetyl-cholinesterases (acetyl-ChE) with cholinoreceptor (ChR) sites (1, 3, 7, 8, 9) predicts a kinetic behaviour of this enzyme analogous to that of ChR; it should be noted that this hypothesis does not link ChR with the esteratic sites of acetyl-ChE. but with the anionic ones, which might be located on the neighbour polypeptide chain of the same macromolecule (4, 5). From this point of view, the kinetics of acetyl-ChE built-in into mouse diaphragm motor endplates was studied under the influence of cholinergic activating or blocking agents, and departures from the Michaelis-Menten kinetics were interpreted in terms of conformational transitions of acetyl-ChE protein (10, 11). Such kinetic experiments were later extended: a) to some more substances, and b) to acetyl-ChE of the frog heart ventricle, i. e., to a tissue responding to acetylcholine (Ach) with the muscarinic effect.

2. METHODS

Acetyl-ChE, built-in into either mouse diaphragm endplates or into the frog heart ventricle, was used as enzyme preparation; its activity was measured by continuous pH-static titration. The technical details were the same as described previously (11), except that Tris — HCl was omitted, and that in experiments with the frog ventricle acetyl-ChE, the NaCl concentration of the reaction mixture was lowered to 6.5 g/l.

3. RESULTS

The main results are summarised in Table 1.

Table 1.

TYPE OF KINETICS OF TWO MEMBRANE-BOUND AND ONE PURIFIED ACETYLCHOLINESTERASE (ACETYL-ChE) PREPARATIONS

Enzyme preparation	Compounds	Michaelis-Menten kinetics	n_H
acetyl-ChE of motor endplates	Ach (8 μM — 0.25 mM)	obeyed	1
	Mch (63 μM — 1 mM)	not obeyed	2
	Ach + TC (1 μM)	not obeyed	2
	Ach + Gal (2.5 μM)	not obeyed	2
	Ach + TEA (1 mM)	not obeyed	2
	Ach + TMA (3 mM)	obeyed	1
	Ach + Amb (2 nM)	obeyed	1
	Ach + E (0.1 μM)	obeyed	1
	Ach + WIN_{7775} (0.1 μM)	not obeyed	2
	Ach + WIN_{3317} (0.1 μM)	obeyed	1
	Ach + Atr (1 μM)	obeyed	1
acetyl-ChE purified (Worthington)	Ach (8 μM — 0.25 mM)	obeyed	1
	Ach + TC (1 μM)	obeyed	1
	Mch (63 μM — 1 mM)	obeyed	1
acetyl-ChE of frog heart ventricles	Ach (8 μM — 0.25 mM)	obeyed	1
	Ach + Atr (0.1 μM)	not obeyed	0.5

Ach: acetylcholine. Mch: acetyl-β-methylcholine. TC: d (+) tubocurarine. Gal: gallamine. Amb: ambenonium. TEA: tetraethylammonium. TMA: tetramethylammonium. E: eserine. WIN_{7775} and WIN_{3317}: for their chemical structures see (6), Atr: atropine. n_H. the Hill coefficient at substrate concentrations lower than about 0.1 mM.

4. DISCUSSION

Our membrane-bound acetyl-ChE preparations are mixtures of various proteins and other molecules; even so the binding of the investigated substances to other molecules than acetyl-ChE is irrelevant since acetyl-ChE activity is the only measured parameter.

Deviations from the Michaelis-Menten kinetics, as listed in Table 1, refer only to the membrane-bound acetyl-ChE while the purified enzyme always obeyed the usual kinetics.

Whit the endplate acetyl-ChE some substances change the Michaelis-Menten kinetics in the sense that the Hill coefficient (n_H) increases from $\sim$ 1 to $\sim$ 2; in other words, the rectangular hyperbola in the activity-substrate concentration plot changes to an S-shaped curve (11). Interpreting the responses of nicotinic ChR, Changeux and Podleski (2) pointed out that a hyperbolic dose-response curve indicates that the affinity of individual subunits for the ligand is independent of whether the neighbour subunits are occupied by the ligand or not ($n_H \sim 1$); an S-shaped curve, on the other hand, is indicative of a conformation where

the binding of the ligand to one subunit enhances the affinity of still unoccupied neighbour subunits for the ligand ($n_H \tilde{>} 2$).

From the first section of Table 1 it can be seen that five out of eleven tested substances cause a change from the hyperbolic ($n_H \sim 1$) to the S-shaped kinetic type ($n_H \sim 2$). In the concentrations used, the same five substances are cholinergic blocking agents, while the remaining six are either cholinergic activators or indifferent to nicotinic ChR. One is tempted to call this relation a rule, especially since such analogue pairs as Ach and Mch, TMA and TEA, WIN_{3317} and WIN_{7775} are included: in spite of the close structural similarity between the two members of each pair, they influence both the kinetics of acetyl-ChE and that of nicotinic ChR differently.

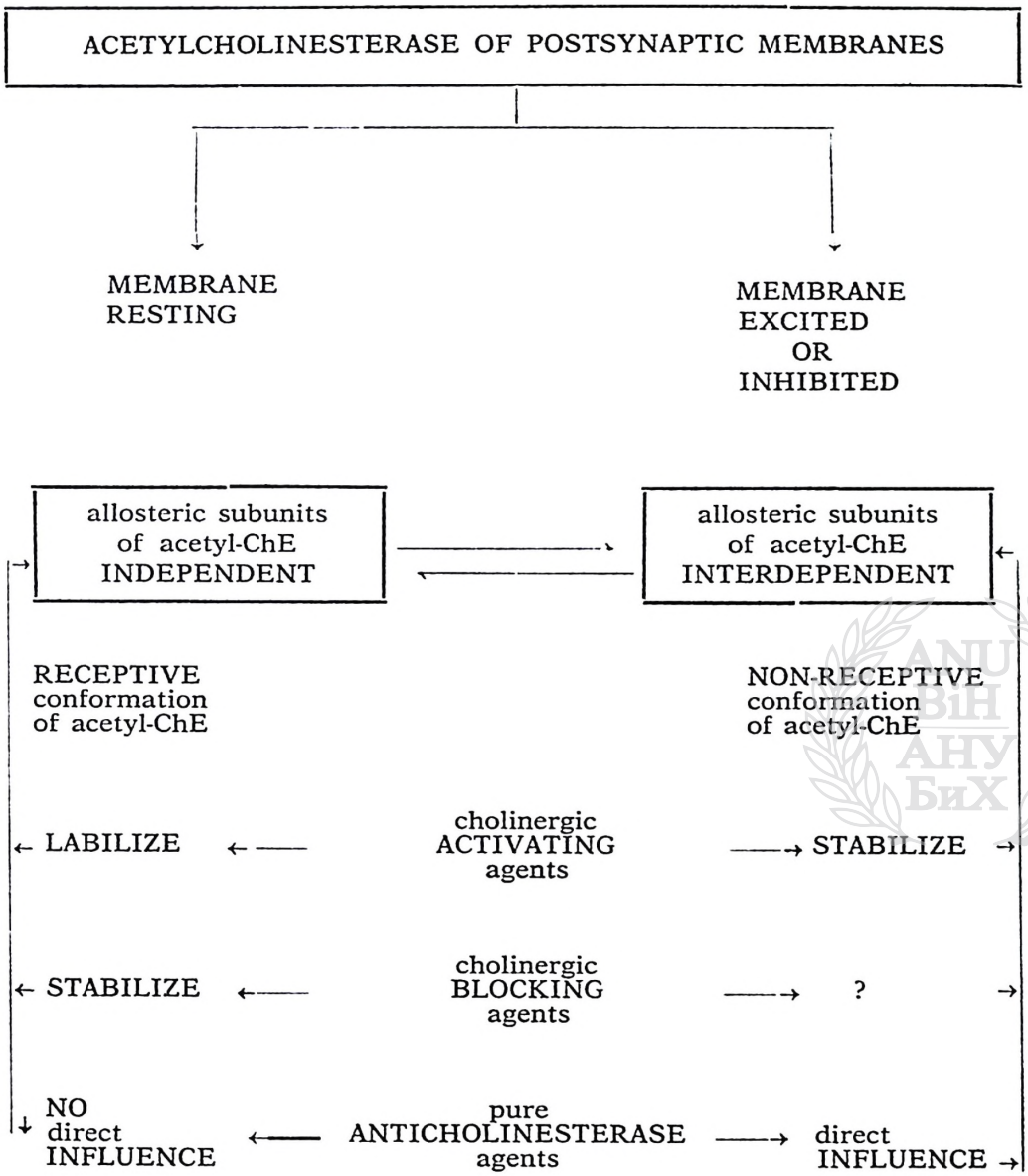
Thus, eleven tested substances influence the kinetics of acetyl-ChE of motor endplates in the same sense as they influence the kinetics of nicotine ChR: cholinergic activators induce a transition from the conformation with coöperatively dependent subunits ($n_H \sim 2$) to the conformation with independent subunits ($n_H \sim 1$); cholinergic blocking agents prevent such a conformational transition ($n_H \sim 2$), while substances with a pure anticholinesterase activity are indiferent to it ($n_H \sim 1$). The ability of cholinergic blocking agent to prevent the conformational transition of the membrane-bound acetyl-ChE protein, is independent of its inhibitor potency for this enzyme. Irrespective of their affinity for the esteratic site of acetyl-ChE, all tested cholinergic activating and blocking agents are bound with their cationic heads also to the anionic sites of this enzyme; we suggest that it is through these sites that cholinergic activating or blocking agents induce or prevent the conformational transition of the receptive enzyme.

Before turning to acetyl-ChE of the frog heart ventricle, it is worth noting that Atr, even in a 10 μM concentration did not affect the kinetics of motor endplate acetyl-ChE. From Table 1 one can see that in the frog ventricle, too, acetyl-ChE with Ach as substrate obeys the Michaels-Menten kinetics ($n_H \sim 1$). Since the heart ventricle responds to Ach with the muscarinic effect, Atr was chosen as cholinergic blocking agent; at a 0.1 μM concentration, Atr altered the kinetics in the sense that n_H was lowered to ~ 0.5.

In our interpretation, this means that the frog ventricle acetyl-ChE protein, before being influenced by Ach, exists in a conformation with interdependent subunits; this interdependance is, however, negatively coöperative, i. e., the binding of the ligand to one subunit lowers the affinity of the neighbour subunits for the ligand. Hence in our model, Atr blocks the effect of Ach on the heart ventricle because it stabilizes the negatively coöperative conformation of acetyl-ChE. So far, it is impossible to decide whether a negatively coöperative allosteric conformation is peculiar to either the muscarinic ChR or inhibitor ChR, or frog heart ventricle ChR. Methodologically, however, it is possible to clear up these alternatives.

The following tentative model of ChR function of acetyl-ChE is suggested (see Scheme 1):

Scheme 1



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A. O. ŽUPANČIČ

MODEL HOLINORECEPTORA OSNOVAN NA KONFORMACIJSKIM PROMJENAMA ACETILHOLINESTERAZNE BJELANČEVINE VEZANE ZA MEMBRANE

KRATAK SADRŽAJ

Kinetičko ponašanje acetilholinesteraza integriranih u postsinaptičke membrane efektora sa nikotinskim ili muskarinskim odgovorom na ACh studirali smo pod uticajem dvanaest supstancija; rezultate smo interpretirali u smislu konformacijskih promjena tog enzima: holinomimetici su inducirali promjenu konformacijskog oblika sa međusobno zavisnim enzimskim podjedinicama u oblik sa nezavisnim podjedinicama; holinolitici su sprečavali ovaj prelaz, dok čiste antiholinesterazne tvari nisu uticale na njega. Zaključujemo da su acetilholinesteraze holinergičkih postsinaptičkih membrana alosteričke bjelančevine s osnovnim svojstvima hipotetičkih nikotinskih, odnosno muskarinskih holinoreceptora.

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