FUNCTIONAL PROPERTIES OF POSTJUNCTIONAL MEMBRANE

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INTRODUCTION

The diverse functions of the postjunctional membrane (PJM) have been associated with the activity of specific receptors. Synaptic receptors are beginning to be viewed not as mere abstractions but as macromolecular complexes that can be isolated and subjected to chemical analysis. In this connection an understanding of how the various functions of the PJM are carried out is essential. The transduction of a signal from a chemical into an electrical form consists of several functional stages. In this study a survey is made of current ideas on the molecular nature, kinetics, and possible interactions of activation stages of PJM. Because some of these topics have already been reviewed (1–12), we consider only the most recent or not yet fully accepted questions. Since the bulk of our knowledge of postsynaptic mechanisms is gained from the study of effects of acetylcholine (ACh), this review is restricted to a discussion of the functional organization of cholinoceptive membranes.

ACh-INDUCED CHANGES OF ION PERMEABILITY AND CLASSIFICATION OF CHOLINORECEPTORS

Ionic mechanisms providing for ACh effects are most diverse. Permeability may increase selectively for Na^+, K^+, Ca^{++}, or Cl^- and decrease for Na^+ and K^+. Direct activation of electrogenic ionic pumps is possible but doubtful. Main approaches to the ionic nature of synaptic potentials have been discussed in detail in the reviews by Ginsborg (6, 13). Permeability of activated PJM shows selectivity, which means that molecular devices determining such ion selectivity (ionic channels) are specific. It is most essential to learn whether these sodium, potassium, and chloride channels are structural elements of appropriate cholinergic receptors (ChRs) or whether the functions of PJM are performed by different but interacting macromolecules. There is no direct answer to this question as yet. We hope, however, that progress in

isolating molecular complexes of cholinoceptive membranes will permit identification and localization of the components of PJM (11, 14) and thus elucidate the molecular structure of all elements of the functional chain from the recognition site to the selective ion filter. In any case, these components differ substantially in function.

Pharmacological variations between different ChRs that are likely to reflect peculiarities in the structure of their recognition sites have long been known. However, the biological meaning of these variants is not clear. It would be interesting to know among other things whether there is a correlation between pharmacological properties of ChRs and ionic mechanisms that bring about the ChR activation, that is, whether variants in the combination of the active center of one or another type of ChR with a definite type of the PJM ionic mechanism are random or regular. The evidence presented in Table 1 does not support such a correlation. ChR of the heart, smooth muscle, cortical neurons, and part of ChR of autonomic ganglion neurons are muscarinic. They do not differ pharmacologically, although the ionic effects of their activation vary significantly. Neurons of mollusks were found to contain three different types of ChR controlling three different types of ionic channels (31, 32). In this case, however, a nicotinic-like ChR operates the chloride channel, and its activation leads to hyperpolarization of the neuron. The solution of the important problem of correspondence between types of ChR and ionic effects of their activation is, among other things, limited by imperfect pharmacological classification of ChR. Their division into nicotinic and muscarinic receptors provides only a basic classification. Nicotinic ChRs, for example, differ in their ability to interact with various bisquaternary compounds (41).

Differences between ChRs can be revealed not only with the aid of classical cholinergic drugs but also with the action of α-bungarotoxin or dithiothreitol. It is known that α-bungarotoxin and other postsynaptically acting neurotoxins (NT) block the effect of ACh on skeletal muscles of vertebrates and their derivatives (electroplax). They are inefficient on muscarinic ChR (21) and not all nicotinic Ch-receptors are susceptible to the action of NT. Although the lamprey heart and somatic muscle contain pharmacologically identical nicotinic ChR (42, 43), neurotoxins block only ChR of the muscle without affecting the heart (44). NT does not block ACh effects in neurons of the rabbit sympathetic ganglia (25). NT produces a peculiar effect on ChR in leech. Responses of the dorsal muscle to such compounds as succinyldicholine and decamethonium are blocked selectively (45–47). NT exerts no influence on ChR of neurons (Retzius cells) in leech ganglia (58).

The effect of dithiothreitol is inverse; only the depolarizing action of ACh on the neurons in leech is blocked (58) while the response of the dorsal muscle is not affected (45). Muscles of lamellibranchs, gastropods and cephalopods, mollusk hearts, muscles of polychaetes, sipunculoids, echinoderms, and ascidians are insensitive to NT, but in most cases dithiothreitol blocks ACh effects (39).

Further work is needed to classify ChR and to interpret the biological significance of variation in their pharmacological properties and ionic mechanisms controlled by ChR.

Table 1 Effects of ACh on different cells[a]

Cells	Effect of ACh	Changes of ionic conductance	Type of ChR	Effect of α-bungaro-toxin	References
Skeletal muscle	d	↑ Na, K	n	+	15–17
Electroplax	d	↑ Na, K	n	+	18, 19
Heart of vertebrates	h	↑ K	m	–	20, 21
Smooth muscle of vertebrates	d	↑ Na (K, Ca)	m	–	21, 22
Sympathetic ganglion:					
Fast EPSP	d	↑ Na, K	n	–	23–25
Slow EPSP	d	↓ K	m	–	25–26
		or ↑ Na, Ca			27
		or including the electrogenic pump			28
Slow IPSP	h	↓ Na			120
Cortical neurons	d	↓ K	m		7
Snail neurons:					
Fast EPSP	d	↑ Na, K	n	–	29–32, 121
	d	↑ Cl			33
Fast IPSP	h	↑ Cl	n	+	30, 34, 35, 121
Slow IPSP	h	↑ K		–	31, 32, 121
Leech neurons	d	↑ Na	n	–	36, 58
	h	↑ Cl	m	–	58
Leech muscle	d		n	– +	45–47
Insect neurons	d	↑ Na	n	–	40
Mytilus muscle	d	↑ Na, K	n	–	37–39

[a] d = depolarization; h = hyperpolarization; ↑ = increase of conductance; ↓ = decrease of conductance; n = nicotinic ChR; m = muscarinic ChR; + = block; – = no block.

SOME PECULIARITIES OF PJM IONIC CHANNELS

Takeuchi & Takeuchi (15, 48) describe some important features in ion permeability of the activated end-plate: 1. Permeability increases only for cations (Na, K, and to a small extent, Ca). 2. The ratio between shifts of Na and K conductance is constant ($\Delta G_{Na}/\Delta G_K = 1.29$) and does not change in any time phase of activation, that is, the Na and K currents are completely synchronous. 3. The PJM conductance is not affected by changes in the electrical field and the ionic content.

In the last few years these postulates (*2* and *3* in particular) have been revised many times. For instance the influence of the increase of $[K]_o$ had been already

shown by Takeuchi (48). Takeuchi also described the influence of the membrane potential on the time course of the end-plate current (e.p.c.) (15). Further analysis of this fact required the revision of the postulate that conductance is independent of the potential (see next section). However, the striking electrochemical behavior of the activated PJM detected by these studies remains irrefutable. Ionic currents of the PJM cannot be described quantitatively by equations commonly used to define resting and action potentials (49). Thus some requirements of the Goldman constant field theory are not fulfilled in the activated PJM. There is a convenient experimental approach for revealing "non-Goldman" behavior of the PJM—a considerable shift of the equilibrium potential by 18–20 mv when $[K]_o$ decreases fivefold. Using this criterion of K = sensitivity we can demonstrate the electrochemical identity of responses to ACh, carbacholine, decamethonium, and succinyldicholine (50). On the other hand, it has been found that the equilibrium potential of responses to suberyldicholine and sebacinyldicholine does not change on lowering of $[K]_o$ (50–52). K = sensitivity disappears as the temperature decreases to 2–3°C (53). These results fit reasonably well with the hypothesis that the peculiar electrochemical behavior of the PJM is due to a very short life span of an open channel during which the concentration profile of a passing ion does not alter significantly (54). Were the time prolonged in some way (by decrease of temperature, action of suberyldicholine) the K = sensitivity as a sign of "non-Goldman" behavior of the PJM would disappear.

The assumption about the longer lifetime of open channels under suberyldicholine and low temperature was later confirmed in experiments analyzing voltage fluctuations induced in the end-plate by cholinomimetics (55, 56). The lifetime of the open state of ionic channels defined by the investigation of membrane noise (msec) substantially exceeds the time (μsec) consistent with the proposed hypothesis of transitional processes. According to this hypothesis the time course should have been determined not by the kinetics of ionic channels but by some other process [changes in ACh concentration in the synaptic cleft or time of existence of the ACh–ChR complex (for details, see section below)].

The independence of K and Na currents is the critical point of the hypothesis of transitional processes. The unitary channel, however, must not show electrochemical anomaly when the life span is short (50). The strict synchronism of changes in Na-K conductance makes it difficult to ascertain whether the cations Na^+ and K^+ move in through the same channel or whether there are selective channels for each of the permeant cations. The ability of the membrane equilibrium potential to alter under the action of some drugs (see Table 2) testifies in favor of separate channels (57). One ChR may control the state of the both Na and K channels so that the kinetics of changes in their conductance depends on the behavior of the common gating mechanism. From this point of view synchronous changes in the Na and K currents upon alteration of the membrane potential level would be more easily comprehended. A shift in the equilibrium potential by the action of certain drugs might be a direct selective effect on one of the channels. The existing facts are not sufficient to advance such a hypothesis.

TIME COURSE OF POSTSYNAPTIC CURRENTS

The use of the voltage clamp technique in the end-plate zone makes it possible to record the time course of changes in the synaptic current (e.p.c.). When the holding potential is –80 mv the maximum rise time for the e.p.c. of frog muscles is 0.5–0.6 msec, and the half-time of the decay is 1.1–1.2 msec.

The course of the decay curve is exponential, but first and last portions of the curve deviate from the exponential (60–63). The time course of the e.p.c. seems to reflect

differences in the rates of successive reactions constituting the activation process, a search for a rate-limiting factor or factors seems to be the best approach. Of the sequence of PJM activation stages (release of ACh, approach of a molecule to ChR, formation of an ACh-ChR complex, activation of the complex, appearance of ionic conductance, passing of ionic currents, elimination of ACh molecules through diffusion and enzyme hydrolysis, decrease of conductance) not all contribute directly to the postsynaptic current but any one may happen to be rate limiting.

In the 35–40 years since the end-plate potential was first recorded, the interpretation of its time course has undergone considerable change. The enzymic hydrolysis by cholinesterase (ChE) initially appeared to be a main factor (64), because the inhibition of ACh prolongs the e.p.c. Later, ACh diffusion in the synaptic cleft was shown to proceed so rapidly that the enzymic hydrolysis could not be regarded as the only limiting factor (65). It was then supposed that the formation and dissociation of the ACh–ChR complex is the slowest process. This hypothesis accounts for changes in the time course of e.p.c. under the influence of some drugs and altered temperature (63, 112), but it is difficult to use it for interpreting a relationship between the time course of the e.p.c. and the level of muscle fiber polarization. The first studies of the e.p.c. showed that hyperpolarization prolongs while depolarization shortens the e.p.c. (15), and this was confirmed repeatedly (60–63, 66–68). Takeuchi & Takeuchi (15) attempted to explain these findings by suggesting that the postsynaptic current affects the effective concentration of ACh in the vicinity of ChR in end-plate zone by iontophoresis. A thorough experimental analysis of this hypothesis by Stevens and collaborators indicated that this explanation was inappropriate (60, 61, 69, 70). The existence of separate ionic channels was hypothesized (57, 66, 71). Simultaneously it was postulated that the kinetics of these two channels are different and that the sodium channels are open for a longer time than the potassium channels. Kordas (72) checked one of the inevitable consequences of this hypothesis, suggesting different time courses for Na and K currents, i.e. a biphasic total current in the equilibrium potential region, and detected no such phenomenon. Against this interpretation, however, is evidence that the time course can be lengthened progressively on increase of hyperpolarization outside E_k zone to 150–200 mv and that the e.p.c. declines exponentially at any holding potential (60). It is clear now that the concept of potential independence of PJM conductance (15) is not valid, though the main principle of electrical inexcitability of PJM (109) still stands.

Table 2 Effects of modifiers of postjunctional membrane

Drug (references)	Time course of e.p.c.		Potential dependence of decay	ACh-noise (life span of channels)	Dependence of e.p.c. amplitude on MP level	Influence on reversal potential	Influence on desensitization kinetics
	Character of decay	Effect on exponential form of e.p.c.					
Procaine (57, 66, 68, 71, 72, 87, 98, 122)	biphasic decay: initial phase faster, final phase slower than normal			complicated	nonlinear	absent	accelerate
Lidocaine (71, 98, 112)	biphasic decay, but small slow phase					absent	accelerate
Diisopropyl-fluorophosphate (76, 77)	biphasic decay		lost			shift to E_K	
Edrophonium (103)	prolonged	hold	hold		normal		
Scopolamine (67, 81)	biphasic decay	only slow phase	only slow phase		nonlinear	absent	
Atropine (67, 91, 113–116)	shortened	hold	hold	shortened	normal	shift to E_{Na}	absent

Table 2 *(Continued)*

Drug (references)	Time course of e.p.c.		Potential dependence of decay	ACh-noise (life span of channels)	Dependence of e.p.c. amplitude on MP level	Influence on reversal potential	Influence on desensitization kinetics
	Character of decay	Effect on exponential form of e.p.c.					
Serotonin (109, 118)	slightly shortened	hold	lost		nonlinear	shift to E_K	absent
Morphine (118)	slightly shortened	hold	lost		nonlinear		
[redacted]arbital (52, 98, 104)	shortened	hold				absent	accelerate
Pentobarbital (98, 111)	shortened	hold	hold		normal	absent	accelerate
Chlorpromazine (52, 98)	slightly shortened					absent	accelerate
SKF-525A (119)	shortened	hold				absent	accelerate
Histrionicotoxin (102)	shortened	hold	decrease		nonlinear	absent	accelerate
N-butanol (78, 52, 98)	prolonged			prolonged			accelerate
Hexanol (78)	biphasic						

A valuable suggestion concerning the kinetics of the PJM activation mechanism was made by Magleby & Stevens (60, 61). Using as the basis a scheme proposed earlier (117)

$$ACh + ChR \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} AChR \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} AChR^*$$

they suggested that the initial stages, i.e. ACh diffusion within the synaptic cleft and formation of the ACh-receptor complex, proceed faster than the activation of this complex. Hence the process of opening and closing ionic channels was chosen as the time limiting factor. The channel may exist only in one of the two states, open or closed. The transition from one state into another takes almost no time, that is, the form of an elementary impulse of the postsynaptic current approaches the rectangular. The constants β and α reflect the mean probability of these transitions for each of a host of channels. These ideas were applied to the analysis of acetylcholine-induced noise (55). The improvement of the recording technique upon clamping of voltage (69) allowed a study of the conductance kinetics of the PJM comparing the simultaneous action of a large number of ACh molecules making up an integral number of quanta (the e.p.c. and m.e.p.c.) to the effects of asynchronous action of single ACh molecules. Good agreement was observed between the time parameters of the e.p.c. and m.e.p.c. on one hand, and elementary ACh-currents, on the other. The kinetics of macro and micro phenomena were similarly dependent on the membrane potential level and the temperature (55, 60, 69), which permits us to consider in more detail the nature of the e.p.c. time course. The formation of the complex results in opening of ionic channels. Since under given assumptions this is a probability process to be described exponentially, the greatest number of channels may open immediately after formation of the ACh-ChR complex. Distribution of the number of channels opening per unit of time depends on the membrane potential level and temperature; with hyperpolarization or decrease of temperature the rate of increase of the number of open channels declines. The duration time of the open state of the channels obeys the exponential law as well. Should all channels making up a population open synchronously, the rise instantaneously to maximum and purely exponential decay of the e.p.c. will reflect the distribution curve of the number of open channels; however, there is a period when the ACh-ChR formation is not completed, the opening of channels is proceeding, and the closing has already started. This period should probably comply with an experimentally measurable period consisting of the rise time and the initial declining portion of the current. Subsequent exponential decay of current depends only on the process of closing the ionic channel, since the average life span of channels can be measured with a good deal of accuracy by determining a time constant of the e.p.c. decline. This parameter also depends on the membrane potential level and the temperature. Such models of the PJM ionic channel kinetics adequately explain experimental evidence obtained earlier, among other things, variations in the nonlinearity displayed by the dependence of the e.p.c. maximum and ACh-induced potential upon the membrane

potential as the latter grows more negative (70). This model may be employed to interpret changes of the e.p.c. time course caused by the action of pharmacological agents such as serotonin (59) and atropine (81), which render the e.p.c. declining phase more rapid without disturbing its exponential form (see Table 2).

The kinetic model of Stevens and collaborators permits a reasonable physical interpretation (61). It suggests a gating molecule that controls a selective ionic channel. The interaction of ACh and ChR decreases the energy barrier of transition of the gating molecule from a state when it bars the passage of ions through the channel into a state admitting them. The electrical field may affect the level of energy barriers if the gating molecule has a dipole. In light of this model the effect of various factors on the kinetics of the PJM conductance changes can be easily explained by the influence on the local surface membrane charge, by direct influence on the gating molecule, or by the influence on its hydrophobic environment. Any of these factors may determine the level of energy barriers of the transition from one state to another.

There are facts, however, that cannot be explained by the two-state model, or that require additional assumptions:

1. The effect of one or another of the agonists is accounted for not only by the affinity of the agonist to ChR but also by the ability, which is different for various agonists, to activate the ChR. In modern molecular pharmacology this ability is defined as *efficacy* (73) or *intrinsic activity* (74). The investigation of membrane noise induced by different cholinomimetics has shown that these drugs vary not only in their different affinities to ChR (judging by effective concentrations) but also by different ways in which the ChR is activated. To date the mean conductance of each channel (γ) and the mean time of open state (τ) have been determined for only a small number of cholinomimetics. In measurements of the noise voltage (56) the mean time of the open state varies from 0.12 msec on activation with acetylthiocholine to 1.65 msec with suberyldicholine. Although the variation of γ is smaller (75) (from 12.8 pmho for phenylpropyltrimethylammonium to 28.6 pmho for suberyldicholine) the authors believe it is great enough to contradict the earlier model (61) according to which the conductance of all channels is identical and invariant once they are opened by different activator agents.

2. The tail of the e.p.c. decay deviates from an exponential time course. This is best seen in the case of hyperpolarization (60–63). A qualitatively identical, but more strongly pronounced, phenomenon may be observed in the presence of some drugs: procaine (57, 68, 71), scopolamine (67, 81), DFP (76, 77), and hexanol (78). There is a biphasic decline of the e.p.c. with different time constants for the initial portion (fast decline) and the tail (slow decline). This phenomenon is difficult to interpret on the basis of the two-state hypothesis (122). If we assume that the relaxation of individual channels obeys the Poisson distribution, only one time constant of the e.p.c. decline should be observed. Biphasic decline could be expected, however, if only a portion of the channels is modified by these agents and if they acquire a longer time constant of relaxation. The biphasic decline may be explained by the fact that both normal and modified channels take part in its formation. In

the presence of procaine and similar substances, however, the initial portion of the e.p.c. decay is faster than normal, but it is difficult to suggest that procaine modifies some of the channels so that they become faster, while others become slower.

3. E. Neher and B. Sakmann (personal communication) compared cholinomimetic-induced current fluctuations on chronically denervated muscle fibers with those on normal end-plates and found the average lifetime of open channels on denervated membrane to be five times longer. At the former end-plate of denervated muscle fibers a response with two components was outlined for which the τ-values correspond to those of the end-plate and extrasynaptic membrane. This could be due to two receptor populations at denervated end-plates.

4. If diffusion of ACh from the synaptic cleft is exceedingly fast and if ChE does not affect the duration of elementary conductance reactions (55, 69) the role of enzymic hydrolysis should be relatively insignificant. Lengthening of the decline phase of e.p.c. and persistence of its potential dependence when ChE is inhibited appears to be certain (60–63). The consistency of this finding with the two-state kinetic model is based on these additional assumptions: (*a*) the proper effect of anticholinesterase drugs on the time course of e.p.c. independent of the inhibition of enzymic hydrolysis (60); (*b*) the repeated binding of ACh molecules to ChR (79); (*c*) a different degree of cooperativity of the effect of ACh in the presence and absence of ChE (80).

5. The non-Goldman behavior of the activated PJM, which cannot be explained by the two-state hypothesis, is a controversial point. It has been suggested, however, that the elementary reaction measured by analysis of ACh noise consists of a great number of short bursts of conductance of a cluster of ionic channels (55, 75). With such an assumption, the above-mentioned hypothesis would agree with the existing evidence. If these channels are separate and open only for microseconds, then a different efficacy of cholinomimetics and a non-Goldman behavior of the PJM becomes explicable.

The nature of the time-limiting factor is not yet fully known. We can only hope that some day true rates of conformational changes will be measured directly in experiments with isolated molecular complexes of the PJM.

PROCESSES OF PJM INACTIVATION

The presence of some peculiar mechanism of slow inactivation in the PJM is beyond doubt. The phenomenon reflecting the existence of this mechanism is known as desensitization. It arises after prolonged action of ACh or cholinomimetics. A reversible inactivation of a great number of functional units of the PJM occurs, which is a sign of a decrease of its sensitivity (82, 83). To interpret the functional properties of the PJM it is necessary to understand the desensitization mechanism. Most hypotheses of desensitization mechanisms suggest either specific changes of a ChR—its transition into an inactive form (82, 84) or blockage of active centers by endogenous inhibitors (85). The question as to which link in the chain of postsynaptic phenomena is subject to inactivation is still open to argument.

A wide variety of effects and factors have been found that can affect desensitization kinetics. The rate of desensitization rises with an increase of $[Ca]_o$ and declines with its decrease (86–91, 97). Some other multivalent ions are able to produce a similar or even stronger effect: Sr^{2+} (90–92), Mn^{2+} (93), UO_2^{2+} (94), Al^{3+} (90), La^{3+} (90, 95). The increase in tonicity of a Ringer solution accelerates desensitization (93). The rate of desensitization declines with the increase of $[K]_o$ but the initial level may be restored by polarization of the fiber. The artificial shift of the membrane potential level results in increase of the rate of desensitization upon hyperpolarization (90), and decrease upon depolarization. The decrease of $[Na]_o$ in the solution also accelerates desensitization (86, 87). An analogous effect is observed when Na^+ is replaced by Li^+ (96). We found a great number of drugs varying in their chemical structure that significantly increased the rate of desensitization. They are local anesthetics [procaine and lidocaine (87, 98)], barbiturates [amobarbital and pentobarbital (98)], some derivatives of diphenylacetic acid [adiphenin (99, 100), mesphenal (91, 99), SKF-525A (117), tropazin (99)] chlorpromazine (98), diphenhydramine (98), promethazine (98), and long-chain alcohols (98). Since all these substances are characterized by an ability to diminish the amplitude of responses to iontophoretic application of ACh at lower concentrations than in the case of m.e.p.p.s., drugs such as hexafluorenium (101), chistrionicotoxin (102), DFP (76, 77), edrophonium (103), and thiopental (104) may accelerate the desensitization. At present it is difficult to conclude whether a common mechanism affecting desensitization exists for such a wide variety of substances. Many of these drugs may be classed with membrane stabilizers (98, 105), and they have similar chemical features: one part of the molecule is a cationic head or an ionizable group while the other is hydrophobic. These drugs probably accelerate the transition of functional units of the postjunctional membrane into an inactive state. To determine the true chemical mechanism of this effect it is essential that these drugs affect only the rate of desensitization without influencing the rate of recovery of the former sensitivity level, i.e. the reactivation kinetics of the PJM functional units. Also important is their ability to affect the rate of desensitization not only when they are added to the bathing fluid but also after intracellular iontophoretic injection inside the muscle fiber in the end-plate zone (106). With the decrease of temperature the rate of onset of desensitization declines considerably more than the rate of recovery of the former sensitivity (107).

The above evidence has led us to advance a hypothesis that the activation of the PJM ion permeability is a limiting factor rather than the reaction of ACh and the recognition site of ChR (87). It was suggested that activation of ionic channels involves changes in the state of Ca in the PJM. If ACh acts for a long time or frequently, Ca ions accumulate near the gates of synaptic ionic channels and disturb the activation mechanism (12, 90–92). Following the physical model proposed by Stevens and collaborators (61) it may be assumed that the state of dipoles controlling changes in the membrane conductance for permeant cations alters. The question whether these changes are "receptory" or "nonreceptory" is open until we know whether a mechanism controlling the state of ionic channels is a part of the protein

molecule interacting with ACh or whether these are different but closely interrelated molecules.

The affinity to ACh of "desensitized receptor" may be higher than normal, as was assumed by Katz & Thesleff (82). Changeux and co-workers (110) recently found an increase of ACh binding by fragments of membrane of electroplax treated by local anesthetics, SKF-525A or high $[Ca]_o$. But this phenomenon was not reproducible with purified receptor protein.

Nastuk & Parsons suppose that Ca acts on the inner side of the PJM. This assumption is supported by the observations that caffeine is able to accelerate desensitization (108). Some metabolic inhibitors promoting the increase of $[Ca]_i$ also accelerate desensitization (F. Vyskočil, personal communication). The blockade of Ca channels by manganese, however, increases desensitization (93).

MODIFIERS OF THE POSTJUNCTIONAL MEMBRANE

Since the discovery of the chemical nature of synaptic transmission, understanding of its intimate mechanisms has progressed through the utilization of new and more perfect chemical tools. Initially, these were so-called cholinergic compounds, i.e. drugs imitating ACh effects (cholinomimetics) and playing the part of competing antagonists (cholinolytics) or drugs inhibiting ChE. Now there is a new class of compounds vital to the consideration of the problem of the PJM modifiers. Possibly these drugs do not affect the reaction of ACh with the recognition site of ChR, or the effect they exert is not the main one in their mechanism of action. They rather affect subsequent links in the activation mechanism of ionic conductance.

Table 2 contains available evidence of the phenomenology of modifying effects of some drugs from this vast class. Comparison of the data reveals that the common feature of these drugs is the ability to influence
kinetics of the PJM ion channels.

Some drugs shorten the open time of channels or prolong it by inducing a biphasic decay of the e.p.c. They influence by different mechanisms the potential dependence of the time course of the e.p.c. Many of these drugs may also affect desensitization by accelerating its onset. No correlation has been found between the effect of these compounds on the activation and inactivation of the PJM. Amobarbital, pentobarbital, chlorpromazine, histrionicotoxin, for example, shorten the time course of the e.p.c. whereas *n*-butanol prolongs it, but all these drugs potentiate desensitization. On the other hand, atropine substantially shortens the time course of the e.p.c. and exerts no influence
ence in the concentration of drugs affecting desensitization and the time course of the e.p.c. Only a small number of drugs can alter the PJM equilibrium potential. Nor is there a correlation between this ability and the effect of the compounds on the time course of the e.p.c. It is sufficient here to compare atropine and histrionicotoxin. Both shorten appreciably the time of the e.p.c. decay, but only atropine affects the equilibrium potential of the e.p.c. Among congeners of atropine there are compounds that produce the same effect on the time course of e.p.c., but they are not able to shift the equilibrium potential. Differences in the behavior of the equilib-

rium potential of natural responses and responses to ionophoretic application of ACh in the presence of atropine are still puzzling (67).

The molecular mechanisms of the modifying actions of these drugs are still unknown in every detail but they may serve as useful tools for elucidating the important functional properties of the PJM.

CONCLUSION

There are many gaps in the general scheme of the functional organization of the cholinoceptive PJM. So far, attempts have been made to construct a single scheme for the end-plate of vertebrate skeletal muscles despite a great variety of cholinergic membranes. It is not clear, however, which of these functional devices (recognition site of a Ch-receptor, gating mechanism, selective ion channel, mechanism of recovery of the initial state, etc) is of major importance in determining the functional properties of the entire PJM. Possibly the diversity of cholinoreceptive membranes is accounted for by various combinations of these functional "bricks." The unabated growth of potentialities of technique and the use of new approaches, for example, elaboration of mathematical models of the basic synaptic processes, enables us to anticipate further gains in this important field of research.

ACKNOWLEDGMENTS

The author is greatly indebted to Professor H. Grundfest and Professor B. Khodorov for reading the manuscript and for helpful criticism.

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