Biophysical Chemistry 29 (1988) 95–101
Elsevier

BPC 01217

Ion channels and postsynaptic potentials

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Accepted 15 October 1987

Ion channel; Postsynaptic potential; Membrane potential; Receptor activation

Neurotransmitters open transmembrane ion channels in target membranes. At the motor endplate, the open time of channels activated by acetylcholine determines the time course of the endplate current. Endplate channels are selective for cations and their conductance and open times are influenced by the nature of the permeating cation. Similar effects have been seen at anion-selective inhibitory synapses. Some 'blocking' drugs that depress postsynaptic responses to neurotransmitters produce effects not consistent with a simple model in which they block open channels. Channels activated by neurotransmitters can show subconductance states that may reflect the fundamental mode of operation of a variety of ion channels.

1. Introduction

It is now generally accepted that, at most synapses, transmission is mediated by a chemical messenger or neurotransmitter that is secreted from the presynaptic terminal, diffuses across the synaptic cleft and activates receptors in the postsynaptic membrane. It is activation of these receptors that generates the postsynaptic potential in the postsynaptic cell. Much of our knowledge of the generation of postsynaptic potentials comes from work at the motor endplate and this paper will therefore focus mainly on the properties of ion channels and postsynaptic potentials at this synapse.

2. Endplate currents and potentials

In a classic series of experiments, Fatt and Katz [1] used the new technique of intracellular recording to define the properties of endplate potentials in frog skeletal muscle fibres. They found that an endplate potential spread electrotronically, being largest and fastest at the endplate and becoming smaller and slower the further the distance from the endplate. They concluded that the active phase of neuromuscular transmission was a localised, impulsive event lasting only a few milliseconds, later confirmed by voltage-clamp experiments in which endplate [2] or spontaneous miniature endplate [3] currents were recorded directly. Takeuchi and Takeuchi [2] noted that the decay of endplate currents was 'approximately exponential', that the rate of decay was influenced by membrane potential and that the Q_{10} of the rate of decay was about 2. Spontaneous miniature endplate currents (mepcs) were found to have a similar time course [3] except that the early part of mepcs was not influenced by the phasic release of transmitter.

It was suggested by Del Castillo and Katz [4] that the sequence of events generating the conductance responsible for an endplate potential could be represented as:

$$T + R \rightleftharpoons TR \rightleftharpoons TR'$$

where T is the neurotransmitter, R the receptor

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molecule in its initial, reactive form, TR an intermediate state and TR′ the state associated with open channels. Magleby and Stevens [5,6] recorded endplate currents with an exponential decay that was influenced by membrane potential and used the above reaction scheme to determine the nature of the process that controlled the rate of decay of endplate currents. In view of the high Q_{10} of the decay and persistent voltage sensitivity in the presence of anticholinesterases, it was concluded that the decay of TR′ was rate-limited by the conformational change from TR′ to TR rather than by the rate of loss of transmitter from the vicinity of the receptors by hydrolysis or diffusion. This hypothesis was supported by the observation that the average open time of endplate channels, obtained by Fourier analysis of current noise, was the same as the time constant of decay of endplate

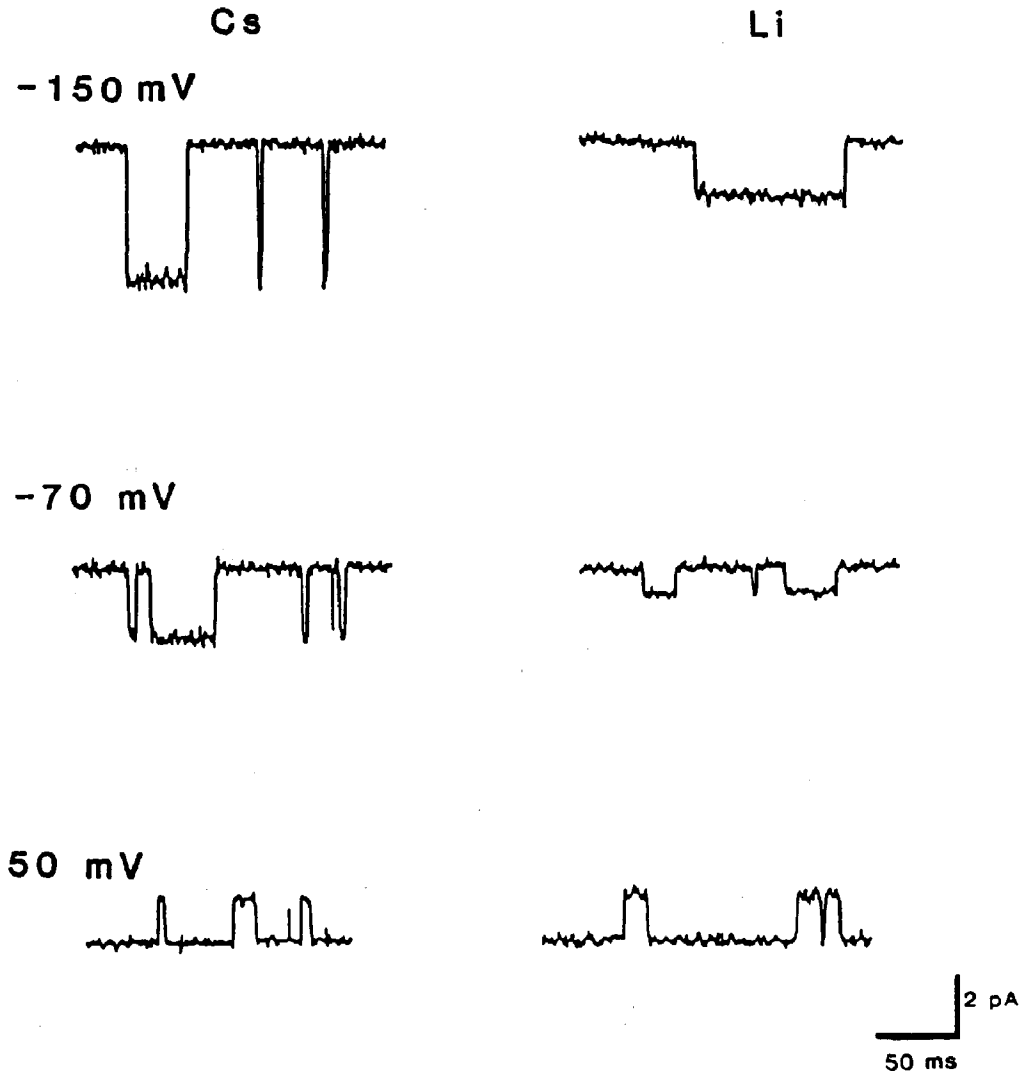

Fig. 1. Single-channel currents activated by acetylcholine in cell-attached patches of denervated toad muscle. Pipette solutions contained CsCl (left traces) or LiCl (right traces) instead of the normal NaCl. Currents were larger in Cs^+ solution than in Li^+ solution at negative potentials (from ref. 22).

currents [7]. In summary, the rate of decay of endplate currents is normally controlled by the rate at which open channels relax to their closed state.

The average open time of channels, reflected in the time constant of decay of currents, influences the amplitude of the postsynaptic response. From knowledge of the passive electrical properties of muscle fibres and the time course of the endplate current in the same fibre, it is possible to predict the amplitude and time course of endplate potentials at any distance from the endplate. Comparison of these predictions with experimental records [8] confirmed the hypothesis of Fatt and Katz [1] that an endplate current can be regarded as a point source of current injected into a passive cable. A consequence of the relatively long time constant of the muscle membrane is that changes in the decay of endplate currents, e.g., prolongation produced by cooling or exposure to drugs such as anticholinesterases, result in changes in the amplitude and time course of endplate potentials. The membrane capacity also plays an important role in determining the amplitude and decrement of endplate potential amplitude along a fibre: the 'space constant' for endplate potentials is less than the d.c. space constant and the amplitude of an endplate potential is less than the product of the peak amplitude of the current and the input resistance of a cell [8].

3. Ion selectivity

Del Castillo and Katz [9] found that the effect of endplate potentials on action potentials reversed at membrane potentials between -10 and -20 mV. In the light of these observations, it was proposed that acetylcholine released from a motor nerve terminal caused a non-selective increase in ion permeability that can be represented as a localised short circuit of the postsynaptic membrane. Takeuchi and Takeuchi [2] also found, by linear extrapolation from the current-voltage curve, that endplate current became zero at a potential between -10 and -20 mV. In later experiments in which it was possible to record endplate currents at positive potentials, the null (zero current) potential has been found to be close to 0 mV. By measuring null potentials for endplate currents when large ions were substituted for Cl^-, Na^+ and K^+ in the extracellular solution, Takeuchi and Takeuchi [10] were able to show that endplate channels were about equally permeable to Na^+ and K^+ but impermeable to Cl^-.

Endplate channels display some selectivity between cations. The first evidence for this was the observation of a decrease in amplitude of mepcs and single-channel currents when Li^+ was substituted for Na^+ in the extracellular solution [11]. Substitution of Cs^+ for Na^+ had the opposite effect. In extracellular solutions containing K^+, Cs^+ or Li^+ substituted for Na^+, peak conductance during an mepc and single-channel conductance (γ) measured from power spectra of current noise followed the sequence $\gamma_K > \gamma_{Cs} > \gamma_{Na} > \gamma_{Li}$ [12]. Null potentials in the Li^+, Na^+, K^+ and Cs^+ solutions (at 8 °C) of -7.3, -5.3, -0.1 and -2.4 mV, respectively, gave the permeability sequence, $P_{Cs} > P_K > P_{Na} > P_{Li}$ [13]. The same permeability sequence has been obtained from null potentials by others [14,15]. The difference between the conductance and permeability sequences indicated that the ions were not moving independently through the channels [13]. These kinds of observations, that have been extended to include a wide range of cations [16], indicate that many permeant ions interact with intra-channel sites. Other studies have shown that conductance saturates as ion concentration is increased [17–19], again indicating interaction of ions with channel sites. Because the channel conductance varies depending on the nature of the permeating cation, current-voltage curves in asymmetric solutions (e.g., in the normal situation) are non-linear, especially when Li^+ is the predominant external cation [12].

Measurements of null potentials, single-channel conductance and the influence of membrane potential on single-channel conductance [11,12] provided sufficient information to test some models of ion permeation. It was immediately apparent that the variation of γ with membrane potential was inconsistent with constant field predictions. An electrodiffusion model provided a good fit to the results [13]. It was assumed that the channel was long compared with the Debye length

within the channel and that cations competed for negative sites or negative groups within the channel. Bulk electroneutrality then requires that anions are also present within the channel but there must be at least one high-resistance barrier for anions to explain their low permeability. Because channel conductance varied with cation concentration, it was concluded that the channel was lined with 'neutral' sites (such as the negative ends of dipoles) rather than 'charged' sites. The model which gave the best fit to the experimental results saw the channel as a diffusion pathway lined with 'high field strength' negative polar groups and containing at least one high-resistance barrier for anions: it was noted that a rate-theory model with several or many binding sites would give similar fits. The relative binding constant sequence for the four ions was found to be a 'polarizability' sequence, $K_{Li} > K_{Cs} > K_{Na} > K_{Li}$.

Lewis [20] sought to explain the influence of Na^+ and Ca^+ concentration on the null potential and γ by taking fixed surface charge into account and using a single-barrier, rate-theory model. Failure of the model suggested that ion interaction probably involved competition for an intra-channel binding site. Lewis and Stevens [21] found that their data could be fitted by invoking such a site. However, in order to account for the observed variation of γ with membrane potential, it is necessary to postulate more than one intra-channel cation-binding site [22].

We have recently used patch-clamp techniques to study directly the characteristics of 'extrajunctional' channels in cell-attached patches of denervated toad (*Bufo marinus*) sartorius fibres [22]. The results were consistent with observations made previously with less direct techniques. An example of the difference in amplitude of single-channel currents at negative potentials when Cs^+ or Li^+ are substituted for Na^+ in the extracellular solution is shown in fig 1.

Null potentials with Na^+, Li^+, Cs^+ and K^+ in the pipette solution were -8.0, -20.5, 3.7 and 1.3 mV, respectively. Relative permeabilities calculated from these null potentials using the generalised null potential equation gave a permeability sequence of $P_{Cs} > P_K > P_{Na} > P_{Li}$. The conductance sequence was $\tau_K > \tau_{Cs} > \tau_{Na} > \tau_{Li}$, as found for junctional channels in amphibian muscle [12,14] and in myotubes [24]. Conductance-voltage curves in solutions containing one of the four monovalent cations could be well fitted by symmetrical channel models based on either the electrodiffusion model or a rate-theory model with four barriers and three binding sites [22].

4. Influence of permeant ions on channel open time

As mentioned above, the time constant of decay of endplate currents reflects the average channel open time. Channel open time can be affected in a wide variety of ways, e.g., changing temperature, membrane potential, a variety of drugs including alcohols and anaesthetics, denervation and the nature of the agonist. The nature of the permeating cation also influences endplate channel open time [11,12]: simple monovalent cations which reduce single-channel conductance increase average open time and there is a reciprocal relationship between the two that produces a constant charge transfer. A plausible explanation for similar effects at an *Aplysia* synapse [25] was that ions that interact strongly with sites within a channel give a low conductance (because of reduced ion mobility) but, by remaining in the channel for a longer time, prevent the channel from closing. However, some ions that reduce channel conductance have little effect on channel open time [16].

The influence of the permeating ion on channel open time is not restricted to cation-selective channels. For example, replacement of Cl^- with other anions changes the time constant of decay of inhibitory postsynaptic currents activated by neurotransmitters such as γ-aminobutyric acid [26] or acetylcholine [23].

5. Channel block

Many drugs such as pentobarbitone reduce the decay time constant of endplate currents and drugs such as local anaesthetics make the decay multiphasic. An explanation for such effects with appealing simplicity is that such drugs can enter open endplate channels and block them. A simple

version of this model is:

$$\text{closed} \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} \text{open} \underset{b}{\overset{fc}{\rightleftharpoons}} \text{blocked}$$

where c is the concentration of the drug and α, β, f and b rate constants.

We have analysed the effects of procaine and pentobarbitone on mepcs and single-channel currents to test this simple model, but several of its predictions are not fulfilled. For example, when a drug produces an mepc with biphasic decay, the model predicts a relationship between the amplitudes and time constants of the two components, such that

$$A = r_s(r_f - \alpha)/\alpha(r_f - r_s)$$

where A is the relative amplitude of the slow phase (as a fraction of the total amplitude), r_f and r_s the rates (reciprocal time constants) of decay of the fast and slow phases, and α is the normal (pre-drug) decay time constant. We were unable to obtain agreement between the amplitudes and decay time constants of the two phases of biphasic mepcs recorded in solutions containing 0.05–0.1 mM procaine [27].

The efficacy of procaine in producing biphasic mepcs is increased at hyperpolarized potentials and a possible explanation is that the concentration of the charged drug at an active site in a channel is influenched by the membrane field. However, mepcs became biphasic and the effect was enhanced by membrane hyperpolarization when the drug was injected intracellularly [28,29]. Under these conditions, membrane hyperpolarization should have reduced ionised drug concentration in the channel. It seems more likely that the membrane field influences the affinity of some membrane site for the drug.

A common explanation for the 'bursting' behaviour of channels produced by ions or drugs is also channel 'block'. However, the characteristics of bursts produced by classical 'blocking' drugs such as procaine and pentobarbitone often do not fit with this model [30,31]. The model predicts that the integral of current and mean blocked time during a burst should be independent of drug concentration but that burst duration should increase with drug concentration. These predictions are violated when channels in cultured muscle cells are exposed to QX222 at concentrations from 40 to 250 μM [30]. Pentobarbitone, at concentrations ranging from 10 to 500 μM [31], also produces effects on channels that are inconsistent with predictions of the model; mean blocked time increases steadily with pentobarbitone concentration but mean burst length is little changed as concentration is increased. The model therefore does not apply, at least for these drugs at these concentrations. Neher could explain his results by invoking more than one blocked state and a pathway from a blocked state not via the open state. However, it may be preferable to look for alternative mechanisms of action. There are many ways of explaining biphasic current relaxations and bursting behaviour of channels, especially as it is becoming clear that most channels have several open and closed states. By changing rate constants between some of these states, drugs could produce faster, slower or multiphasic current relaxations. Indeed, pentobarbitone produces bursting behaviour in single-channel currents activated by γ-aminobutyric acid but slows the decay of inhibitory postsynaptic currents of unchanged amplitude. The bursting behaviour is qualitatively consistent with channel block but the large increase in the integral of the current cannot be explained in this way. Bursting behaviour is clearly not synonymous with channel block as described by the above model.

6. Subconductance states

Many channels display current levels of smaller amplitude than the maximum current level, although usually transiently and infrequently (see, for early examples, refs. 24, 32 and 33); i.e., a channel can adopt conductance states less than the maximum conductance. In fact, many channels exhibit a large number of subconductance states [34–37]. An analysis of multiple subconductance states that were prominent in large Cl^- channels in cultured pulmonary alveolar cells showed that there were six equally spaced conductance states other than the closed state, and that the probability of any state was consistent with

binomial predictions for six independent 'co-channels', each with the same probability of being open [37]. The subconductance states could be distinguished from contributions from several independent channels because transitions between the maximum conductance and the closed state were much more frequent than predicted from several channels opening and closing independently. Similar observations have been reported for K^+-selective channels in renal tubules [38] in which there appear to be four co-channels forming a four-barrelled channel, very similar to the double-barrelled mechanism postulated for Cl^- channels from *Torpedo californica* electroplax reincorporated into lipid bilayers [39].

It now appears that channels activated by glycine, γ-aminobutyric acid [40] or glutamate [41,42] can also have multiple (at least five) subconductance states. Subconductance states in large Cl^- channels in pulmonary alveolar cells [37], K^+ channels in renal tubules [38], channels activated by γ-aminobutyric acid in spinal cord neurons [40], channels activated in cerebellar neurons by *N*-methyl-D-aspartate [42] and channels activated

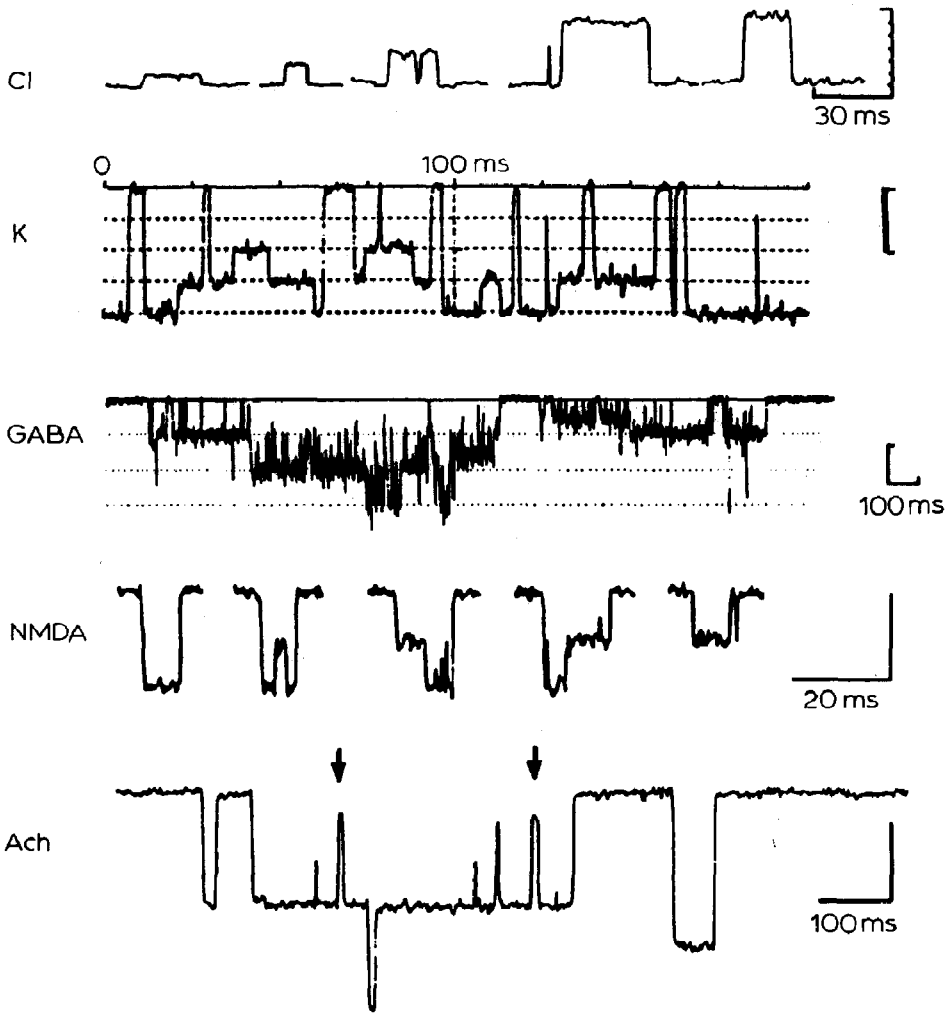

Fig. 2. Subconductance states in (from top to bottom): Cl^- channels in pulmonary alveolar cells (Cl [37]), K^+-activated channels in renal tubular cells (K [38]), γ-aminobutyric acid-activated channels in spinal cord neurons (GABA [40]), *N*-methyl-D-aspartate-activated channels in cerebellar neurons (NMDA [42]) and acetylcholine-activated channels in denervated toad muscle (Ach [22]).

by acetylcholine in denervated toad muscle [22] can been seen in fig. 2.

A possible explanation for this behaviour is that the channel can adopt several different conformations, each associated with a different conductance. However, in many tissues, the subconductance levels are very evenly spaced [37–39]. It would be a remarkable coincidence if the cross-sectional area of a channel should vary in equal steps during conformational changes. An alternative explanation proposed by Krouse et al. [37] is that different subconductance states represent the contributions of different numbers of cooperating subunits of similar conductance and that clusters of these subunits or co-channels form a channel that is characterised by synchronised opening and closing of co-channels from time to time.

It has been proposed [43] that there is a series of animo acids on a membrane-spanning α-helix of the a-subunit of the F_0F_1-ATPase that can form a proton channel if rotation of the helix brings an aspartate on a neighbouring helix of the c-subunit into a gap in the chain of amino acids on the a-subunit. It has been demonstrated by techniques of site-directed mutagenesis that each of five amino acids (four on the a-subunit, one on the c-subunit) is essential for proton transfer. If the assemblages of proteins that form ion channels contain several such parallel pathways for ions that open and close independently as rotation of adjacent, membrane-spanning helices produces uninterrupted paths of amino acids, these may be the structural correlates of the subconductance states that are observed.

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