This view is further supported by experiments where channels were activated by ACh at concentrations >2 μM where single channel currents appear in bursts. Bursts reflect sequential open–close transitions of the same AChR channel. In embryonic muscle they fall into two main classes with respect to the size of their current pulses, corresponding to the two classes observed at low ACh concentration (Fig. 3a). Transitions between these two main current levels were never observed between these two main current levels were never observed during a burst. However, transitions between the main level and the sublevel do occur, as shown in Fig. 3b. The upper trace shows a burst of current pulses at low time resolution, consisting of 25 well separated current events. At high time resolution two events have a complex shape characterized by a transition from the main level to the sublevel. One of these events is illustrated in the lower trace of Fig. 3b. The average probability of this channel being in the substate (given the channel is in the open state), as determined from eight consecutive bursts, was 0.08. This experiment shows directly that a single AChR channel complex, in the presence of constant ACh concentrations, fluctuates between at least three states: a main open state, a substate which is adopted at low probability, and the closed state(s).

The AChR complex purified from Torpedo electroplaque is a pentameric protein complex comprised of five homologous subunits which span the membrane. In lipid bilayers it can form a unit conductance channel which has a similar conductance to that of the AChR channel in rat muscle. The observation that ACh-activated channels in embryonic muscle cells can adopt several conductance states could indicate that these subunits rearrange themselves to form the different open states of a channel. Similarly the two independent classes of channels may represent two different aggregation states of the same set of subunits.

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Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels

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Acetylcholine-like drugs cause ion channels in the skeletal muscle endplate to open briefly, producing, at random intervals, rectangular pulses of current with constant amplitude but random duration, that can be recorded by the patch clamp method. However, even when the agonist concentration is so low that channel activations are very well separated, we have observed, with high resolution methods, that openings may be interrupted by short periods (gaps) so brief that they are very unlikely to arise from two independent channel activations. This sort of behaviour has been predicted on the basis that two or
more openings might occur during the time for which the receptor remains occupied by agonist\(^2\). If this were correct, important new information about agonist activation of ion channels could be obtained from measurements of the gaps between openings. However, short gaps could arise in other ways: for example from brief blockage of the ion channel, perhaps by the agonist itself. We now present results obtained with the acetylcholine-like agonist, suberyldicholine (SubCh, 20–100 nM), which suggest that the brief gaps do not result from ion channel block by the agonist itself, but which are consistent with a mechanism in which the channel opens and closes several times during a single agonist receptor occupancy. We have also observed that the number of short (<1 ms) current pulses is greater than we expected.

Single ion channel currents from the perisynaptic region of normal frog (Rana temporaria) cutaneous pectoris muscle were recorded at 10–13 °C (ref. 4). The Ringer solution contained (mM) NaCl 115, KCl 2.5, CaCl\(_2\) 1.8 and HEPES buffer 3, pH 7.2.

Figure 1a shows the typically low frequency of single channel currents (always <3 s\(^{-1}\)). Individual currents are shown, with high time resolution, in Fig. 1b–c; they are clearly interrupted by brief gaps. During most of these gaps the current does not reach the resting value; however, the frequency response of the system is such that the channel would have to be shut for nearly 300 μs for this to be attained. We assume that the gaps represent brief, but complete, closures of the ion channel, and Fig. 1d–g show how the duration of such closures was estimated. In good records, events with a duration of 50–70 μs could be clearly resolved.

The entire record was digitized so that the duration of all resolvable gaps, as well as openings, could be measured. After this measurement, a safe value was chosen for the minimum resolvable duration, and the record was revised by concatenation of adjacent gaps and openings separated by intervals less than this chosen resolution. Thus an idealized record, with consistent resolution throughout, was obtained for the construction of histograms. All distributions of time intervals were fitted with the sum of one or more exponential functions by the method of maximum likelihood (that is, the actual measured durations were used, not the histogram frequencies).

The distribution of all gaps is exemplified in Fig. 2. In Fig. 2a, the time scale extends up to 2 s, and the gap durations are fitted well by an exponential component with a mean of 352 ms. This is presumably the mean interval between independent activations of ion channels. However, the first bin (up to 50 ms) extends a long way off the graph; the distribution of short gaps is shown in more detail in Fig. 2b, in which the time scale extends up to only 500 μs. There is a clear exponential component of the gap distribution with a mean, in this example, of 45 μs, which corresponds to the short gaps illustrated in Fig. 1. Values of 45–70 μs were consistently observed in nine other experiments. This fast component represented, in this case, 77 per cent of the total area of the gap duration distribution, corresponding to a total number of 2,231 short gaps (although many of these would...
This occurrence of openings in quick succession was called the Nachschlag phenomenon when it was first observed, and this term seems appropriate, whatever the mechanism responsible for the phenomenon. Figure 3 shows the distribution of burst durations; in Fig. 3a the time scale extends to 35 ms, and there are obviously too many short bursts to be fitted by a single exponential distribution. The double exponential distribution shown has a slow component with mean of 10.2 ms, and a fast component with mean of 0.15 ms. The total area under the distribution represents 717 bursts and the slower component corresponds to 73% of the total area in this example, with 20 nM SubCh. Thus we infer, for the experiment illustrated in Figs 2 and 3, that there are, on average 2.251/717 = 3.1 gaps per burst. Similar values (2.4) were found in 10 experiments.

The origin of the short openings illustrated in Fig. 3 is obscure. They may represent a separate type of channel altogether, or perhaps brief openings by channels with only one agonist molecule bound. We shall explore these hypotheses elsewhere.

The most important question that arises is whether the brief gaps result from ion channel block by the agonist, SubCh, itself. Decamethonium (in much higher concentrations) is known to do this. If this were the mechanism, the number of gaps (blockages in this case) per burst should be directly proportional to the agonist concentration. However, the number of gaps per burst with 100 nM SubCh, relative to that with 20 nM, was 0.97 ± 0.11 (three experiments at each concentration), and showed little sensitivity to membrane potential. Therefore, ion channel block by SubCh, in the low concentrations used, cannot explain the brief gaps that we observe. Although it is conceivable that brief closures of the channel might arise from block by some endogenous muscle constituent, or from a mechanism connected with ion permeation, the most plausible alternative to ion channel block is that the closures arise from multiple openings during a single receptor occupancy. Suppose, for example, that two agonist molecules must bind sequentially before the channel can open (see, for example, ref. 9). Then (see ref. 6), at low agonist concentration, the mean length of a gap should be approximately $\beta + 2k_2^{-1}$, and the number of gaps per burst should be approximately $\beta/2k_2$, where $\beta$ is the rate constant for opening of a doubly occupied receptor-channel complex, and $k_2$ is the microscopic rate constant for dissociation of an agonist molecule. Our results, if interpreted in this way, thus suggest preliminary estimates for $\beta$ of the order of 10,000–15,000 s$^{-1}$, with $k_2$ roughly 2,000 s$^{-1}$. This interpretation of our results with SubCh is not compatible with the common assumption that the agonist binding step is very fast, and that the open–shut conformation change is rate-limiting. We are now investigating whether or not the same can be said of agonists other than SubCh. The noise spectrum expected if this interpretation were correct would consist predominantly of a single component, with a time constant close to the mean length of the burst (slow component), that is, 10 ms in the experiment shown in Fig. 3. But this time constant would not correspond, as is often assumed, to the true lifetime of the open state. In the above example the average burst consists of roughly four openings in quick succession, so the mean open lifetime $1/\alpha$ (where $\alpha$ is the channel closing rate constant) would be only about 2.5 ms.

Results similar, in some respects, to ours have recently been found (S. G. Cull-Candy and I. Parker, in preparation) for glutamate-operated channels in locust muscle.

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