

## The Influence of Membrane Patch Isolation on Single Acetylcholine-Channel Current in Rat Myotubes

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### 1. Introduction

One great experimental advantage of the patch-clamp technique is that it permits the recording of single-channel currents from isolated cell-free patches of membrane. By appropriate manipulations, it is possible to obtain cell-free patches in which either the normal external surface of the membrane is towards the bath solution, termed an outside-out patch, or the cytoplasmic surface of the membrane is towards the bath solution, termed an inside-out patch (Hamill *et al.*, 1981). So far, inside-out membrane patches have been employed to study the effects of various intracellular ions on both current flow through open channels (Horn and Patlak, 1980) and channel gating (Marty, 1981; Colquhoun *et al.*, 1981; Yellen, 1982). Although outside-out membrane patches have been used to a lesser extent, they provide a convenient means of studying the effects on channels of various drugs (including neurotransmitters) applied to the outside of the membrane.

Although cell-free patches are clearly an important investigative tool in the analysis of ion-channel function, it is somewhat uncertain whether the procedure of patch isolation itself alters channel properties in any significant manner. In this chapter we address this question for the acetylcholine-activated cationic channels in the membrane of cultured rat myotubes. Single ACh channel currents have been compared using recordings from cell-attached membrane patches and isolated inside-out and outside-out membrane patches. Our results suggest that patch isolation has little effect on the current-voltage relationship or the single-channel conductance of open ACh channels. Single-channel current kinetics also appear unchanged in going from a cell-attached patch to the inside-out recording mode (using mean open burst duration as the criterion, see below). However, formation of

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an outside-out patch does appear to significantly alter ACh channel gating, causing a roughly twofold decrease in mean burst duration.

## 2. Results

As the kinetics of the ACh-activated ion-channel gating reaction are fairly complex, certain complications are introduced into the analysis of channel properties (see Colquhoun and Sakmann, 1981; Chapters 16 and 17). Figure 25-1 illustrates some typical ACh-channel current records obtained from the rat myotubes and some of the methods of analysis that we have employed. The top two traces (Fig. 25-1A) show ACh-channel currents from a cell-attached patch. Like the ACh currents recorded at the frog end plate (Colquhoun and Sakmann, 1981), the square channel openings are sometimes interrupted by brief closing events, which appear as upward spikes. The channel-current records were analyzed in detail by measuring the duration of all closed intervals and plotting the distribution of the closed intervals as a frequency histogram. The distribution for the currents recorded in Fig. 25-1A could be fitted by the sum of two exponential functions. One component was quite rapid, with a decay time constant of 0.79 msec, and corresponds to the very brief closures. The second component was much slower, with a time constant of 351 msec, and corresponds to the long closed intervals between successive channel openings. The initial portion of the closed-time distribution with the fitted fast exponential component is shown in Fig. 25-1E.

According to Colquhoun and Sakmann (1981) (also see Chapter 16 but cf. Chapter 17), this kinetic behavior arises in the following manner. Once an ACh receptor-channel complex has bound agonist and opened, it can then shuttle between the open and closed states several times before the agonist eventually dissociates from the receptor. The brief closings that appear to interrupt the channel openings in Fig. 25-1A thus reflect this cycling of the channel between the open and closed states. The long periods of closure represent the intervals between independent channel openings and reflect the low probability of channel opening in the presence of a low concentration of agonist. The period of repeated channel openings interspersed between the relatively long intervals of closure has been termed the channel burst duration. In general, the burst duration ( $t_b$ ) is longer than the true mean open lifetime of the channel ( $t_o$ ), since each burst is composed of at least one, and possibly several, openings.

In our analysis here, we have used the mean burst duration to characterize the ACh channel current kinetics. Figure 25-1C illustrates one method for determining mean burst duration. A burst was taken to be the total period of channel opening that occurs between two long periods of channel closure. In Fig. 25-1C, we chose a critical length of 3 msec to define a period of "long" closure, so that a burst is defined as any series of channel openings that contains no closing gaps longer than 3 msec. This critical length was chosen to be long compared with the mean duration of the brief closures within a burst (0.79 msec) but short compared to the mean duration of the long closures between two successive bursts (351 msec).