Biochimica et Biophysica Acta, 1063 (1991) 253–258

ADONIS 0005273691001593

BBAMEM 75175

The temperature dependence of some kinetic and conductance properties of acetylcholine receptor channels

James P. Dilger, Roger S. Brett †, David M. Poppers and Yi Liu

Departments of Anesthesiology and Physiology and Biophysics, State University of New York, Stony Brook, NY (U.S.A.)

(Received 17 September 1990)

Key words: Acetylcholine receptor; Channel kinetics; Temperature; Arrhenius plot

We examined the temperature dependence of single-channel properties of the nicotinic acetylcholine receptor channel from clonal BC3H-1 cells over a range of 10–40 °C. We found temperature sensitivities (Q_{10} values) of 2–4 for the mean channel open time. The Q_{10} did not depend strongly on voltage and the voltage dependence of the mean open time was temperature-independent. The Q_{10} of closing rate of the long-lived open state was 3–4 but the Q_{10} of closing rate of the brief open state was independent of temperature. The duration of brief closures could be measured only between 10 and 25 °C. Since this approached the limit of the experimental time resolution, an accurate determination of the Q_{10} could not be made. The current decay due to desensitization after rapid application of high concentrations of agonist varied with a Q_{10} of about 2. The conductance of single channels (the inverse of the ion translocation rate) had a Q_{10} of 1.3–1.5. We found no obvious nonlinearities in the Arrhenius curves for any of the measured properties.

Introduction

The nicotinic acetylcholine receptor-channel is the prototypical transmitter-activated ion channel and currently the best understood member of its class. Our image of this protein is a composite picture derived from biochemical, electrophysiological, structural and genetic experiments.

One early insight into the understanding of the molecular functioning of the receptor ion channel complex was the concept that agonist binding and channel gating are distinct kinetic steps. Support for this idea came from the observation that the rate of decay of end-plate currents was quite sensitive to temperature – suggesting that the decay rate reflected the intrinsic closing rate of the channel rather than the diffusion of acetylcholine out of the synapse [1,2]. Determination of the temperature sensitivity of rates continues to be a useful tool in the understanding of ion-channel kinetics. The essential idea is that the temperature sensitivity of diffusion is fairly low – a ten degree increase in temperature speeds up diffusion by less than 50%. Enzymes, on the other hand, can be very sensitive to temperature – a ten degree increase in temperature can change the rate of reaction of an enzyme several-fold. Implicit in the classification of rates on the basis of their temperature sensitivity is the assumption that the rate being considered arises from a single molecular process – an assumption which is not easily proven. In electrophysiology, however, single-channel recording techniques can potentially reveal reliable information about individual molecular events.

Methods

BC3H-1 cells, which express nicotinic acetylcholine receptors [3], were cultured for electrophysiological experiments [4]. The culture medium was replaced with an 'extracellular solution' consisting of (in mM): NaCl (150), KCl (5.6), $CaCl_2$ (1.8), $MgCl_2$ (1.0) and Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid) (10), pH 7.3. Patch pipettes were filled with a solution containing KCl (140), EGTA (5), $MgCl_2$ (5) and Hepes (10), pH 7.3 and had resistance of 4–6 MΩ. An outside-out patch [5] with a seal resistance of 10 GΩ or greater was obtained and moved into position at the outflow limb of the perfusion system. This perfusion system consisted of a reservoir containing extracellular solution plus 200 nM acetylcholine, a modified Peltier cooling/warming stage (Sensortek TS4, Clifton, NJ, U.S.A.)

† Deceased.

Correspondence: J.P. Dilger, Department of Anesthesiology, State University of New York, Stony Brook, NY 11794-8480, U.S.A.

acting as a heat exchanger and plastic tubing (i.d. = 1.27 mm) – one end of which was inserted into the culture dish. The perfusate flowed at 5 ml/min and its temperature was varied between 10 and 40 °C as measured by a thermocouple (type T needle microprobe) inserted into the perfusion tubing 2 cm upstream from the excised patch. A few experiments were performed on cell-attached patches. For these experiments, 200 nM acetylcholine was included in the pipette solution and the perfusion system was used to change the temperature of the 1–2 ml of extracellular solution in the culture dish as measured by a thermocouple located in the dish. An additional set of experiments were performed on outside-out patches inserted into a rapid solution exchange apparatus [6]. In these experiments, extracellular solution containing 0.1–2 mM acetylcholine was cooled to 10–12 °C as it flowed through stainless steel tubing inserted into an ice bath.

Single-channel currents were measured with a patch-clamp amplifier (EPC7, List Medical Electronics, Darmstadt, F.R.G.), filtered at 3 kHz (902LFP, Frequency Devices, Haverhill, MA, U.S.A.), digitized at 50 μs per point (Sensorium, Burlington, VT, U.S.A.) and stored on the hard disk of a laboratory minicomputer (Micro 11-73, Digital Equipment Corp., Maynard, MA, U.S.A.). Currents obtained during rapid perfusion were detected with the patch-clamp amplifier, filtered at 2 kHz and digitized at 200 μs per point. Data analysis was performed off-line with our own computer programs.

In considering the distribution of the dwell times of channels in open and closed states, we use the following methods and conventions. Several selected traces of raw channel data (2048 digitized points) containing relatively long duration openings were used to assemble an initial amplitude histogram; the distance between the baseline peak and one-channel-open peak was taken to be the approximate single-channel current. The entire 4–10 second segment of raw data (obtained at a particular temperature and voltage) was then examined. An opening transition was assumed to take place when two or more consecutive digitized points were above a threshold value of one half the approximate single-channel current above the baseline. Closing transitions were assumed to occur when at least one digitized point fell below the threshold current. Thus, for the purposes of kinetics, the resolution for open and closed durations was 100 and 50 μs, respectively. A second assessment of the open duration was made based on the calculated amplitude of that particular event. One of the authors or a trained assistant made the final decision to accept or reject the computer's assessment.

At temperatures below 25 °C, the distribution of closed times could usually be fit with two exponentials. However, the time constant of the fast exponential approached the limit of temporal resolution. This was expected since single-channel kinetic studies of acetylcholine receptors from BC3H-1 cells at 11 °C reveal that the predominant component of brief closures has a time constant of about 50 μs [7]. Thus, at all but the very coldest temperatures examined, we were unable to detect most of the brief closing events. Consider the following model of ACh receptor activation:

$$R \overset{K_1/2}{\rightleftharpoons} AR \overset{2K_2}{\rightleftharpoons} A_2R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2R^* \qquad (1)$$

In this model, two agonist molecules (A) sequentially bind to two equivalent sites on the receptor (R) (equilibrium binding constants K_1 and K_2) to form a doubly-liganded closed state (A_2R). This state then undergoes isomerization to an open state (A_2R^*) at a rate β, which subsequently closes at a rate α. In terms of this model, we often cannot distinguish between a single opening and a burst of single openings separated by brief closures. Although we use standard techniques [7] to correct the channel 'open time' for missed brief closures, it is possible that this 'open time' may not provide a precise measure of the closing rate α particularly at high temperatures.

The distribution of open times, containing 200–1000 events, for our data below 35 °C could be fit by two exponentials. The time constant of the brief openings, 100–400 μs, was well within our temporal resolution. This component accounted for 20–40% of the total openings after correction for missed openings [8]. At temperatures above 35 °C, we could not separate the distribution into two components, probably because the time constants of the two components were similar.

For one patch, we examined a wide range of voltage as well as temperatures. The open time results obtained from this patch (Fig. 3 and Table I), refer to the arithmetic mean of all (both brief and long duration) openings since many of the data files contained too few events to warrant a two-exponential fit of the open time distribution.

The temperature dependence of a rate constant, $k(T)$, can be characterized either in terms of a temperature coefficient, Q_{10}, or an activation enthalpy, E_a. The Q_{10} is determined from the slope of a plot of log $k(T)$ vs. temperature ($Q_{10} = 10^{10 * \text{slope}}$). The activation enthalpy is defined by the Arrhenius equation:

$$k(T) = A \exp(-E_a/RT) \qquad (2)$$

where A is a constant, R is the gas constant and T is absolute temperature. Although imprecise, the Q_{10} is a convenient parameter since it can be used to quickly assess the steepness of the temperature dependence of a rate and classify the process as being diffusional ($Q_{10} <$ 1.6) or enzymatic. Similarly, the magnitude of the activation enthalpy can be used for this classification

(E_a < 35 kJ/mol or 8 kcal/mol). Data were fit to Eqn. 2 using a simplex algorithm to minimize the squared deviation. Confidence intervlas were established by finding the range of E_a values which increased the squared deviation by no more than 1 [9].

Results

The qualitative effects of temperature on single nicotinic acetylcholine receptor channels are readily apparent upon examination of single-channel current records (Fig. 1). As the temperature is increased from 13 to 23 to 35 °C, channel openings become more frequent, briefer in duration and larger in amplitude. These effects can be understood in terms of the acceleration of molecular rates by increased temperature: an increase in agonist binding and/or opening rate β giving rise to more frequent openings, an increase in agonist dissociation and/or closing rate α producing a shorter open or burst duration and an increase in permeant ion (Na^+ or K^+) mobility leading to a greater flux of ions through the channel.

Fig. 2 presents our results for the mean channel open time in one patch over a range of temperatures (12.8 through 40.7 °C) and voltage (100 through −120 mV). The exponential relationship between mean open time and temperature and mean open time and voltage is apparent in Fig. 2 since the mean open time is plotted on a logarithmic scale. The linear regression fits of log (mean open time) vs. temperature and vs. voltage are essentially parallel. This suggests that the Q_{10} of the mean open time is voltage-independent and the voltage sensitivity of the mean open time is temperature-independent. The quantitative results of this analysis are presented in Table I. There are small shifts in both the voltage and temperature sensitivity of the mean open time but this was not consistently found in every patch.

The temperature dependence of both brief and long channel open durations at −100 mV for six outside-out patches is shown in Fig. 3 in an Arrhenius plot. The open symbols represent the long duration openings. Analysis of this data gives an activation enthalpy of 93 ± 10 kJ/mol (Q_{10} = 3.6 ± 0.5). Substitution of the mean burst durations and the durations of the all openings for the long open time results in activation enthalpies of 105 (Q_{10} = 4.3) and 100 (Q_{10} = 4.1), respectively. For individual outside-out patches, the Q_{10} of long openings varied between 3.0 and 4.2. We also examined the temperature dependence of the mean open time for two cell-attached patches and found Q_{10} values of 2.1 and 2.9 (temperature range 15–35 °C).

The duration of brief openings (the faster time constant of the two exponential fit to the open duration histogram) is shown in Fig. 3 with closed symbols. At high temperatures, only one component could be resolved – the long-duration open time approaches the duration of brief openings. In contrast to the long-dura-

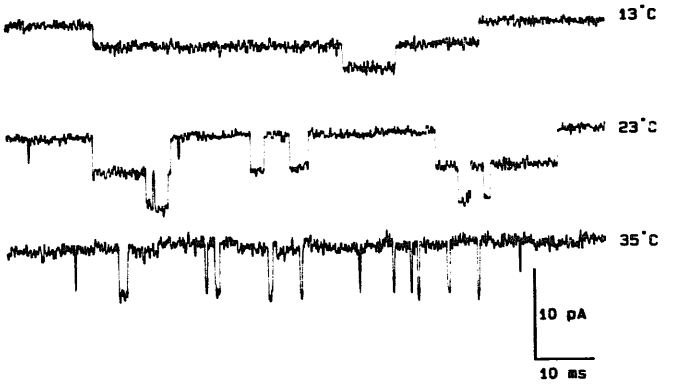

Fig. 1. Examples of single acetylcholine receptor channels at three temperatures (13, 23 and 35 °C) from one of the outside-out patches examined in this study. The patch was held at −100 mV; channel openings are shown as downward current transitions.

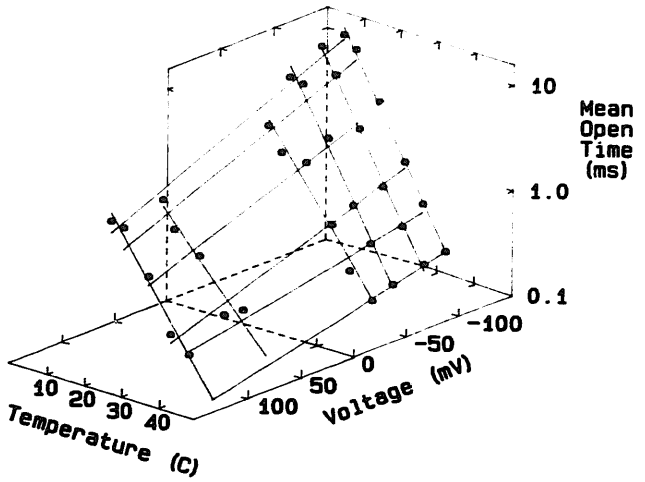

Fig. 2. A three-dimensional view of the logarithm of the mean open time of acetylcholine receptor channels as a function of voltage and temperature for a single patch. Data were obtained from this patch at six different temperatures and voltages (see Table I). Linear regression fits for log(mean open time) vs. temperature at each voltage and log(mean open time) vs. voltage at each temperature are superimposed on the data.

TABLE I

Temperature and voltage dependencies of the mean channel open time

Quantitative results of the linear regression fits presented in Fig. 2. V_e, the potential required to produce an e-fold change in the mean open time, was determined at six different temperatures. Q_{10}, the factor by which the mean open time changes for a 10 °C change in temperature, was determined at six different voltages.

T (°C)	V_e (mV)	V (mV)	Q_{10}
12.8	100	−120	4.5
16.3	100	−100	4.4
23.0	110	−70	4.4
29.6	120	−50	3.2
34.6	210	50	2.5
40.7	170	100	3.4

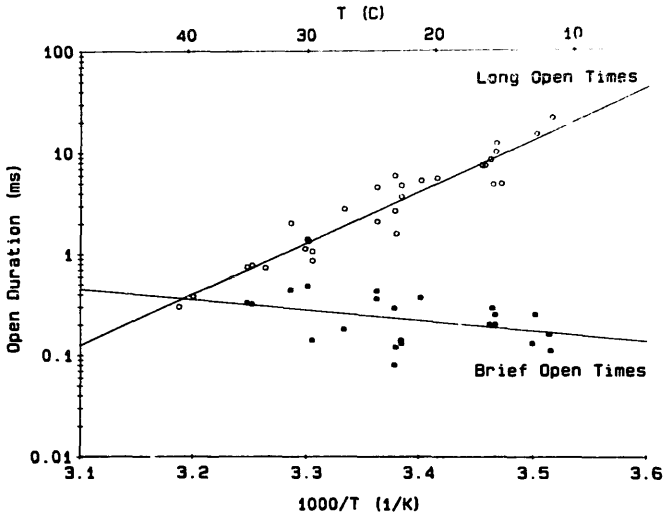

Fig. 3. Arrhenius plot of the temperature dependence of channel-open duration at −100 mV for six outside-out patches. Open and closed symbols represent the time constants of the long and brief components, respectively, of the open time distribution. The lines are drawn based on fitting the data to the Arrhenius equation. For long open times, the fit gave an activation enthalpy of 93 kJ/mol. Linear regression of an Arrhenius transformation of the brief open time data resulted in an insignificant correlation at the 95% confidence level.

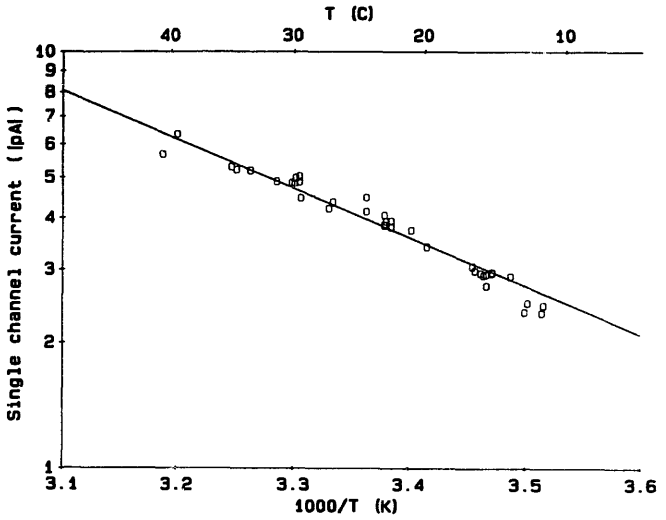

Fig. 4. Arrhenius plot of the temperature dependence of the absolute value of the single-channel current at −100 mV for six patches. The line is drawn based on fitting the data to the Arrhenius equation and is characterized by an activation enthalpy of 23 kJ/mol (Q_{10} = 1.4).

tion open time, the duration of brief openings is essentially temperature-independent ($P > 0.05$). The fraction of brief events decreased with temperature; from about 0.4 at temperatures lower than 20 °C, to 0.2 at temperatures between 20 and 30 °C. The origin of the brief open time component is not fully understood. Several studies have shown that the fraction of brief openings decreases from nearly 1.0 to 0.2 as the acetylcholine concentration is raised from 5 to 200 nM (refs. 10, 11, and unpublished results from our laboratory). This would be consistent with channel activation schemes in which singly-liganded receptors can open, but have a briefer open duration than doubly-liganded receptors. In such a scheme, the fraction of brief openings should decrease to zero as the agonist concentration is raised further. However, the fraction of brief openings remains constant at about 0.2 at high agonist concentrations. Our finding that this residual brief open component is unexpectedly insensitive to temperature, suggests that much remains to be discovered about this component (or group of components).

Fig. 4 shows an Arrhenius transformation of single-channel current amplitude at −100 mV for the six outside-out patches studied. The line is the best fit of the data to Eqn. 2 and gives an activation enthalpy of 23 ± 4 kJ/mol ($Q_{10} = 1.4 \pm 0.1$). For individual patches the Q_{10} ranged from 1.3 to 1.5; in the two cell-attached patches the Q_{10} was 1.3 and 1.6. For three of the outside-out patches, we had sufficient data at different voltages to calculate the channel conductance from a current voltage plot. The Q_{10} values for conductance for these patches were in the range of 1.3 to 1.5. The reversal potential was close to 0 mV at all temperatures.

For one patch, we estimated a Q_{10} of 1.7 for the number of openings per unit time. In most patches, however, there was a gradual decrease in activity over time which precluded such an analysis. Since these experiments were performed with a relatively low concentration of agonist (at 200 nM acetylcholine the open probability is less than 10^{-3}, [12]), the number of openings per unit time is dominated by agonist binding and the total number of channels rather than the channel opening rate itself.

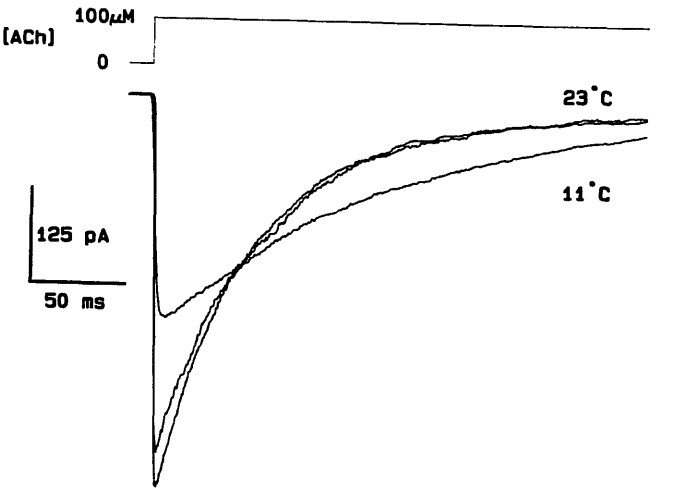

Fig. 5. Macroscopic current decay induced by rapid perfusion of 100 μM acetylcholine to an outside-out patch at 23.5 °C, 11 °C and return to 23.5 °C. The time course of the concentration change is shown at the top of the figure; the return to agonist free solution is not shown. The patch pipette potential was −50 mV. Peak currents and relaxation time constants were −515 pA, 47 ms (23.5 °C); −292 pA, 125 ms (11 °C) and −470 pA, 56 ms (23.5 °C, return). Each trace is the ensemble mean of 10 to 15 individual records.

Examples of the current decay after rapid perfusion of 100 μM acetylcholine are shown in Fig. 5 for 23.5 and 11°C. The current response at the lower temperature has a smaller amplitude ($Q_{10} = 1.5$) and a longer relaxation time constant ($Q_{10} = 2.2$). This patch contained about 250 channels, 90% of which are opened by 100 μM acetylcholine at 20–22°C [12]. The Q_{10} of the decrease in peak current amplitude is similar to the Q_{10} of the single-channel current. Since the peak current amplitude is proportional to the product of the single-channel current and the open-channel probability, this result indicates that the open-channel probability is not sensitive to temperature. Indeed, determination of the open-channel probability at 11°C from ensemble analysis [12] gives $P \approx 0.9$. At higher agonist concentrations (0.5, 1 and 2 mM acetylcholine) the temperature sensitivities of the current amplitudes and relaxation times were 1.5 and 3.0, respectively. The Q_{10} values of the current amplitudes and relaxation times exhibited no voltage dependence over the range of +50 to −50 mV.

Discussion

The starting point for any discussion of the temperature sensitivity of acetylcholine receptor kinetics and conductance must be the classic work of Magleby and Stevens [1,2] on end-plate current decay and of Anderson and Stevens [13] on end-plate current noise. These authors established a connection between the end-plate current decay time constant, the end-plate current noise spectrum cut-off frequency and the closing rate of the channel. They found the closing rate, α, to be exponentially dependent on voltage according to:

$$\alpha(V) = \alpha(0) \exp(V/V_e) \quad (3)$$

where V_e is the voltage needed to change the rate e-fold. The temperature sensitivity of α ($Q_{10} = 2.8$) was consistent with there being a conformational change in the channel protein upon channel closure.

These first studies were done on frog muscle. Subsequent studies found similar voltage and temperature relationships for α in other receptor preparations including mouse muscle, chick myoballs and rat muscle (see Ref. 14 for a critical review of this literature). The introduction of single-channel recording techniques provided a more direct measure of α – the inverse of the channel open time. Using single channel methods, several investigators have verified the voltage dependence of Eqn. 3 (Refs. 7, 10, 11, and our data in Table I). Our data also demonstrate that the temperature sensitivity of the channel open time is similar to the temperature sensitivity of the end-plate current decay and noise.

The long-duration open time may give a biased estimate of the channel closing rate, α. If there are many unresolved brief closures at high temperatures, the open time is actually an estimate of the burst duration, $(1 + \beta/k_{-2})/\alpha$, where k_{-2} is the agonist dissociation rate constant (this is also true for the decay of the end-plate current). This would be a particular problem if β were more sensitive to temperature than k_{-2} and it would also indicate that the measured single channel conductance at high temperatures is an underestimate. It will be difficult to resolve this problem without improvements in the time resolution of single-channel recording or independent measurements of the kinetic parameters. For now, we note that the Q_{10} of the measured burst duration is indistinguishable from that of the long-duration open time.

Studies of the temperature dependence of channel conductance have produced less consistent results. Again, we start with Anderson and Stevens [13] who deduced conductance from noise measurements. They found no dependence of temperature on conductance between 8 and 18°C, but suggested that their experiments may not have been sensitive to the small temperature dependence which would be expected. Other investigators using noise analysis reported values of Q_{10} in the range of 1.3–1.5 [14,15], consistent with the temperature sensitivity of ions in solution [16]. Finally, there are some reports of Arrhenius plots of conductance in which the slope changes abruptly near 20–25°C [17,18]. A number of explanations have been advanced to account for these latter results including lipid phase transitions and temperature dependent conformational changes. To these we would like to add the idea that the presence of subconductance levels of the acetylcholine receptor channel, particularly in chick myoblasts [19], invalidate assumptions inherent in noise analysis.

There is more uniformity in the results of conductance measured from single channel experiments. Several studies indicate that the temperature sensitivity of single-channel conductance is similar to that of diffusion of ions in solution $Q_{10} = 1.3$–1.5 (Refs. 4, 20, and our present results) while Hoffmann and Dionne [21] reported somewhat higher values ($Q_{10} = 1.97$). In one study [20], the Arrhenius plot was slightly steeper at lower temperatures (5–11°C) but did not exhibit the dramatic nonlinearities mentioned above. Similarly, when we analyzed our data of Fig. 4 separately for conductances measured above and below 25°C, we obtained activation enthalpies of 18 and 31 kJ/mol for high and low temperatures, respectively ($Q_{10} = 1.26$ and 1.55).

Magazanik and Vyskocil [22] explored the temperature sensitivity of desensitization in frog muscle between 2 and 22°C. They found a Q_{10} of 1.9, similar to what we observe. It should be noted, however, that the time scale of 'desensitization' is seconds for Magazanik and Vyskocil [22] and less than 100 ms in our experiments so the two studies may have been dealing with

different phenomena. It should be noted that the current decay rate is generally not due to a single kinetic process but may be influenced by the probability of being in a pre-desensitized state and the rate of return from the desensitized state. In the present case, however, the decay rate is independent of concentration above 100 μM acetylcholine and the extent of desensitization is $> 90\%$ [12]. This suggests that the current decay rate is a good indicator of the kinetic rate of desensitization.

It seems appropriate to ask whether temperature-dependence studies have taught us anything new about ion channels. In most cases, these investigations have not progressed beyond the point of cataloging results. One notable exception is a study in which the temperature sensitivity of the single-channel conductance was used to discriminate between models of ion translocation through the channel [21]. The reason that temperature experiments have not also benefitted modeling of channel kinetics may be that the channel closing rate is really the only kinetic parameter which has so far yielded information. Future studies on other rates, the opening rate in particular, may help remedy this situation.

Acknowledgements

We thank Ms. Ana Maria Vidal for assistance in analyzing single-channel data, Ms. Claire Mettewie for culturing cells and Mr. Yashpal Singh for computer programming. This study was supported by grant GM42095 from the National Institutes of Health.

References

1 Magleby, K.L. and Stevens, C.F. (1972) J. Physiol. 223, 151–171.
2 Magleby, K.L. and Stevens, C.F. (1972) J. Physiol. 223, 173–197.
3 Schubert, D., Harris, A.J., Devine, C.E. and Heinemann, S.F. (1974) J. Cell Biol. 61, 398–413.
4 Sine, S.M. and Steinbach, J.H. (1984) Biophys. J. 45, 175–185.
5 Hamill, O.P., Mary, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
6 Brett, R.S., Dilger, J.P., Adams, P.R. and Lancaster, B. (1986) Biophys. J. 50, 987–992.
7 Sine, S.M. and Steinbach, J.H. (1986) J. Physiol. 373, 129–162.
8 Colquhoun, D. and Sigworth, F.J. (1983) in Single-channel recording (Sakmann, B. and Neher, E., eds.), pp. 191–263, Plenum Press, New York.
9 Colquhoun, D. and Ogden, D.C. (1988) J. Physiol. 395, 131–159.
10 Colquhoun, D. and Sakmann, B. (1985) J. Physiol. 369, 501–557.
11 Papke, R.L. and Oswald, R.E. (1986) in Nicotinic Acetylcholine Receptor (Maelicke, A., ed.), pp. 243–257, Springer Verlag, Berlin.
12 Dilger, J.P. and Brett, R.S. (1990) Biophys. J. 57, 723–731.
13 Anderson, C.R. and Stevens, C.F. (1973) J. Physiol. 235, 655–691.
14 Lewis, C.A. (1985) J. Gen. Physiol. 85, 137–156.
15 Sachs, F. and Lecar, H. (1977) Biophys. J. 17, 129–143.
16 Hille, B. (1984) Ionic channels of excitable membranes, Sinauer Associates, Sunderland, MA.
17 Dreyer, F., Muller, K.D., Peper, K. and Sterz, R. (1976) Pflügers Arch. 367, 115–122.
18 Fischbach, G.D. and Lass, Y. (1978) J. Physiol. 280, 527–536.
19 Sachs, F. (1983) in Single-channel recording (Sakmann, B. and Neher, E., eds.), pp. 365–376, Plenum Press, New York.
20 Quartararo, N. and Barry, P.H. (1988) Pflügers Arch. 411, 101–112.
21 Hoffman, H.M. and Dionne, V.E. (1983) J. Gen. Physiol. 81, 687–703.
22 Magazanik, L.G. and Vyskocil, F. (1975) J. Physiol. 249, 285–300.