Br. J. Pharmac. (1982), **77**, 395–404

FAST DESENSITIZATION OF THE NICOTINIC RECEPTOR AT THE MOUSE NEUROMUSCULAR JUNCTION

P. PENNEFATHER & D.M.J. QUASTEL

Department of Pharmacology, Faculty of Medicine, The University of British Columbia, 2176 Health Sciences Mall, Vancouver, B.C., V6T 1W5, Canada

1 When low concentrations of carbachol (2–20 μM) were applied by local superfusion to mouse diaphragm endplates, there occurred a rapid decrease (within seconds) in the height of miniature endplate currents (m.e.p.cs) in addition to the increase of muscle membrane conductance.

2 With 2, 5, 10 and 20 μM carbachol, m.e.p.c. heights were diminished by 5, 10, 30 and 50% respectively. A subsequent slow decrease in height took place at a rate corresponding to that reported for the slow desensitization produced by bath-applied carbachol (see Adams, 1975).

3 The effect of carbachol on m.e.p.c. height was not affected by poisoning of acetylcholinesterase (AChE). After poisoning of AChE, 4 μM acetylcholine (ACh) depressed m.e.p.c. height by 23%.

4 At 20 μM carbachol, both the onset and offset of the effect on m.e.p.c. height lagged behind the subsynaptic conductance change, and the calculated change of subsynaptic agonist concentration, by about 3 s; the onset rate was at least ten times faster than expected for slow desensitization.

5 When the conductance responses produced by carbachol were corrected for fast desensitization, the slope of the log-response log-dose line (Hill coefficient) was increased from 1.7 to 2.0.

6 The Hill coefficient for fast desensitization was 1.4. The data were compatible with a cyclic model for fast desensitization, with receptor activation not a prerequisite for desensitization of receptors.

7 The failure of AChE poisoning to affect m.e.p.c. height during desensitization suggests that desensitized receptor associated with exogenous agonist can continue to bind quantal ACh.

Introduction

Maintained exposure of nicotinic acetylcholine (ACh) receptors to an appropriate agonist results in desensitization, i.e., a reduction in the response to the agonist (Thesleff, 1955; Katz & Thesleff, 1957). This is now known to proceed in at least two phases. With superperfusion of the frog motor endplate with carbachol, the conductance change associated with opening of ionic channels reaches a maximum in several seconds and then declines at a rate proportional to the concentration of the drug; with carbachol this rate is about 500 $M^{-1}s^{-1}$ (Adams, 1975). A more rapid component of desensitization becomes apparent when iontophoretic pulses of carbachol are applied to the endplate in conjunction with bath application (Feltz & Trautmann, 1980; Clark & Adams, 1981), when iontophoretic pulses are applied in rapid succession (Anwyl & Narahashi, 1980), or when amplitude of endplate currents are used as an index of subsynaptic sensitivity (Feltz & Trautmann, 1982). The bursts and clusters of bursts of single channel events recorded by Sakmann, Patlak & Neher (1980) in the presence of high concentrations of ACh also imply at least two components of desensitization.

The presence of a rapid component of desensitization complicates the interpretation of response-concentration curves for agonist, since the peak responses which are measured may occur at times when appreciable fast desensitization has already developed. In the present experiments we have used the amplitude of miniature endplate currents (m.e.p.cs) as an index of the extent of receptor desensitization induced by relatively low concentrations (2–20 μM) of carbachol (cf. Feltz & Trautmann, 1982), in order to obtain response-concentration curves 'corrected' for fast desensitization. The data indicate that such correction brings the Hill coefficient for carbachol to a value of 2.0. However, the Hill coefficient for fast desensitization is less, about 1.4. The rate of recovery from fast desensitization is similar to the rate of development, indicating a cyclic process as postulated by Katz & Thesleff (1957).

In addition, the decrease of m.e.p.c. height by carbachol contrasts with that observed with receptor blockade by tubocurarine or α-bungarotoxin in that it is not antagonized by poisoning of acetylcholinese (Pennefather & Quastel, 1981). This suggests that fast desensitization may *not* be associated with reduction of binding of quantal ACh by receptors, even in the presence of exogenous agonist.

0007-1188/82/110395-10 $01.00

Methods

Mouse hemidiaphragms were maintained *in vitro* at room temperature (25–27°C), mounted on silastic in a shallow chamber. Bathing solutions were applied as described by Cooke & Quastel (1973); the system of local superfusion permitted changing of the bathing solution at superficial endplates within a few seconds. The control bathing solutions had the following composition (mM): $NaNO_3$ 125, KNO_3 10, $Mg(NO_3)_2$ 1, $Ca(NO_3)_2$ 2, NaH_2PO_4 1, $NaHCO_3$ 24, tetrodotoxin 10^{-4}, glucose 11, equilibrated with 95% O_2 and 5% CO_2. The K^+ concentration was raised to 10 mM in order to increase m.e.p.c. frequency to a rate of about 5/s, which was convenient for recording purpose and NO_3 rather than Cl^- was used as the principal anion in order to reduce resting conductance of the muscle membrane and hence improve the signal to noise ratio of recorded m.e.p.cs (Linder & Quastel, 1978).

The method of point voltage-clamping at endplates was essentially conventional and has been described in detail elsewhere (Linder & Quastel, 1978). M.e.p.cswererecorded both on paper, using a Mingograf ink-jet recorder, concurrent with the d.c. record of holding current, and by a PDP-12 computer. Averages of m.e.p.cs were made by the computer after elimination of artifacts and m.e.p.cs that were 'unusual' and particular care was taken to perform the averaging in such a way (Pennefather & Quastel,

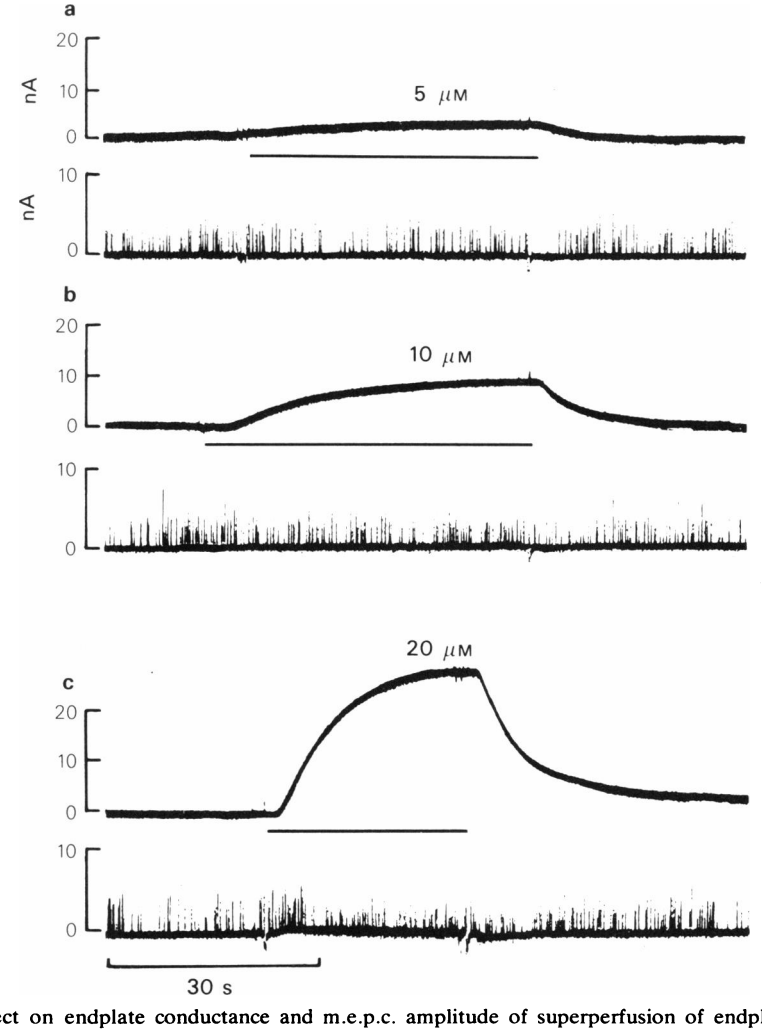

Figure 1 Effect on endplate conductance and m.e.p.c. amplitude of superperfusion of endplate region with carbachol. Concentration of carbachol: (a) 5 μM; (b) 10 μM; (c) 20 μM. In each section the upper trace is a d.c. record of the membrane current and the lower trace is an a.c. record of the m.e.p.cs. Solid bar indicates duration of superperfusion with solutions containing carbachol at concentrations indicated.

1981) as not to introduce artifacts due to a noisy baseline, as occurred when m.e.p.cs were recorded in the presence of exogenous agonist. Average m.e.p.c. amplitude was determined either from the m.e.p.cs recorded by the computer, or from the records on paper. The results of the two methods coincided closely; the paper records were used for resolution of the time course of m.e.p.c. amplitude change within a time frame of seconds.

In some of the experiments acetylcholinesterase was poisoned by exposing the muscle for 4 min to 4 μM paraoxon, an irreversible agent. Other drugs used were carbachol (Aldrich) and acetylcholine (K & K).

Results

Effects of carbachol on m.e.p.c. height

Figure 1 shows typical effects of superperfusion with carbachol; in each section of Figure 1 the upper portion is the d.c. record of the holding current, while the lower portion shows an a.c. record of m.e.p.cs. Together with the increase in membrane current, carbachol also caused a depression of m.e.p.c. height; the time course roughly paralleled the change in current (see Figure 1c).

The effect on m.e.p.c. height tended to be obscured by the spontaneous variation of m.e.p.cs and became much more obvious when they were averaged. Figure 2 shows averages of m.e.p.cs recorded before applying carbachol and during superfusion of 2, 5, 10, and 20 μM carnachol, at two different junctions. In each case, m.e.p.cs were averaged over the period between 15 and 120 s after switching to a given concentration of carbachol. During this time the membrane current was fairly constant. With concentrations of carbachol greater than 20 μM, the membrane current was not constant during this period and m.e.p.cs were generally too noisy to record. At the second junction, illustrated in the lower panel, acetylcholinesterase (AChE) had previously been inactivated by paraoxon; the effect of carbachol on m.e.p.c. height was much the same. In Table 1 are shown data from 45 junctions, recorded in 5 separate experiments. The mean percentage depression of m.e.p.c. height by carbachol was not significantly affected by AChE poisoning at any of the carbachol concentrations tested (2–20 μM). However, it may be noted that no effect of AChE poisoning to increase m.e.p.c. height was apparent in the presence of 5–20 μM carbachol.

In a few cells the effect of ACh on m.e.p.c. height was tested. Before AChE inactivation the response to ACh was extremely variable and concentrations of ACh as high as 30 μM often had little effect on m.e.p.c. height or membrane current. After exposure of the diaphragm to 4 μM paraoxon for 4 min, the action of ACh became reproducible from one end-

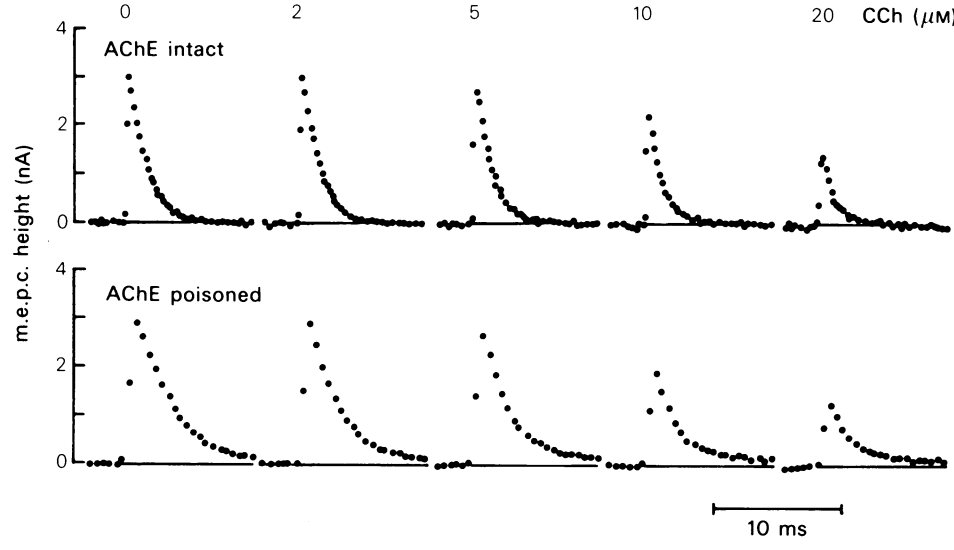

Figure 2 Averages of m.e.p.cs during short exposure to carbachol (CCh). Each average is the mean of about 50 individual m.e.p.cs recorded as described in Methods during the period between 15 s and 60–120 s following the change to solutions containing carbachol. Average m.e.p.cs in the upper part of the figure were derived from m.e.p.cs recorded, at one endplate, in the presence of 0, 2, 5, 10 and 20 μM carbachol. Acetylcholinesterase (AChE) was intact at this endplate. In the lower part of the figure, m.e.p.cs were recorded at another endplate under similar conditions; AChE was poisoned by previous exposure to the irreversible agent, paraoxon (4 μM for 5 min).

Table 1 Effect of brief (30–120 s) superfusion with acetylcholine and carbachol on m.e.p.c. height

Agonist	Concentration (μM)	Before paraoxon		After paraoxon	
		nA	Control	nA	Control
Carbachol	0	2.86 ± .09 (15)	100	3.13 ± .08 (28)	100
Carbachol	2	2.74 ± .10 (9)	95 ± 1	3.02 ± .08 (5)	97 ± 1
Carbachol	5	2.64 ± .10 (11)	91 ± 1	2.63 ± .10 (8)	87 ± 2
Carbachol	10	2.05 ± .10 (12)	71 ± 3	2.09 ± .08 (10)	68 ± 1
Carbachol	20	1.60 ± .07 (7)	51 ± 3	1.61 ± .12 (5)	52 ± 6
Acetylcholine	1	—	—	3.09 ± .16 (4)	101 ± 2
Acetylcholine	2	—	—	3.21 ± .14 (4)	104 ± 1
Acetylcholine	4	—	—	2.39 ± .16 (5)	77 ± 4

plate to another; the potency of ACh in increasing the membrane current and reducing m.e.p.c. height was several times that of carbachol. At 4 μM ACh caused 23% depression of m.e.p.c. height. However, lower concentrations (1 or 2 μM) actually increased m.e.p.c. height to a small extent (Table 1). The latter effect presumably corresponds to the transient potentiation of m.e.p.c. height with low concentrations of either carbachol or ACh in frog muscle (most obvious at 5°C and nearly absent at 24°C) reported by Feltz & Trautmann (1980, 1982).

The effect of carbachol on m.e.p.c. height developed almost as rapidly as the effect on the net current. In Figure 3 are shown data for 10 μM car-

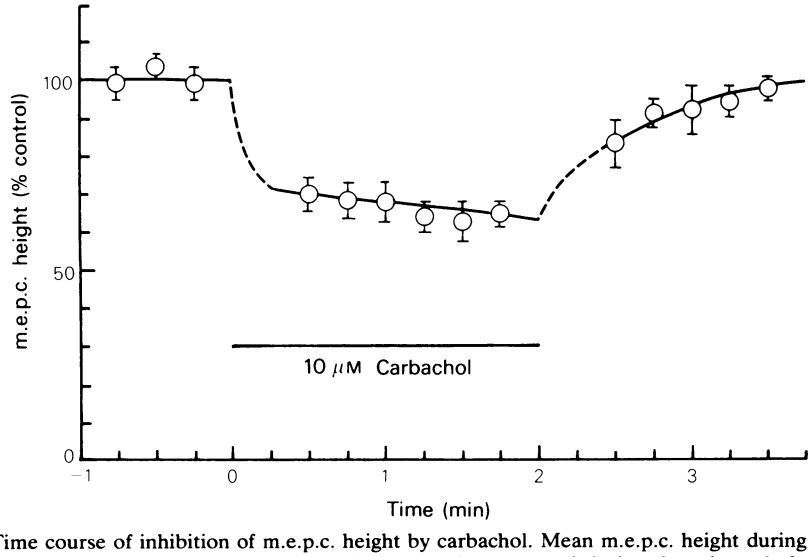

Figure 3 Time course of inhibition of m.e.p.c. height by carbachol. Mean m.e.p.c. height during 30 s periods is expressed as a percentage of the mean height of m.e.p.cs that occurred during the minute before exposure to carbachol. Each point is an average of data from 5 different cells. Solid horizontal bar indicates duration of superperfusion with 10 μM carbachol. Vertical bars indicate s.e.mean. Line drawn through points between 30 s and 105 s exposure to carbachol is line of best fit by linear regression. Other lines drawn by eye.

bachol averaged from 5 endplates. On this time scale, depression of size was seen to be nearly complete within 30 s; a slight downward trend in m.e.p.c. height during the subsequent 2 min period of recording presumably represents the slow component of desensitization, which would appear to have a time course similar to that found at the frog neuromuscular junction (Adams, 1975); with the periods of exposure and concentration of agonists used in these experiments it was generally small enough to ignore.

Dose-response relationship

The concentration-response relationship for the conductance increase produced by carbachol is shown in Figure 4. Carbachol 10 μM caused a peak increase in current of 6.4 ± 1.0 nA ($n = 9$) at -60 mV; this corresponds to a conductance increase of about 100 nS. Responses to 2, 5 and 20 μM carbachol, expressed as a fraction of the response in the same cell to 10 μM carbachol, show a relation between the log-response and the log-concentration that is linear and has a slope of 1.7 (solid line Figure 4). However, when the

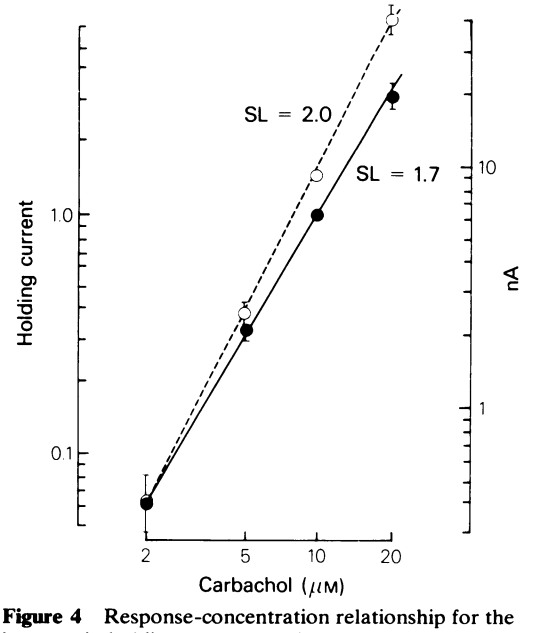

Figure 4 Response-concentration relationship for the increase in holding current produced by carbachol corrected for fast desensitization. Data were obtained from 10 endplates. Vertical bars indicate s.e.mean. (●) Means of the increase in membrane current expressed as a fraction of the increase at 10 μM carbachol. Solid line is the linear regression line of these points (on a log-log plot) and has a slope of 1.7. This slope corresponds to the Hill coefficient since all responses were small compared to the expected maximum. (○) Obtained by correcting the response for fast desensitization. These are fitted by a line with a slope of $1.96 \pm .06$.

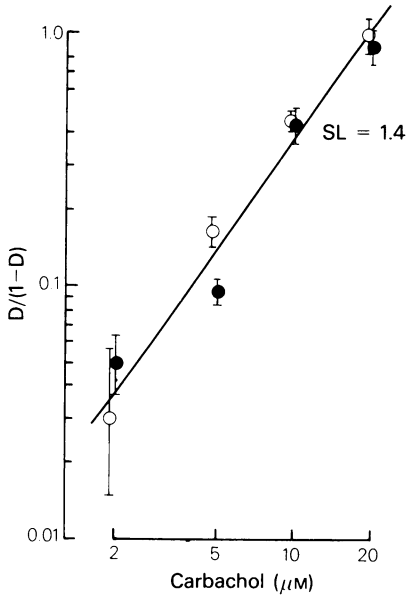

Figure 5 Hill plot of the relation between fast desensitization and carbachol concentration. The amount of fast desensitization produced by carbachol was calculated from data in Table 1. (●) Endplates where AChE is intact; (○) endplates where AChE has been poisoned by paraoxon. D is defined in appendix and is an estimate of the fraction of receptor desensitized. The Hill coefficient is 1.4.

responses to carbachol were 'corrected' for desensitization by dividing them by the average m.e.p.c. height during the response, expressed as a fraction of control m.e.p.c. height (dashed line, Figure 4), then a slope of 2.0 was obtained. Provided the reduction of m.e.p.c. height is proportional to the reduction in the number of activatable receptors, this correction is valid no matter what the mechanism by which desensitized receptors are generated (see Appendix). Although the correction yields a Hill coefficient of 2.0 for the change in membrane current, the Hill coefficient for fast desensitization was 1.4 (Figure 5).

The total number of ACh receptor ionophores at the mouse neuromuscular junction is about 10^7 (Matthews-Bellinger & Salpeter, 1978) each giving a conductance of about 25 pS (Colquhoun, 1978). Thus, the conductance change produced by 10 μM carbachol, 106 ± 16 nS ($n = 9$) corresponds to activation of 0.042% of all receptors, which is 0.06% of activatable receptors (since 30% are desensitized). If we assume that both binding sites on the receptor bind agonist independently, these values give for K_a (the receptor-carbachol dissociation constant) a value of 400 μM. This value for K_a is similar to values (300–1,000 μM) reported for carbachol (again assuming independent binding) on the basis of

response recorded under conditions when fast desensitization should be minimal (ionic fluxes—Neubig & Cohen, 1980; Moore & Raftery, 1980; ionophoresis—Dreyer, Peper & Sterz, 1978; Dionne, Steinbach & Stevens, 1978). If there exists positive cooperation in agonist binding, the K_a for association of the first agonist molecule will be greater while the K_a for association of the second agonist molecule will be less.

Time course of the depression of m.e.p.c. height

At several junctions we attempted to resolve the time course of the rapid effect of carbachol on m.e.p.c. height. The endplate area was repeatedly superperfused with carbachol for 20–30 s periods, and the heights of m.e.p.cs occurring in 3 or 4 s 'bins' were averaged over successive trials; at least 30 m.e.p.cs were obtained for each bin. Figure 6 shows a typical result. The depression of m.e.p.c. height by carbachol at each time has been expressed as a fraction of the maximum depression of m.e.p.c. height, obtained at the end of the exposure to carbachol. It is evident that there was a lag of about 4 s between the change in total current (solid line) and the reduction of m.e.p.c. height (histogram) and the time lag between the return of the current to baseline and the return of the m.e.p.c. height to control values was no greater. The time course of change of carbachol concentrations at receptors could be calculated by correcting the response (change in net current) for desensitization, as estimated from the m.e.p.c. size, and taking the square root (cf. Figure 5). The inset in Figure 6 shows that the resulting function corresponded to a single exponential process, with a time constant of 6 s, for the increase of carbachol concentrations during the period of application. The dotted lines in Figure 6 are drawn assuming a 6 s time constant for both entry of carbachol to the receptor region and for wash out of carbachol on changing to control solution, i.e., we have disregarded the slow phase of recovery of holding current, which may well reflect Na^+ gain and loss of K^+ from the muscle during the action of carbachol (see Lester, 1978). For example, in a 30 μm diameter fibre, an inward current of 10 nA would correspond to internal reduction of $[K^+]$ at about 0.1 mM/s if the change were uniform over a 1 mm length. The consequent change of holding current can be calculated by summing the inward membrane currents resulting from shift of the K^+ equilibrium potential at various

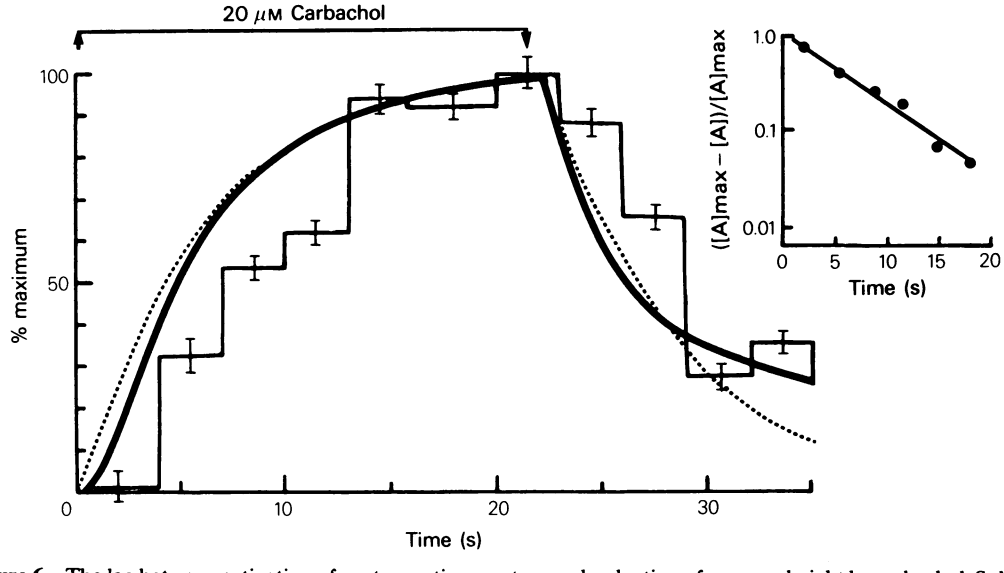

Figure 6 The lag between activation of postsynaptic receptors and reduction of m.e.p.c. height by carbachol. Solid bar in upper part of the figure indicates the duration of superperfusion with 20 μM carbachol. Solid line is the change in membrane current, expressed as a percentage of the peak change during and following 5 repeated exposures of carbachol at the same endplate. Histograms represent the mean reduction of m.e.p.c. height expressed as a percentage of the peak reduction, over the averaging periods indicated. The dotted line represents the local carbachol concentration calculated as proportional to the square root of membrane current 'corrected' for desensitization, and expressed as a percentage of maximum. The line for fall of carbachol concentration is drawn on the assumption that it followed the same time course as the rise. Inset shows that the accumulation of carbachol at the endplate follows a simple exponential process. A is the calculated concentration of carbachol at the endplate and A_{max} is the concentration at 21 s.

distances from the endplates; values up to about 5 nA (depending mainly on fibre diameter) after 500 nC of Na^+ influx (see Figures 1 and 6) are predicted by this mechanism. The existence of this current during carbachol application should have no effect on m.e.p.cs and negligible effect on dose-response curves.

The observed time lag for development and recovery of desensitization corresponds to a time constant of the process of about three seconds. It will be less if some of the total response to carbachol was generated by relatively quickly accessible extrajunctional receptors. The 3 s value is similar to that observed by Katz & Thesleff (1957) for the desensitization produced by ionophoretic applications of ACh and for the fast phase of desensitization by bath applied ACh or carbachol (Feltz & Trautmann, 1982), at the frog endplate. The present data are entirely compatible with the cyclic model proposed by Katz & Thesleff (1957), namely:

$$\begin{array}{ccc} A + R & \underset{}{\overset{K_a}{\rightleftharpoons}} & AR \\ k_2 \uparrow\downarrow k_{-2} & & k_{-1} \uparrow\downarrow k_1 \\ A + R' & \underset{K_a'}{\rightleftharpoons} & AR' \end{array} \quad \text{(cyclic model)}$$

the only difference being that in our scheme AR does not represent activated receptor. Here, A is the agonist, R is activatable receptor, and R′ and AR′ are desensitized receptors. K_a and K_a' are dissociation constants while k_1, k_{-1}, k_2 and k_{-2} are rate constants. The form AR is presumably an intermediate in the generation of the active A_2R species. If k_{-1} and k_{-2} are small enough to be ignored on the time scale of the observations, the rate of formation of desensitized receptors is $k_1[AR]$, and the rate at which desensitized receptors revert to the activatable form is $k_2[R']$. The rate constant of the equilibrium of the system is given by the equation

$$\tau^{-1} = k_1[AR]/([AR] + [R]) + k_2[R']/([AR'] + [R'])$$

At equilibrium ($k_1[AR] = k_2[R']$), and with 20 μM carbachol half of all receptors are desensitized ($[AR] + [R] = [R'] + [AR]$). Hence, at 20 μM carbachol,

$$k_1/(1 + K_a/[A]) = k_2/(1 + [A]/K_a') = \tau^{-1}/2 \simeq 0.15\,s^{-1}$$

With K_a about 400 μM (see above) this gives $k_1 \simeq 3\ s^{-1}$, which is comparable to the corresponding rate ($2\,s^{-1}$) found by Sakmann, Patlak & Neher (1980) for the limitation of single channel bursts (in frog at 11°C with 20 μM ACh). With the present techniques, carbachol was not washed out sufficiently fast to permit a good estimate of k_2; the data are compatible with a wide range of k_2 and k_a'. The results of Sakman *et al.* (1980) indicate $k_2 \simeq 5\ s^{-1}$. With this value, K_a' will be $\simeq 0.7$ μM, and 97% of desensitized receptors will be associated with agonist at 20 μM carbachol.

Discussion

The reduction of m.e.p.c. height produced by low concentrations of carbachol develops and recovers too rapidly to be accounted for by the same process responsible for the slow decay of the steady state response produced by bath-applied agonist (see Rang & Ritter, 1970; Adams, 1975). However, it may well correspond to the desensitization observed with iontophoretic application of agonist (Katz & Thesleff, 1957; Magazanik & Vyskocil, 1976), which has similarly rapid rates of onset and offset.

There is convincing evidence that activation of a nicotinic receptor-ionphore at the neuromuscular junction normally requires 'cooperation' of two molecules of agonist (rev. by Adams, 1981). But, with bath application of carbachol or ACh, the log response-log concentration line typically has a slope of 1.5–1.7 (present data, Rang, 1971; Colquhoun, Dreyer & Sheridan, 1979; Pennefather & Quastel, 1980), which can be interpreted as suggesting that channels may sometimes be opened by only one molecule of agonist attached to the receptor (Dionne *et al.*, 1978). An alternative explanation for a Hill coefficient of less than 2.0 is that fast desensitization acts to distort the response-log dose curve; the present finding that correction for fast desensitization (see Appendix) increases the slope of the log response-log concentration line from 1.7 to 2.0 suggests that cooperation is indeed a prerequisite for activation.

Another consequence of fast desensitization is that it will lead to an apparent dissociation constant for agonist that is far removed from any one of the dissociation constants of the various receptor species. When the receptor system is examined in terms of agonist binding on a time scale long relative to k_1 and k_2, an *apparent* dissociation constant (K_c) will be defined by the relation:

$$[A]/K_c = ([AR] + [AR'])/([R] + [R'])$$

At 20 μM carbachol half the receptors are desensitized. [AR] is small, and so is [R′] if K_a' is small; K_c is therefore about 20 μM. For *Torpedo* electroplaques Weiland & Taylor (1979) have reported a progressive increase of apparent affinity of nicotinic receptors for carbachol; the apparent dissociation constant changes from 20–50 μM at about 15 s, to 0.2–0.5 μM upon 30–60 min of incubation. The increase presumably corresponds to the development of slow desensitization (Cohen, 1978; Weiland & Taylor 1979; Sine & Taylor 1980; Boyd & Cohen, 1980a, b). Since it is clear that the activated state must have a still lower affinity for carbachol than the 'low affinity' state observed at 15 s (see Neubig & Cohen 1980; Boyd & Cohen, 1980b), the latter may well correspond to the fast desensitization cycle.

The failure of AChE poisoning to increase m.e.p.c. height in the presence of 5 μM or more carbachol stands in contrast to the 16% increase in height which is observed normally, or the increase of about 40% when m.e.p.c. height is depressed (to about 50%) by tubocurarine, α-bungarotoxin or myasthenic immunoglobulin (Pennefather & Quastel, 1980; 1981). The implication is that in the presence of an appreciable number of desensitized receptors, blockade of ACh hydrolysis does not increase the number of ACh molecules that combine with activatable receptors during the rising phase of the m.e.p.c. i.e., the efficiency of capture of quantal ACh (see Pennefather & Quastel, 1981) does not appear to be reduced by fast desensitization. The simplest explanation for this result is that fast desensitization is produced by a change that takes place at only one of the agonist binding sites on the nicotinic receptor; the other site continuing to be able to bind nerve-released ACh. This concurs with the observation by Sine & Taylor (1980) that the two agonist binding sites, found on nicotinic receptors of cultured muscle cells, have different affinities for both agonists and antagonists; the change in affinity of these binding sites produced by exposure to cholinoceptor agonists, and presumed to correspond to desensitization (Cohen, 1978), can occur independently at the two sites and independently of receptor activation. The concept that fast and slow desensitization reflect distinct processes occurring at two different binding sites implies the possibility of transition from a fast-desensitized to a doubly desensitized state (Sine & Taylor, 1980) and a time course of recovery from desensitization dependent upon the duration of application of agonist (Feltz & Trautmann, 1982). Slow desensitization, of course, results in an equilibrium depression of postsynaptic sensitivity much more profound than that which occurs with fast desensitization alone (Feltz & Trautmann, 1982).

The fact that the Hill coefficient for the concentration-response relationship of fast desensitization is 1.4 and not 2.0 excludes the possibility that channel opening is a prerequisite for desensitization. The fact that it is greater than unity does not necessarily indicate that the fast desensitized state is sometimes derived from an A_2R state. If either development or recovery steps in the cyclic scheme represents an essentially irreversible process (as in the first scheme suggested by Katz & Thesleff, 1957), then [R'] becomes an increasing function of [A] and total desensitized receptor ([R'] + [AR']) increases more than linearly with [A]. Within a more complete scheme which incorporates slow desensitization (Feltz & Trautmann, 1982) such an irreversible step would explain why the final plateau response, observed at a time when slow desenitization is fully developed, may become smaller the higher the agonist concentration (see Adams, 1975; Lester, Changeux & Sheridan, 1975). In addition it would explain the transient increase in the response sometimes seen when superperfusing solutions containing desensitizing concentrations of agonist are replaced by control solutions (i.e., the 'humps' observed by Adams, 1975).

This work was supported by grants from the Muscular Dystrophy Association of Canada and the Medical Research Council of Canada.

Appendix

(a) *Correction of response to agonist for desensitization*

In any particular model of receptor activation and desensitization there will be in the steady state an equilibrium between the various states of the receptor. The amount of receptor activated (R^*) by a given concentration of agonist (A) will be equal to the amount of activatable receptor (R) multiplied by a function of agonist concentration. The same will be true for the amount of receptor that is desensitized (R'), or in other states (R^o), e.g. forms intermediate in the formation of R* from R.

$$\begin{aligned} R^* &= f(A) \cdot R \\ R' &= g(A) \cdot R \\ R^o &= h(A) \cdot R \\ \text{total receptor, } R_t &= R + R^* + R' + R^o \end{aligned}$$

where $f(A)$, $g(A)$ and $h(A)$ depend upon the particular model.

$$\begin{aligned} \text{Thus: } R/R_t &= 1/[1 + f(A) + g(A) + h(A)] \\ R^*/R_t &= f(A)/[1 + f(A) + g(A) + h(A)] \\ R'/R_t &= g(A)/[1 + f(A) + g(A) + h(A)] \\ R^o/R_t &= h(A)/[1 + f(A) + g(A) + h(A)] \end{aligned}$$

Provided the height of the m.e.p.c. is proportional to the density of activatable receptors (R) at the moment when a quantum of ACh is discharged into the synaptic cleft, then

$$\text{m.e.p.c.}_{carb}/\text{m.e.p.c.}_{con} = R/R_t = [1 + f(A) + g(A) + h(A)]^{-1}$$

Hence, the response to bath applied agonist (proportional to R^*) can be corrected for desensitization, since,

$$R^* \times \text{m.e.p.c.}_{con}/\text{m.e.p.c.}_{carb} = R_t \times f(A).$$

Note (i) the corrected response is itself proportion-

al to f(A) – there is no need for further correction for receptor saturation, and (ii) the possibility that R^o may be activatable has been omitted.

(b) *Calculation of Hill coefficient for desensitization*

It is desired to obtain points relating g(A) and A. If one defines D as the fractional reduction of m.e.p.c. height by carbachol, i.e.:

$$D = (1 - \text{m.e.p.c.}_{carb}/\text{m.e.p.c.}_{con})$$

since $$\text{m.e.p.c.}_{carb}/\text{m.e.p.c.}_{con} = [1 + f(A) + g(A) + h(A)]^{-1}$$

then $$D = [f(A) + g(A) + h(A)]/[1 + f(A) + g(A) + h(A)]$$

With 20 μM carbachol the change in membrane current was 20 nA at -60 mV; the conductance change was about 3×10^{-7} S. This is about 0.1% of 2.5×10^{-4} S (10^7 channels $\times$ 25 pS/ channel), the conductance expected for opening of all channels at the endplate. Thus, at all concentrations used f(A) was small enough to be negligible and also neglecting h(A),

$$D = g(A)/[1 + g(A)]$$
$$g(A) = D/(1 - D)$$

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(*Received February 23, 1982.*
Revised May 19, 1982.)