Desensitization and Recovery at the Frog Neuromuscular Junction

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ABSTRACT The time course of carbachol-induced desensitization onset and recovery of sensitivity after desensitization have been compared at the frog neuromuscular junction. The activation-desensitization sequence was determined from input conductance measurements using potassium-depolarized muscle preparations. Both desensitization onset and recovery from desensitization could be adequately described by single time constant expressions, with τ_{onset} being considerably shorter than $\tau_{recovery}$. In nine experiments, τ_{onset} was 13 ± 1.3 s and $\tau_{recovery}$ was 424 ± 51 s with 1 mM carbachol. Elevating the external calcium or carbachol concentration accelerated desensitization onset without changing the recovery of sensitivity after equilibrium desensitization. Desensitization onset was accelerated by a prior activation-desensitization sequence to an extent determined by the recovery interval that followed the initial carbachol application. The time course of return of τ_{onset} was closely parallel to, but slower than the time course of recovery of sensitivity. These results are consistent with a cyclic model in which intracellular calcium is a factor controlling the rate of development of desensitization.

INTRODUCTION

When acetylcholine or other depolarizing compounds (agonists) are applied to the end-plate region of skeletal muscle fibers, the postjunctional membrane undergoes a rapid increase in ionic conductance which is slowly reversed if the agonist is allowed to remain at the end plate. During this time the postjunctional membrane is said to become "desensitized" (Thesleff, 1955).

In recent years the process of end-plate desensitization has been studied extensively and the influence of many factors such as type of agonist, agonist concentration, composition of the ionic environment, and the presence of pharmacologically active agents has been examined. Nevertheless, the molecular mechanisms responsible for desensitization remain a matter of speculation and both "receptor" and "extrareceptor" mechanisms have been proposed to explain the desensitization process (Katz and Thesleff, 1957; Magazanik and Vyskocil, 1970, 1975; Rang and Ritter, 1970; Nastuk and Parsons, 1970; Cochrane and Parsons, 1972; Adams, 1975; DeBassio et al., 1976).

In contrast to the large number of studies concerning the onset of desensitization, only a limited amount of information is available concerning the time course of recovery from desensitization (Katz and Thesleff, 1957; Rang and Ritter, 1970; Magazanik and Vyskocil, 1975; Adams, 1975). A more complete

characterization of the recovery process may provide insight into the mechanisms that are responsible for receptor desensitization. Consequently, we have examined the time course of desensitization and the time course of recovery of sensitivity after desensitization as well as the influence of agonist concentration and external calcium concentration on these processes.

Our results demonstrate that desensitization is accelerated by increasing agonist or calcium concentration while the recovery of sensitivity is independent of these manipulations. It was also found that the time course of desensitization onset is dependent not solely on the extent of the agonist-induced conductance change but also on history, as prior exposure to carbachol appears to influence desensitization onset time constant.

A preliminary description of some of our observations has been presented elsewhere (Scubon-Mulieri and Parsons, 1975).

MATERIALS AND METHODS

General Methods

All experiments described in this report were performed in vitro on the sartorius muscle of the frog *Rana pipiens* at temperatures ranging between 14° and 18°C during the period March to July. The muscles were dissected and maintained in a Tris-buffered Na^+-Ringer solution (mM: NaCl, 120; KCl, 2.5; $CaCl_2$, 1.8; tris(hydroxymethyl)aminomethane, 1.0; pH = 7.0) for at least 2 h. After this equilibration period the resting membrane potentials were measured. Preparations in which two out of three fibers had membrane potentials less than −85 mV were discarded. After this, the muscle preparations were transferred to a depolarizing, isotonic potassium solution (mM: K propionate, 122.5; $CaCl_2$, 1.29; Ca propionate, 0.51; Tris, 1.0; pH = 7.0) and allowed to equilibrate for at least 30 min before the experiments were begun. All experiments were done during a subsequent 60-min exposure to the high potassium solution. Muscles were kept in a 12-ml bath with a 50 ml/min flow of fresh bathing solution. During the experimental period there was no progressive change in the control input conductance of the fibers. In this elevated potassium solution the resting membrane potential of individual fibers ranged from +5 to −5 mV. The potassium-depolarized preparation was considered appropriate for the present experiments because it eliminates the change in membrane potential which is associated with receptor activation in polarized fibers. Since desensitization onset is influenced by membrane potential (Magazanik and Vyskocil, 1970; Anderson and Stevens, 1973) we felt that potential might also be a factor influencing the time course of the recovery of sensitivity after desensitization.

Carbachol (Sigma Chemical Co., St. Louis, Mo.), dissolved in the isotonic potassium solution, was microperfused onto the end-plate region by hydrostatic pressure from a 100-μm diam pipette placed within 50 μm of the intracellular micropipettes to produce activation of the end-plate receptors (Manthey, 1966; Johnson and Parsons, 1972). Junctional regions of individual muscle fibers were located visually by use of transmitted light under a magnification of 300 by following the nerve fibers to the last node of Ranvier. When this technique was used on polarized muscle fibers the rise times of miniature end-plate potentials were generally less than 1 ms, indicating close proximity to the junction (Nastuk and Parsons, 1970).

Standard electrophysiological techniques were employed to measure membrane potential and membrane input conductance (Nastuk and Parsons, 1970; Manthey, 1972; DeBassio et al., 1976). Current pulses were injected at a frequency of 1 Hz and were

sufficiently long (20–50 ms) that the membrane capacity was fully charged and the voltage had plateaued to a steady level. The amplitude of the current pulses was adjusted to produce an ~20 mV change in membrane potential. The microelectrodes used in this study were filled with 3 M KCl and had resistances of 10–15 MΩ.

Measurement of Postjunctional Membrane Sensitivity, Rate of Desensitization Onset, and Recovery of Sensitivity

The data were analyzed and interpreted by the simple parallel conductance model shown in Fig. 1.

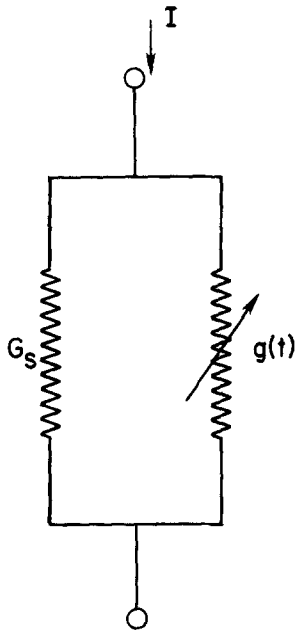


FIGURE 1. The schematic model used to analyze the experimental data where g(t) represents the time varying end-plate conductance and G_s represents a constant parallel shunt conductance. In the simplified model the total input conductance, $G_T(t)$, is inversely proportional to the change in transmembrane voltage $\Delta V_m(t)$ produced by the constant current pulses, I.

For this model the total input conductance, $G_T(t)$, is the sum of the parallel shunt conductance, G_s, assumed constant, and the end-plate conductance, g(t):

$$G_T(t) = G_s + g(t). \tag{1}$$

End-plate sensitivity was calculated as the maximum increase in input conductance (mho × 10^{-6}) produced by the application of carbachol in such a manner that:

$$\text{sensitivity} = \Delta G_T = G_{T(\text{maximum})} - G_{T(\text{initial})} = g(t)_{(\text{maximum})} - g(t)_{(\text{initial})},$$

with $g(t)_{(\text{initial})}$ assumed zero in the absence of carbachol. The time course of end-plate desensitization during sustained perfusion with carbachol was determined from the rate of decrease of the input conductance change after the initial peak.

The recovery of sensitivity after desensitization was determined by the following procedure. The end-plate activation-desensitization sequence was induced by the local application of carbachol. After desensitization was fully developed, i.e. when the input conductance had returned to the initial (precarbachol) level, the perfusion was terminated by raising the perfusion pipette and the end plate was allowed to recover for a variable interval, at which time carbachol (at the same concentration) was reapplied and the increase in input conductance was measured. The extent of recovery of sensitivity after desensitization was determined by expressing the test carbachol-induced conductance change as a function of the maximum input conductance change produced by the first carbachol perfusion. The initial application was maintained for 5 min. Subsequent perfusions were maintained only until the conductance had plateaued at the preperfusion level (i.e. 100% return).

In estimating the time course of recovery of sensitivity for an individual fiber, the end plate was repeatedly exposed to carbachol. Consequently, an obvious concern was that the maximum sensitivity to the agonist might change during the experiment. An internal check was used in these experiments to test for such a change. In a series of multiple applications with varying recovery intervals, the sensitivity after a 3-min recovery interval was periodically checked. The maximum peak-to-peak variation in sensitivity was less than 20% and showed no progressive trend during an experiment.

Altered Calcium Concentration

In some experiments the calcium concentration was changed to test its influence on the time course of desensitization and recovery of sensitivity. Calcium was elevated (from 1.8 to 10 mM) by increasing its concentration in the perfusion medium so that altered calcium was present only during carbachol application and only in the region of the end plate.

Statistical Analysis

The data were fitted to mathematical expressions by use of a nonlinear optimatization method developed and computerized by R. K. Wright (Department of Mathematics, University of Vermont).[1] In the presence of carbachol, the end-plate conductance increases, remains at a peak value for a brief period, and then declines as desensitization occurs (Fig. 2). During the early phase of the response, we assume that agonist concentration is equilibrating so that changes in both activation and desensitization occur; after this the response is seen to decline as a single exponential function of time. To determine the decay time constant, τ_{onset}, data points closest to the peak were successively eliminated until the early nonrandom deviation from the exponential fitted to the later portion of the curve disappeared.

Statistical significance was determined with a Student's t-test, values of $P \leq 0.05$ being regarded as significant. All values are expressed as mean ± standard error of the mean (SEM).

RESULTS

Comparison of the Time Course of Desensitization Onset with the Time Course of Recovery of Sensitivity

Application of carbachol onto the end-plate region produced an increase in input conductance which reached a peak value and then declined toward the precarbachol levels in the continued presence of agonist. The rate of this conductance decline is an index of the time course of desensitization (Manthey,

[1] Submitted for publication.

1972; DeBassio et al., 1976). The time course of desensitization onset is well described by a single exponential decay under all the conditions used in the present study; i.e. under different carbachol and calcium concentrations. Two examples appear in Fig. 3 which illustrate this exponential onset of desensitiza-

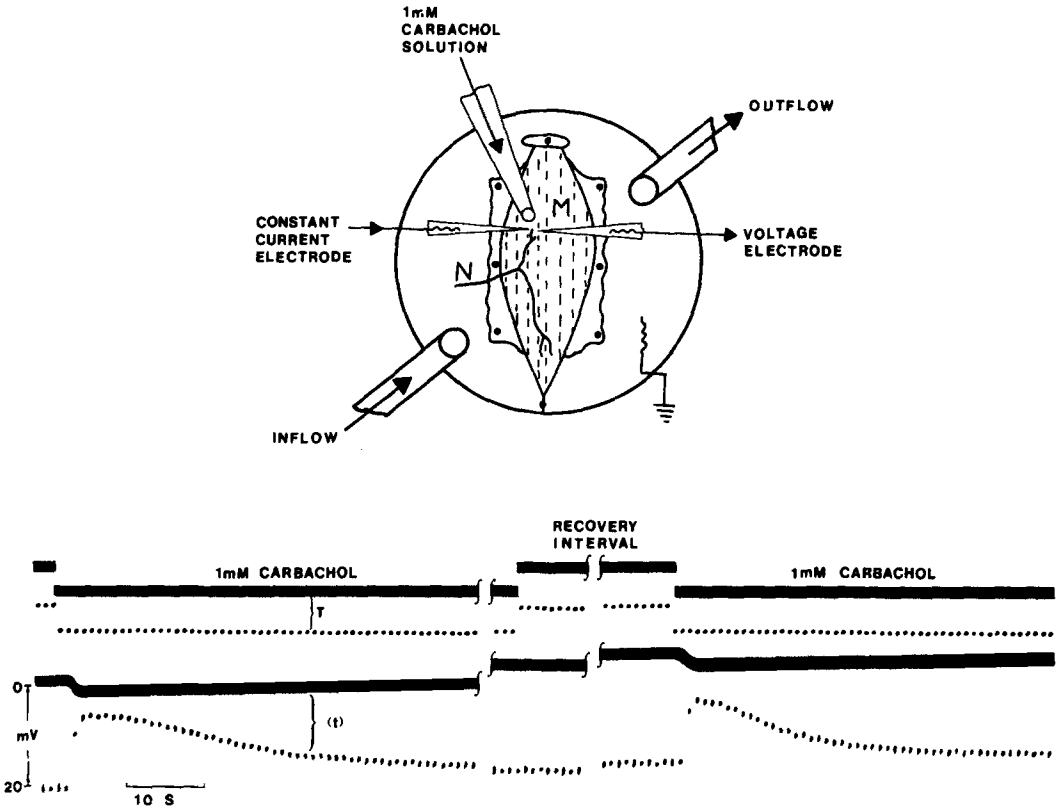


FIGURE 2. Schematic illustration of apparatus used to study end-plate desensitization and recovery of sensitivity after desensitization. A constant flow of bath solution was maintained by gravity feed of precooled solution through inflow and suction removal through outflow. Input conductance was monitored by passing constant current pulses through one microelectrode while monitoring potential change, ΔV, with a second microelectrode. Carbachol 1 mM was applied by rapidly lowering a 100-μm diam perfusion pipette through the bath-solution interface to within a few micrometers of the end-plate region. B, Sample record. Top dashed trace is a tracing of the current record with constant amplitude, 50-ms duration pulses delivered at a frequency of 1 Hz. Downward offset in the current base line indicates presence of carbachol perfusion. The bottom dashed record is a tracing of the voltage record showing the transient hyperpolarizations produced by the injected current pulses.

tion. These responses represent (Fig. 3 A) one of the slowest onsets observed at 1 mM carbachol (τ_{onset} = 15.6 s) and one of (Fig. 3 B) the fastest onsets at 10 mM carbachol (τ_{onset} = 2.0 s).

Like desensitization onset, the recovery of sensitivity occurred expontentially,

but with a time constant, $\tau_{recovery}$, considerably greater than τ_{onset}. The time course of the recovery of sensitivity was determined by the experimental protocol illustrated in Fig. 2. In this example, the end-plate region was locally perfused with 1 mM carbachol for 5 min to allow the activation-desensitization

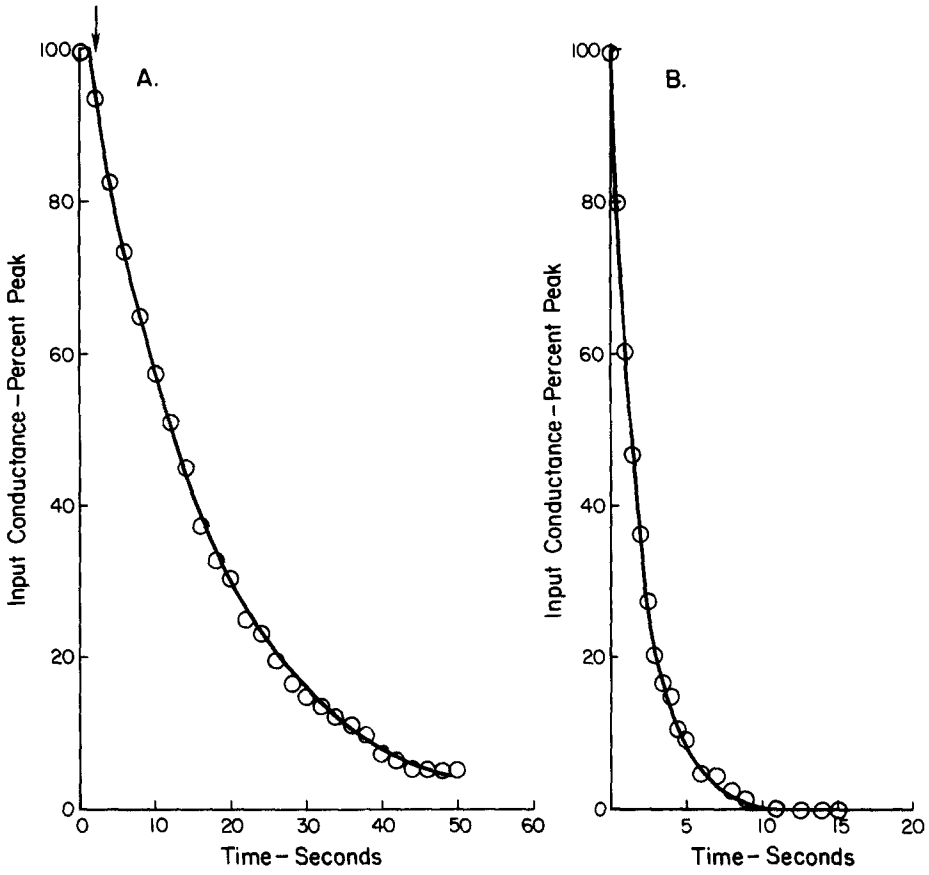


FIGURE 3. Examples from two different fibers illustrating the exponential onset of desensitization. In these examples the decline of the input conductance, expressed as a percent of the maximum value during carbachol perfusion, is plotted as a function of time. A, The slowest onset of desensitization was observed with 1 mM carbachol, τ_{onset} = 15.6 s. B, One of the fastest onsets of desensitization was obtained with 10 mM carbachol, τ_{onset} = 2 s. In example A, the exponential function was fitted only to the data points following the arrow.

sequence to develop fully; the agonist was then removed while bath flow continued for various intervals, after which carbachol was reapplied. With this approach the time course of recovery of end-plate sensitivity after desensitization was measured in the same end plate as was used to determine desensitization. The top pair of traces shows the amplitude of the current pulses which were injected: the DC shifts of the I-trace show the onset and termination of agonist perfusion. The bottom pair of traces shows the resting potential (approximately 0 mV in this preparation) and the pulsatile changes in potential caused by the

current pulses. When the carbachol was perfused onto the end-plate two changes occurred: (*a*) a small hyperpolarization (less than 2 mV in this fiber); and (*b*) a diminution of the amplitude of the voltage pulses reflecting the increase in input conductance. In the continued presence of agonist the membrane potential slowly returned toward the preperfusion level and the amplitude of the voltage pulses slowly increased to the preperfusion level. During the recovery period (6 min in this example) there was no further increase in amplitude of the voltage pulses although there was a small change (about 1 mV) in the membrane potential which may be due to electronic drift. The reapplication of 1 mM carbachol after the 6-min wash period again caused membrane conductance to increase to a maximum value and to decline toward the precarbachol value. The conductance increase induced by the second carbachol perfusion was 58% of that obtained in the initial application, indicating that end-plate sensitivity in this fiber had not recovered completely after a 6-min wash period.

The results from such an experiment are presented in Fig. 4 so that the time course of desensitization onset and time course of the recovery of sensitivity after desensitization can be compared. In this experiment the end plate was activated by 1 mM carbachol application a total of 14 times. During the initial carbachol exposure, desensitization developed rapidly with the time course shown on the lefthand side of Fig. 4 B (τ_{onset} = 11 s). In contrast, the recovery of sensitivity, shown on the righthand portion of Fig. 4 B, proceeded more slowly ($\tau_{recovery}$ = 317 s). The time course of recovery of sensitivity was determined by plotting activation (denoted as a percent of the conductance increase obtained during the initial carbachol exposure) as a function of the recovery interval. The data points have been fitted on the assumption of a single exponential approach to 100% recovery. To insure that the carbachol response was not changing with time, the extent of the agonist-induced conductance change after a 3-min wash interval was determined at six different times (the 2nd, 4th, 6th, 9th, 11th, and 14th perfusions) during this experiment. These results are presented in Fig. 4 A and demonstrate that no progressive decline occurred with time or repeated activation.

Similar results were obtained in nine experiments. In these fibers, the mean time constant of desensitization onset with the first application of 1 mM carbachol was 13.3 ± 1.3 (range 9.3–20.6) s whereas the mean time constant of recovery of sensitivity after desensitization was 424 ± 51 (range 276–673) s. The time course of recovery of sensitivity with data pooled from these same nine fibers is shown in Fig. 5 A. For each fiber, subsequent responses at the different recovery intervals were normalized as a percentage of the conductance change produced by the initial carbachol exposure in that particular fiber. The solid curve in Fig. 5 A represents an exponential approach to complete recovery with the time constant recovery sensitivity, $\tau_{recovery}$, being 370 s.

Carbachol Concentration and External Calcium Concentration Affect the Time Course of Desensitization Onset but Not of Recovery of Sensitivity after Desensitization

Katz and Thesleff (1957) and Magazanik and Vyskocil (1975) have shown that the time course of recovery of sensitivity after desensitization was independent of

the level or onset time course of desensitization. Similar findings were obtained in the present investigation. We have altered the time course of desensitization onset by two manipulations: (*a*) changing the carbachol concentration; and (*b*) increasing the external Ca^{2+} concentration.

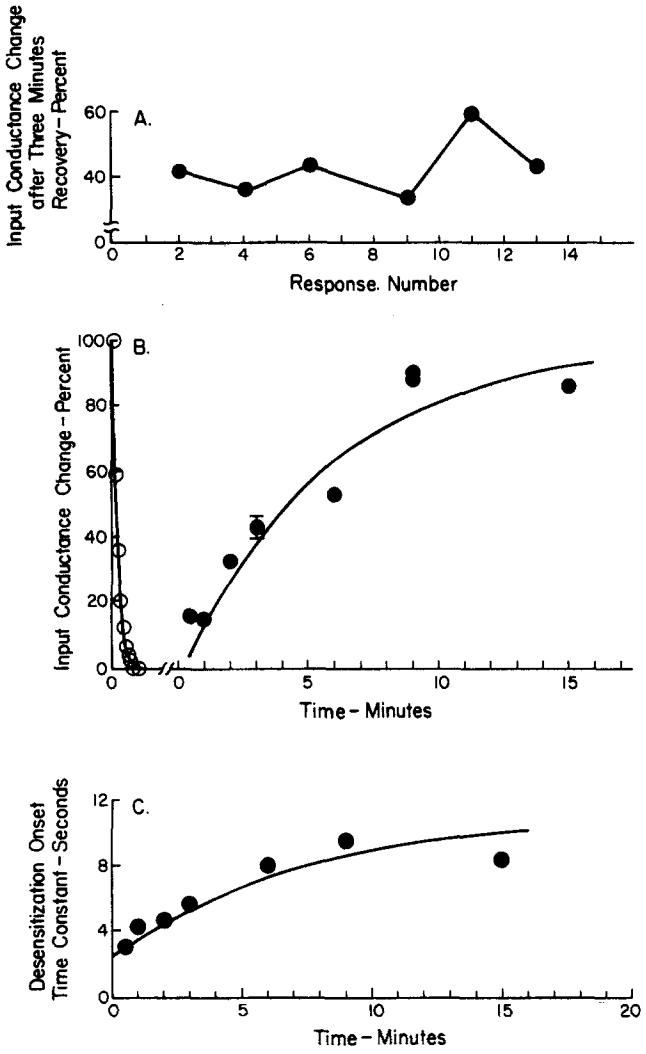


FIGURE 4. Results from one fiber comparing the time course of desensitization onset, recovery of sensitivity, and return of τ_{onset}. A, Input conductance change produced by application of 1 mM carbachol after 3 min of recovery plotted as a function of the perfusion number. This fiber was exposed to carbachol 14 times during the experiment. B, Time course of desensitization onset (open circles) during the initial carbachol exposure and time course of recovery of sensitivity (solid circles). The time scale is identical for desensitization onset and recovery, both of which developed exponentially with τ_{onset} for the initial carbachol exposure = 11 s and $\tau_{recovery}$ = 317 s. C, The time course of return of τ_{onset}. In this example, τ_{onset} recovered towards its initial value exponentially with a time constant = 480 s.

When the carbachol concentration was raised from 1.0 to 10 mM, the extent of the agonist-induced conductance change and the rate of desensitization onset were both significantly increased (Table I A). In contrast, the extent of the recovery of sensitivity after a 3-min wash period was not significantly different with 1.0 or 10 mM carbachol-induced desensitization.

Desensitization onset was accelerated by elevating the Ca^{2+} concentration in a 1 mM carbachol-perfusion solution (Table I B) without significantly altering the initial carbachol-induced conductance change. Again, the extent of the recovery of sensitivity after a 3-min wash interval with either 1.8 or 10 mM Ca^{2+} was not changed.

From an inspection of the data presented in Table I, it is apparent that there are noticeable differences between the two sets of control values for initial sensitivity, desensitization onset time constant, and 3-min extent of recovery of sensitivity. This illustrates one of the difficulties noted in preliminary experiments, that there was considerable variability among the results obtained at different times. Consequently, each series of experiments reported in Table I was done in a prescribed time with separate controls. The influence of carbachol concentration was studied in the Spring (March–April) whereas the calcium experiments were done in Summer (July). The differences between the two control groups may reflect a seasonal factor although this has not been tested.

Desensitization Onset is Accelerated as Sensitivity Recovers

For subsequent agonist exposures, τ_{onset} was significantly shortened to an extent determined by the recovery interval after the initial activation-desensitization sequence. This progressive decrease of τ_{onset} reversed as end-plate sensitivity recovered. An example of this phenomenon is presented in Fig. 6. For this fiber, τ_{onset} for the initial and sixth activation (after a 3-min wash interval) was 13.5 s and 4.2 s, respectively. At this time τ_{onset} was 31% of the initial value and end-plate sensitivity had recovered 24%.

The results from another experiment are presented in Fig. 4 C to illustrate in more detail the time course of decline of τ_{onset}. In this particular fiber, τ_{onset} was 11 s during the initial carbachol perfusion and 3 s after a 1-min wash interval, and it slowly returned toward the initial value as the duration of the wash period increased. This change could be approximately described by an exponential expression, with the time constant in this example being 480 s.

The time-dependent change of τ_{onset} with data taken from the same nine fibers studied to determine the time course of sensitivity recovery is shown in Fig. 5 B. To pool the data from the different fibers, values of τ_{onset} were computed individually as a percentage of the τ_{onset} during the initial carbachol application for each fiber. The curve represents an exponential expression having a time constant of 486 s drawn if one assumes 100% recovery of τ_{onset}.

The time course of recovery of sensitivity and return of τ_{onset} could be compared quantitatively in seven experiments. As indicated in Table II, in four experiments desensitization onset recovered considerably slower than sensitivity, in two fibers this difference was less marked, and in one experiment sensitivity recovered more slowly than desensitization onset. The mean time constant for recovery of these two processes was significantly different, suggesting that sensitivity recovers before τ_{onset}.

DISCUSSION

Desensitization at the motor end plate of skeletal muscle fibers is a well-documented phenomenon whose molecular mechanisms are not yet established. To gain more information about desensitization we have quantitatively

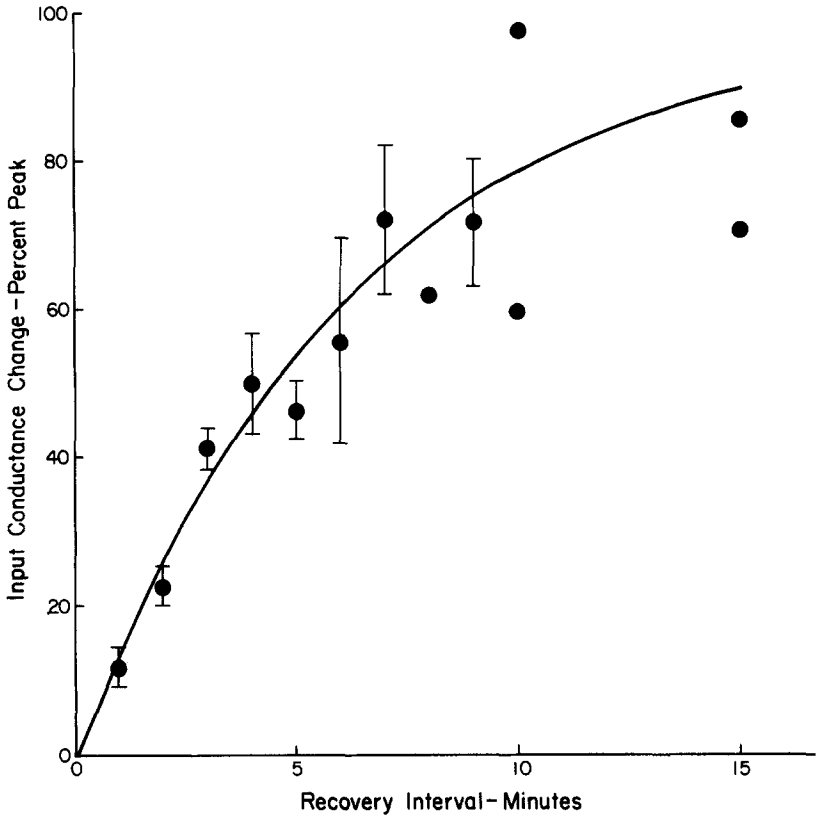


FIGURE 5A

FIGURE 5. Time course of recovery of sensitivity (A) and the time course of return of τ_{onset} (B) using data points pooled from nine experiments. A, The responses at various recovery intervals from each individual experiment are stated as a percentage of the peak conductance change during the initial application of 1 mM carbachol to that fiber. The curve is a single time constant expression with $\tau_{recovery}$ = 370 s. B (opposite), Values of τ_{onset} at different recovery intervals are expressed as a percentage of the initial τ_{onset}. The solid curve is a single time constant expression with a time constant = 486 s. Data points without standard error bars represent single observations.

compared the time course of desensitization onset and sensitivity recovery after full desensitization. Our results demonstrate that desensitization onset is considerably faster than recovery of sensitivity in the potassium-depolarized preparation. Both onset and recovery could be described by a single time constant exponential expression with τ_{onset} being considerably shorter than $\tau_{recovery}$.

The time constants for desensitization onset obtained in the present study are comparable to those recently reported by Adams (1975) with bath-applied agonist in voltage-clamped fibers, and previously reported values by Katz and Thesleff (1957) and Magazanik and Vyskocil (1970) using iontophoretic agonist application. In contrast, the time constants for recovery of sensitivity obtained in the present study, although similar to that reported by Rang and Ritter (1970),

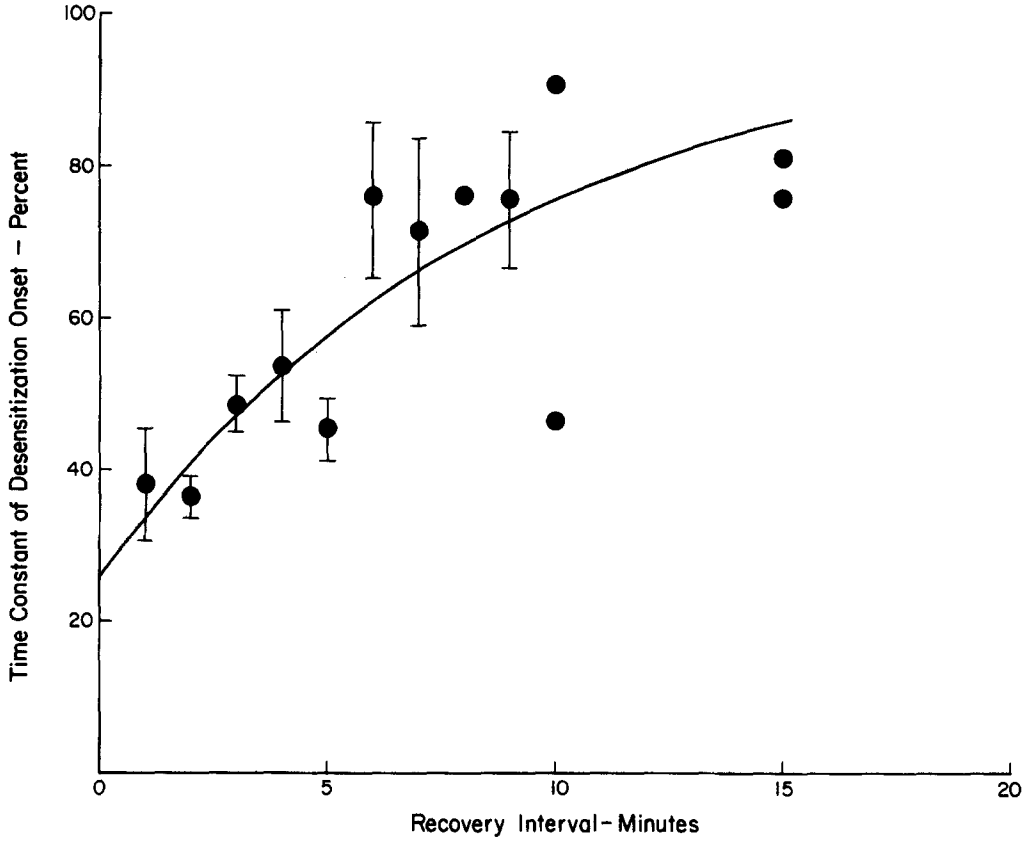


FIGURE 5B

are considerably greater than those reported previously by Katz and Thesleff (1957) and Magazanik and Vyskocil (1970, 1975). The marked difference between our results and those of the latter may be due to differences in experimental conditions or may reflect differing processes being investigated. However, Nastuk and Wolfson (1976) have recently reported that even with the iontophoretic technique under conditions where desensitization can be produced within a few seconds, complete recovery to normal sensitivity requires many minutes. Adams (1975) has suggested that recovery may proceed in two phases, a rapid phase which he suggested might correspond to that seen in the iontophoretic experiments of Katz and Thesleff (1957), and a slow component comparable to

TABLE I

INFLUENCE OF CARBACHOL AND CALCIUM CONCENTRATION ON END-PLATE SENSITIVITY, DESENSITIZATION ONSET TIME CONSTANT, AND RECOVERY OF SENSITIVITY AFTER A 3-MIN WASH PERIOD

Experimental series	Carbachol concn	Calcium concn	Initial sensitivity ΔG_T	Desensitization time constant (I)*	Desensitization time constant (II)*	3-min recovery % initial sensitivity	No. fibers
	mM	*mM*	*mho* × 10^{-6}	*s*	*s*		
A	1.0	1.8	14.9±1.3	12.2±1.1	6.4±0.7	45.2±4.3	5
	10.0	1.8	21.5±0.6	7.0±0.8	2.6±0.4	45.8±6.8	4
B	1.0	1.8	21.3±2.1	17.9±2.3	6.9±1.0	52.1±6.5	8
	1.0	10.0	21.4±4.0	9.4±1.8	5.1±0.6	58.6±10.9	6

* I, τ_{onset} during first perfusion; II, τ_{onset} during second perfusion done after the 3-min recovery interval.

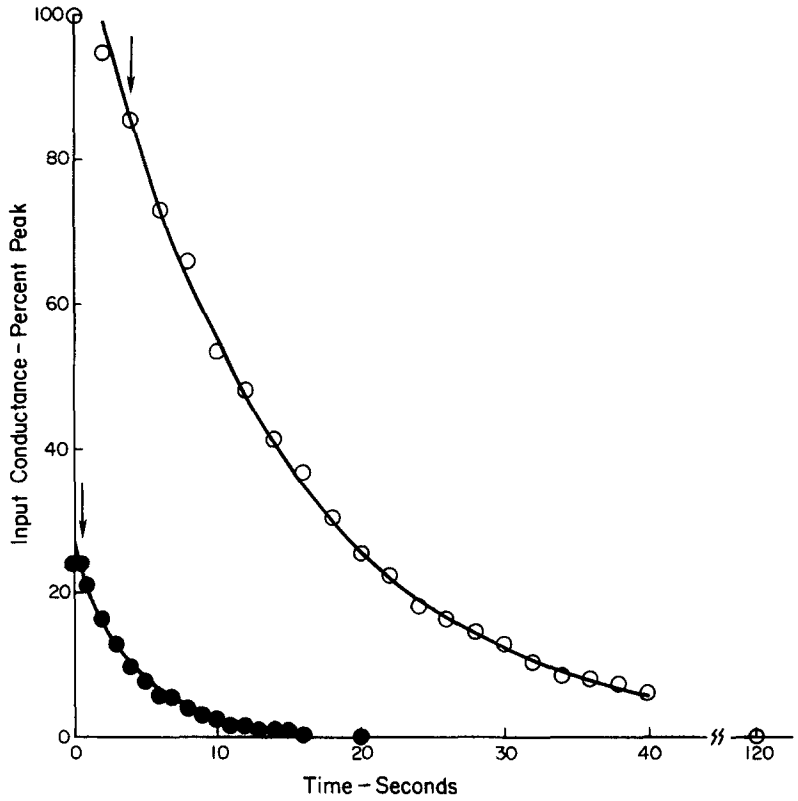


FIGURE 6. Demonstration of the acceleration of desensitization observed at one end plate with repeated exposure to 1 mM carbachol (1.8 mM calcium). Solid circles show data points and fitted curve (τ_{onset} = 13.6 s) for the first activation. Solid squares show data points and fitted curve (τ_{onset} = 4.2 s) for the sixth activation done after a 3-min recovery interval. Only the data points after the arrows were used to generate the respective exponential curves. Final values are shown by symbols.

that reported by Rang and Ritter (1970). However, we found no indication that recovery proceeded in two phases under our experimental conditions.

In the present study, large perfusion pipettes and a relatively high concentration of agonist were used in an effort to achieve the most uniform concentration of agonist possible over the end-plate region consistent with rapid application. However, as with all commonly used techniques of drug application, some degree of nonuniformity of agonist concentration over the end-plate region must have occurred, since the neuromuscular junction may extend many hundreds of micrometers along the fiber (Kuno et al., 1971; McMahon et al., 1972). This problem introduces errors of unknown magnitude in quantifying the time course of desensitization onset, for the onset rate is dependent on agonist concentration (Katz and Thesleff, 1957; Nastuk and Parsons, 1970; Adams,

TABLE II

COMPARISON BETWEEN THE TIME CONSTANT OF RECOVERY OF SENSITIVITY AND TIME CONSTANT OF RECOVERY OF DESENSITIZATION ONSET IN SEVEN INDIVIDUAL EXPERIMENTS*

Experiment	Time constant for recovery of sensitivity	Time constant for recovery of desensitization onset	No. of Carbachol applications
	s	*s*	
1	600	1,920	7
2	462	678	11
3	317	480	12
4	540	648	9
5	624	258	9
6	312	1,038	13
7	342	570	7
Mean±SEM	456.9±50.9‡	798.9±206.9‡	

* Carbachol concentration was 1 mM.

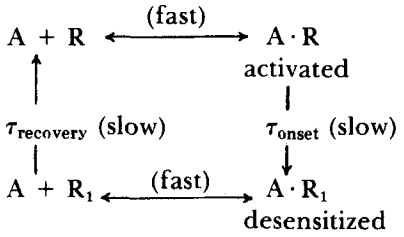
‡ Values are statistically significant ($P < 0.05$) by paired *t*-test.

1975). However, this problem should not affect estimation of the recovery time course which is independent of agonist concentration (Katz and Thesleff, 1957; also see Results).

Another possible source of artifact which can arise when agonist is locally applied is that different populations of receptors may be activated in the initial and subsequent perfusions. Two observations suggest that this did not occur to any large degree. First, the rise times of the responses were not different for the initial and subsequent activations, suggesting that subsequent responses were not obtained from receptors distant from those initially desensitized. Second, in those experiments with 10 mM carbachol, a concentration which produces a maximal conductance change under these conditions (Parsons, 1975), the results were similar to those with 1 mM carbachol.

It has been shown with the iontophoretic application techniques that the rates of desensitization onset and recovery are not similarly affected by environmental changes (Katz and Thesleff, 1957; Magazanik and Vyskocil, 1975). Similarly, we have observed that desensitization onset can be changed by Ca^{2+} and agonist

concentration without influencing the recovery from desensitization. These observations suggest that the onset and recovery of desensitization may involve independent processes. Consequently, of the various models proposed, a cyclic scheme such as that presented below, initially proposed by Katz and Thesleff (1957), is an appropriate model to describe the desensitization onset-recovery process.



Note that no attempt has been made to separate the receptor from subsequent steps in the permeability-activation system (see Lapa et al., 1974; Katz and Miledi, 1975) as the site undergoing change during desensitization. Both receptor (Katz and Thesleff, 1957; Rang and Ritter, 1970) and extrareceptor (Magazanik and Vyskocil, 1970, 1975; Nastuk and Parsons, 1970; Cochrane and Parsons, 1972) sites have been proposed. However, the results presented here do not allow us to distinguish between these alternatives. In this scheme, the activated A·R complex is slowly converted to an inactive state A·R_I with prolonged exposure to agonist. The recovery of sensitivity after desensitization would occur as A·R_I dissociated and R_I reverts to R with the time constant $\tau_{recovery}$ and is complete when all receptor has returned to the free R configuration. In addition we have assumed for simplicity, as did Katz and Thesleff (1957), that only the activated end-plate receptor-ionophore complex undergoes desensitization whereas inactive intermediate complexes do not, although an inhibition by certain antagonists having some properties similar to desensitization has been described (Hancock and Henderson, 1972).

An unexpected and very interesting observation in the present study is that desensitization onset was accelerated by a prior activation-desensitization sequence (Figs. 4 C, 5 B). The time course of recovery of τ_{onset} was somewhat slower than the time course of recovery of sensitivity. Two examples of mechanisms which may explain the change in τ_{onset} by prior activation have been considered. These illustrate an intrinsic vs. an environmental basis for the observed change in τ_{onset}. First, one may assume that there is a spectrum of desensitization onset and recovery rates within the population of receptor-ionophore complexes. If onset and recovery are closely related so that rapidly desensitizing receptors also recover quickly, then the first complexes to recover would be those with the fastest intrinsic desensitization onset rates. A following challenge would cause this selected population of receptors with faster onset rates to desensitize more quickly and so explain our observations. However, we have shown that when τ_{onset} is increased by elevating agonist or calcium concentration, there is no accompanying change in recovery. Consequently, as an alternative we suggest

that the intrinsic rate of desensitization (τ_{onset}) may be altered by changes in the internal ionic constitutents, e.g., Ca^{2+}, so that:

$$\tau_{onset} = f([Ca^{2+}]_i).$$

Such an alteration might involve either a specific receptor effect or some nonspecific change in the vicinity of the receptor-ionophore complex, but in any case desensitization onset could be affected in the manner we have observed. Support for this type of mechanism's being responsible for the observed change in τ_{onset} is derived from the following work.

The onset of desensitization is accelerated by calcium (Mathey, 1966). Further, evidence has been presented indicating that it is the interaction of intracellular ionized calcium at some site on the inner surface of the end-plate membrane which accelerates desensitization onset (Nastuk and Parsons, 1970; Cochrane and Parsons, 1972; DeBassio et al., 1976). In the above scheme, then, we suggest that an elevation of internal Ca^{2+} may accelerate the transition between the activated $A \cdot R$ complex to the inactive state, $A \cdot R_I$, that is, increased τ_{onset}. Normally, the concentration of intracellular ionized calcium is maintained at very low levels but rises in the vicinity of the end plate during the action of carbachol which causes not only an increase in sodium and potassium permeability but also in calcium permeability (Takeuchi, 1963; Parsons and Nastuk, 1969; Parsons et al., 1973; Manthey, 1974). If it is postulated that the rate of calcium sequestration is slower than recovery of sensitivity, then the time course of desensitization onset would be accelerated during recovery of sensitivity. A second application of carbachol during the period when the internal level of ionized calcium is still elevated would increase the rate of desensitization onset. Evidence that internal calcium concentration in muscle may decay as slowly as our model requires is given by the observation that twitch potentiation decays with half-times as long as 5 min after tetanic stimulation (Connolly et al., 1971).

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