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THE EFFECT OF TEMPERATURE ON DESENSITIZATION KINETICS AT THE POST-SYNAPTIC MEMBRANE OF THE FROG MUSCLE FIBRE

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SUMMARY

1. The time course of acetylcholine (ACh) potentials during development of desensitization was prolonged when a double-barrel ACh pipette was used for evoking desensitization. When a single-barrel ACh pipette was used, no change in the time course of ACh potential was to be seen during desensitization.

2. The recovery after desensitization did not depend on the rate of onset or on its level when a single ACh pipette was used. The half-time of recovery had a constant value of about 5·8 sec in the presence of chlorpromazine or SKF-525A in a muscle bath at 22° C.

3. Unlike the rate of onset, recovery from desensitization does not depend on the membrane potential.

4. The rate of onset of desensitization, i.e. time taken for reduction of ACh potentials to half-way between the initial amplitude and final steady value, decreased when temperature of the muscle bath was lowered.

5. Q_{10} of desensitization onset was found to be 1·5 for a change of temperature from 32 to 22° C, 1·9 from 22 to 12° C, 2·6 from 12 to 5° C and 3·3 from 12 to 2° C.

6. A similar temperature effect was observed in the case of desensitization recovery, the Q_{10} being 1·2 for temperature changes from 32 to 22° C, 1·3 from 22 to 12° C and 2·36 from 12 to 2° C.

7. Intracellular application of quaternary methiodide of SKF-525A or chloropromazine caused more rapid desensitization by ACh. The rate of desensitization onset depends on the ACh dose and on the frequency of application. The rate of recovery, however, has a constant value with a half-time of 5·5–5·7 sec at 22° C.

8. Both the rate of onset and the rate of recovery changed with temperature in the case of intracellular potentiation of desensitization, in a similar manner to that observed after extracellular application of these drugs.

9. The onset of desensitization can thus be influenced by different substances as well as by changes in temperature. Recovery apparently has a different mechanism from the onset, because its time course can be altered only by changes in temperature of the muscle.

INTRODUCTION

It is well known that many processes in biological membranes are affected by temperature. In particular, this has been shown for electrogenic events at the activated post-junctional membrane of the neuromuscular junction (Katz & Miledi, 1972; Kordaš, 1972; Magleby & Stevens, 1972*a*, *b*; Bregestovski, Chailachjan, Dunin-Barkovski, Potapova & Veprintsev, 1972).

Naturally, the question arose, whether the desensitization of the postsynaptic cholinergic membrane to acetylcholine (ACh), as a phenomenon opposite to activation (Thesleff, 1955; Katz & Thesleff, 1957; Nastuk, 1967; Magazanik & Vyskočil, 1970), is also temperature dependent. In the first experiments, unequivocal results were obtained indicating that the progressive decline of sensitivity during prolonged application of ACh (onset of desensitization) changes with temperature (Magazanik & Vyskočil, 1973). In this paper further analysis of the effect of temperature, not only on the onset of desensitization, but also on the recovery of sensitivity to the previous level, is described. The recovery was studied by single-barrel ACh pipettes which make it possible not only to desensitize the post-junctional membrane but also to obtain the whole restitution curve of ACh sensitivity. It was found that, with the exception of temperature, the recovery is not influenced by factors which promote the onset of desensitization. In the first part of the results the use of two methods of local desensitization induction is discussed, namely the double-barrel and single-barrel ACh pipette techniques.

METHODS

Experiments were performed on the isolated sartorius muscle of the frog *Rana temporaria*. Lightly stretched muscles were fixed on the bottom of a translucent perfusion chamber (5 ml.) and washed by Ringer solution of the following composition (mM): Na^+ 117·0; K^+ 2·5; Ca^{2+} 1·8; Cl^- 120·5; HCO_3^- 2·4; pH = 7·2–7·4. A Peltier semiconductor cooling device mounted in the bottom of the chamber was used for maintaining the temperature of the bath at a given level.

Standard glass micro-electrodes filled with 2·75 M-KCl (resistance 10–15 MΩ) were used both for intracellular recording of the membrane potential and for

passage of polarizing current through the muscle fibre membrane in the end-plate region.

Similar electrodes filled with 2 M-AChCl (Merck) were used for ionophoretic application of ACh as well as double-barrel micropipettes, prepared from *Simax*-glass (Kavalier, Czechoslovakia). Details of the ionophoretic technique have been published by Nastuk (1953), Katz & Thesleff (1957) and Magazanik & Vyskočil (1970).

The diethylaminoethyl ether of diphenylpropylacetic acid (SKF-525A), the quaternary methiodide of SKF-525A and chlorpromazine (Spofa) were used in the experiments presented here.

RESULTS

Comparison of two methods of desensitization induction: double-barrel and single-barrel ACh pipettes

The use of double-barrel pipettes provides a good method for measuring the rate of onset of desensitization and for following the effects of different factors on it (Katz & Thesleff, 1957; Magazanik, 1968; Magazanik & Vyskočil, 1970).

On the other hand, one meets some difficulties when a double-barrel pipette is used: for example we found, as did Katz & Thesleff (1957, see Table 1, p. 70 in their paper) that there exist unpredictable variations of the rate of recovery together with the fact that recovery rate seems to be unaffected by the factors that speed up the onset of desensitization.

It has previously been shown that desensitization takes place in the presence of many substances even when the single-barrel ACh pipette is used for evoking ACh potentials. Short (5–20 msec) rectangular ionophoretic pulses with frequencies of 0·2–1 Hz (Magazanik, 1970; Magazanik, 1971; Magazanik & Vyskočil, 1973) were used in these experiments. Desensitization is 'visible' as a progressive decline of the amplitude of ACh potentials (Fig. 1) which reaches a plateau after a sufficiently long train of impulses. When the pulse ionophoresis is switched off for some time there is a gradual restoration of amplitude to the original level. The longer this period, the more complete was the recovery and eventually the full response amplitude was obtained. This was practically identical to the first one in the train. The time course of this restoration apparently reflects the process of recovery after desensitization. In contrast to the experiments with double-barrel pipettes the original sensitivity is always regained: this provides an opportunity to obtain the whole recovery curve (Fig. 1). The effect of intermittent, rather than continuous, ACh application on the onset rate is discussed later (p. 16).

One of the reasons why the time course of desensitivity and recovery is often not reproducible when a double-barrel pipette is used may be due to the fact that zones of ACh action on the muscle membrane are not identical under the two barrels from which conditioning and test doses

of ACh are applied. This can also be supported by the fact that during desensitization produced by double-barrel pipettes the subsequent test potentials are prolonged (Fig. 2 *B*), as was originally observed by Katz & Thesleff (1957). A similar slowing down of the responses was also observed in the case of the single ACh pipette, when its tip was not in close contact

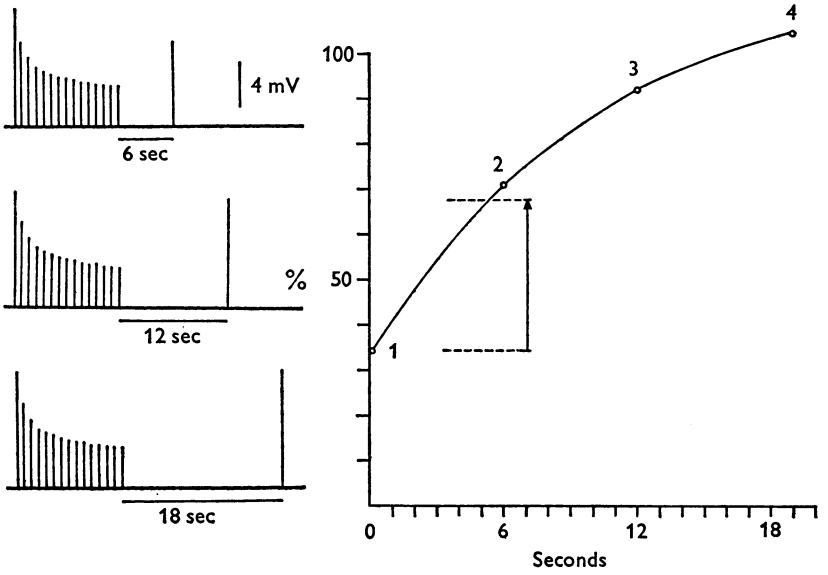

Fig. 1. Desensitization and recovery of sensitivity on the post-synaptic membrane after application of a train of constant ionophoretic impulses from a single acetylcholine micropipette. On the left, oscillographic records of three trains of ACh potentials evoked at the same site at 60 sec intervals. After each train the period of rest (6, 12 and 18 sec) was followed by a single test pulse, indicating the recovery of sensitivity. On the right, the curve of recovery. Abscissa, the time in sec between last pulse in the train and test pulse; ordinate, amplitude of the last pulse in the train (1), and of test pulses after 6 sec (2), 12 sec (3) and 18 sec (4) rest period (100 % = amplitude of the first ACh potential in the series). Arrow indicates the time at which the recovery reaches 50 % of the difference between desensitization level (1) and maximal sensitivity (100 %, point 4) (half-time of recovery, *t*). Temperature 22° C.

with the membrane and a greater amount of ACh was applied. The zone of ACh action may change (increase) if ACh accumulates during a series of doses. Changes in the time course of ACh responses during desensitization cannot therefore be considered as a typical feature of this process. When a single pipette was placed focally, and good contact with the sensitive part of the membrane was established in the presence of substances which enhance desensitization (SKF-525A or chlorpromazine)

(Fig. 2*A*), no prolongation of responses in the train was observed during desensitization.

Only one method exists whereby the onset of desensitization can be controlled in experiments with double-barrel pipettes: the regulation of the conditioning dose of ACh. The use of single-barrel pipettes gives us two methods of regulating desensitization: either by changing the amount

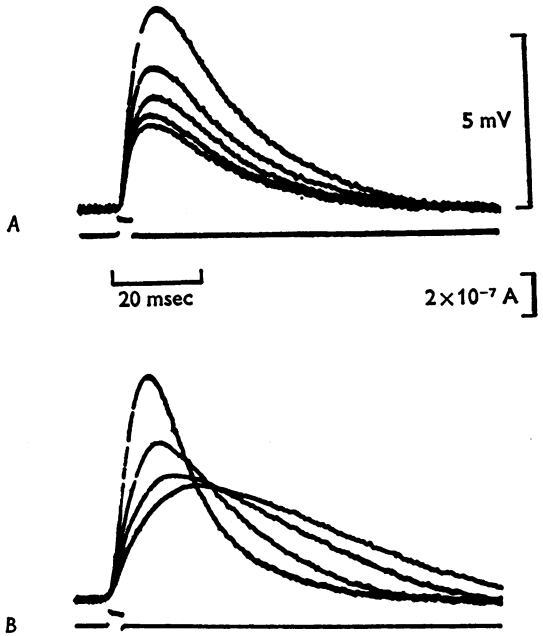

Fig. 2. Different time course of ACh potentials during desensitization with ACh applied by single ionophoretic micropipette (*A*) or double-barrel micropipette (*B*). The potentials were evoked every 2 sec and superimposed responses were photographed. The pulse on the lower line of both records monitors the ionophoretic current. *A* and *B*: two different muscle fibres with comparable level of ACh sensitivity (about 10 mV/nC, Miledi, 1960). Chlorpromazine 10^{-7} M was present in the bath. Temperature 22° C.

of ACh applied by each pulse in the train, or by changing the frequency of the responses and keeping constant the amount of ACh released by each pulse (Fig. 3). The results indicate that the rate of onset of desensitization is dependent, under constant conditions, only on the total amount of ACh which is applied on the post-junctional membrane (amount per pulse × frequency). According to some preliminary calculation (G. Brožek & F. Vyskočil, unpublished) both the onset and recovery, measured by using a single ACh pipette follows in most cases a simple exponential

curve; half-time values between initial amplitude and steady desensitization level in the train were therefore used for estimating the changes in the rate of onset (t_0). An analogous procedure was used for evaluating the rate of recovery (t_r) (see Fig. 1).

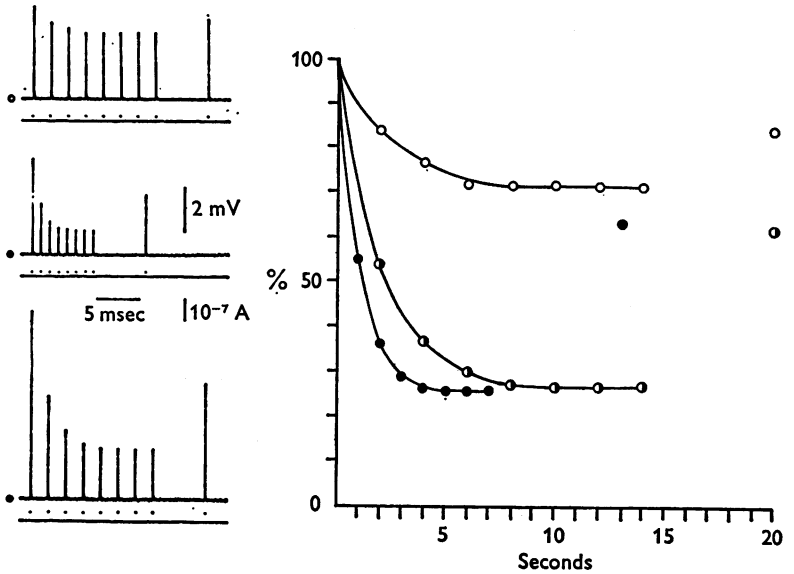

Fig. 3. Dependence of the desensitization on the amplitude of ACh responses and frequency of pulse application. On the left, oscilloscope records of three ACh-potential trains evoked at the same site on the post-junctional membrane. A single ACh-pipette was used for ionophoretic microapplication of ACh at a frequency of 0·5 Hz (○ and ◑) and 1 Hz (●). On the bottom left-hand record the amount of ACh ejected in the ionophoretic pulses was increased to obtain twice the amplitude of the first potential in the upper train. As is shown in the right diagram, an increase of frequency as well as amplitude of the responses lead to a faster onset and a more pronounced desensitization. Where the rate of onset (t_0) was calculated as the time for the amplitude to reach a point half-way in-between the initial value and final steady value, t_0 equals 1·8 sec (○), 1·2 sec (◑) and 0·8 sec (●). Abscissa, time in sec after the beginning of the train; ordinate, the amplitudes of the following ACh responses as a percentage of the first one in the train. Each train was followed by a 6 sec period of rest and one test response, in all three cases, was approximately half the difference between the amplitude of the first and last response in the train. This indicates that the rate of recovery is independent of the rate and level of desensitization. 5×10^{-7} M chlorpromazine, 22° C.

The influence of some factors affecting onset of desensitizaion on the recovery process

Recovery after desensitization does not depend on the rate of onset or the level of desensitization (Table 1, Fig. 3). The dose, or the type, of desensitization potentiating agents are also ineffective in promoting recovery. It has been found that the half-time of recovery (t_r) has a constant value of about 6 sec in experiments in which either (*a*) 5×10^{-7} or $1{\cdot}5 \times 10^{-6}$ M chlorpromazine ($t_r = 5{\cdot}88 \pm 0{\cdot}4$ sec, seven experiments, $t_r = 5{\cdot}82 \pm 0{\cdot}1$ sec, five experiments respectively), or (*b*) 1×10^{-7} or 5×10^{-7} M SKF-525A ($t_r = 5{\cdot}79 \pm 0{\cdot}1$ sec, five experiments) was present

TABLE 1. The non-dependence of half-time recovery (t_r) on the level of desensitization

1·7 Hz 25–35 % t_r (sec)	0·5 Hz 50–60 % t_r (sec)	0·3 Hz 70–80 % t_r (sec)
6·0	5·9	6·1
6·0	6·0	6·0
5·8	5·5	5·8
5·4	5·8	5·8
5·8	6·0	5·9
mean ± S.E. 5·80 ± 0·11	5·85 ± 0·09	5·92 ± 0·06

Five fibres were examined with single-barrel ACh-pipettes. On each fibre three frequencies (Hz) of ACh application were used, giving three different levels of desensitization, expressed in percentage of the first response in the train (cf. Figs. 1 and 3). The recovery curve was obtained similarly as is shown in Fig. 1 and t_r was then read out for every experiment. 5.10^{-7} M chlorpromazine, temperature 22° C. Each experiment was performed on a different fibre.

TABLE 2. Effect of polarization of the post-junctional membrane on the half-time of recovery from desensitization

No.	t_r (sec)	Membrane potential (mV)	t_r (sec)	Membrane potential (mV)
1	5·5	90	5·5	60
2	5·8	90	5·5	60
3	6·0	90	6·1	60
4	6·2	90	6·0	60
5	5·9	85	6·0	60
6	5·8	85	5·9	60
Mean ± S.E.	5·87 ± 0·10		5·83 ± 0·11	

The recovery from desensitization was measured in six muscle fibres at two levels of membrane potential by the single-barrel pipette method. The second intracellular micro-electrode (2 M-K citrate) was used for polarization of the muscle fibres. t_r = half-time of recovery in sec. Temperature 22° C.

in the muscle bath. The values of half-time of recovery are very close to those obtained by Katz & Thesleff (1957) with double-barrel pipettes, but without any potentiating drugs.

Using the single-barrel pipette we confirmed the earlier observation (Magazanik & Vyskočil, 1970) that the rate of desensitization onset depends on the polarization of the fibre (Fig. 4). The lower the transmembrane potential is, the slower the rate of desensitization and vice versa. However, when the recovery from desensitization was estimated (Table 2), no difference was observed in the half-time of recovery between two different levels to the onset of desensitization, recovery is not influenced by polarization of the post-junctional membrane, t_r being about 6 sec at 22° C.

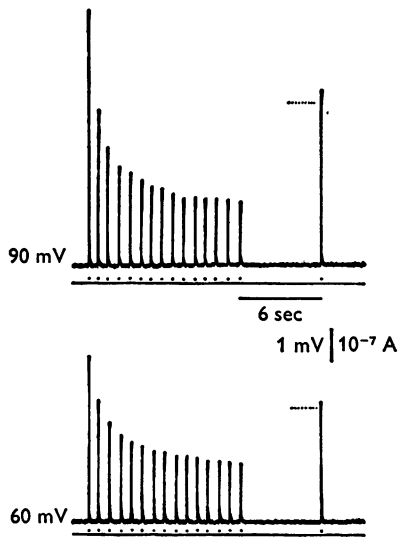

Fig. 4. Effect of the membrane potential level on desensitization. The trains of ACh potentials were elicited ionophoretically using a single ACh-pipette with a frequency of 1·3 Hz and the test potential was applied after a 6 sec period of rest. The muscle fibre was polarized from its original level of −86 to −90 mV and −60 mV by current from another microelectrode (2 M-K citrate) inserted approximately 50 μm from the recording electrode. During depolarization, the rate of onset (t_o) changed from 1·2 sec (−90 mV) to 1·9 sec (−60 mV), but no change in the rate of recovery (t_o) was observed, being 5·5 sec in both cases. Interrupted line = 50 % of recovery. 5×10^{-7} M chlorpromazine was present in the bath and the temperature was of 22° C.

Desensitization and temperature

The kinetics of desensitization and recovery were studied at five temperature levels, namely 32, 22, 12, 5 and 2° C. From our experience, the cooling and/or heating of the preparation over such a wide range of

temperatures very often leads to the dislocation of the ionophoretic and sometimes also of the recording micro-electrode. This is caused by passive mechanical changes of both muscle and micro-electrode. Therefore, in most experiments, the desensitization processes were measured and compared only at two different temperatures, for example 22 and 12° C, 32 and 12 or 5 and 2° C. In several experiments, results were obtained at three temperatures (Fig. 5). It is clear from this Figure that decrease of temperature markedly prolongs the onset of desensitization. This effect can be

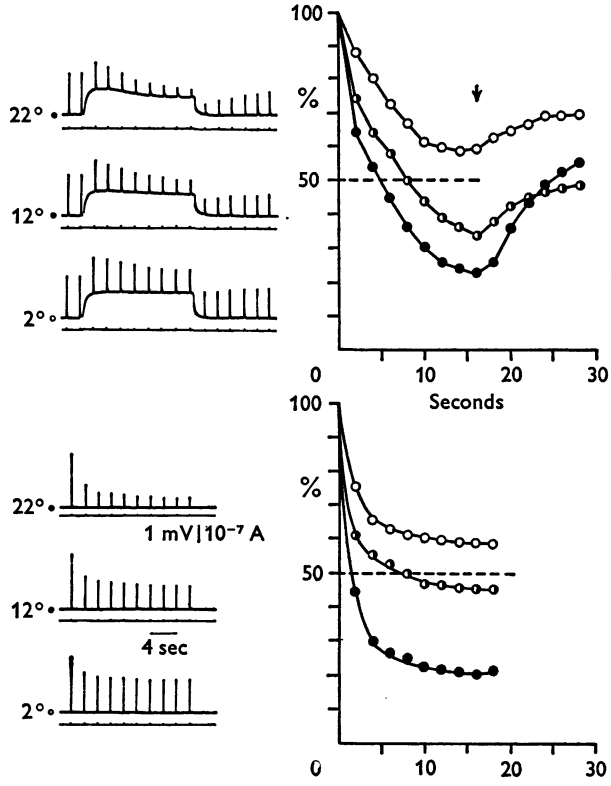

Fig. 5. Effect of temperature on the time course of desensitization. Upper part, oscilloscopic records and curves constructed from the test ACh potential amplitudes during desensitization produced by prolonged conditioning ACh-pulse from one barrel of a double-barrel micropipette at 22 (●), 12 (◑) and 2° C (○) respectively. Abscissa, time in seconds from the beginning of conditioning pulse. Arrow indicates when this pulse is switched off. This is followed by partial recovery of test potentials. Ordinate, relative amplitude of test potentials as a percentage of the first potential in the train. Lower part, desensitization at different temperatures during subsequent ACh pulses elicited from single-barrel pipette in the presence of 5 to 10^{-7} M-SKF-525A.

demonstrated by both methods, using double as well as single-barrel micropipettes and without any potentiating drugs or even in their presence.

From these experiments (three to ten in each temperature range) the Q_{10} of the onset of desensitization was calculated; these were $1{\cdot}5 \pm 0{\cdot}3$ (S.E. of mean) for 32–22° C, $1{\cdot}9 \pm 0{\cdot}2$ for 22–12° C, $2{\cdot}6 \pm 0{\cdot}2$ for 12–5° C and $3{\cdot}3 \pm 0{\cdot}5$ for 12–2° C. The reference points used for calculating the Q_{10}

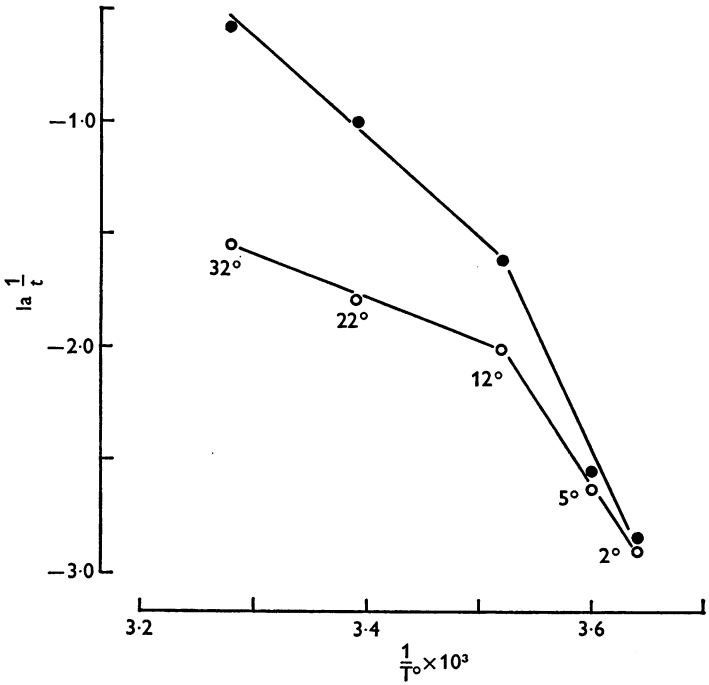

Fig. 6. Arrhenius relationship for the rate of onset (●) and recovery (○) of desensitization (t_o and t_r) during temperature changes between 32 and 2° C. Abscissa, reciprocal of the absolute temperature (T°). Ordinate, ln $(1/t_o)$ or ln $(1/t_r)$. The values of recovery rate are based on data from Table 3, values of onset rate were obtained from temperature coefficients (Q_{10}) for each temperature range. The mean rate of onset (t_o = 2·7 sec, twenty fibres) was used as reference point for reconstruction of the whole curve. Each point represents the mean of three to twenty experiments on different fibres.

of onset of desensitization was the time in sec at which the desensitization curve crossed the point of 50 % in between the initial amplitude response and the final steady value. It has been found that Q_{10} of the onset differs in different temperature ranges, and increases markedly when the temperature is lowered below 12° C. When the Arrhenius relationship (Arrhenius, 1889) was calculated and the natural logarithm of t_r was

plotted against the reciprocal of the absolute temperature (Fig. 6, filled circles), the linearity of the curve showed a break of 12° C.

The changes in the rate of recovery of desensitization might be considered as the most interesting aspect of the temperature effect. The marked slowing of the recovery process was also observed when double-barrel pipettes were used without any potentiating drugs (Fig. 5*A*), but the most convincing results were obtained when recovery was measured by the single-barrel micropipette in the presence of chlorpromazine or other similarly acting agents (SKF-525A, or its quaternary methiodide). The latter technique provided the opportunity to measure the rate of recovery in absolute time units (sec) (see Fig. 1). In Table 3 the values of t_r obtained at five temperatures are presented. It can be seen from these Figures that here also the non-linear effect of temperature exists: in the range 32–12° C the Q_{10} was little changed, being 1·25–1·36, whereas it rose to 2·36 between 12 and 2° C. This increase does not follow the simple Arrhenius equation (Fig. 6, open circles), there exists a break at 12° C, similar to the case of the onset.

When the Q_{10} values of onset and recovery are compared (Fig. 7) one can see a practically identical course of the temperature dependence of both processes. The Q_{10} of recovery is always smaller than the Q_{10} of the onset, but the ratio between them remains the almost same throughout the whole temperature range.

TABLE 3. The effect of temperature on half-time recovery (t_r) from desensitization

Temperature (°C)	t_r (sec)
32	4·73 ± 0·12 (3)
22	5·91 ± 0·05 (20)
12	7·76 ± 0·04 (16)
5	13·69 ± 0·14 (13)
2	18·14 ± 0·13 (10)

Numbers in parentheses – number of fibres studied.

At least three separate measurements were performed on each fibre at different levels of desensitization. 5×10^{-7} M chloropromazine was present in the bath. Mean ± S.E.

Intracellular application of desensitization potentiating drugs and the effect of temperature

It has been shown previously that drugs which are able to accelerate the onset of desensitization also reveal this potentiating effect when they are administered into the muscle fibre in the ACh-sensitive part (Vyskočil & Magazanik, 1972; Magazanik & Vyskočil, 1972). To elucidate the mechanism of their action it is important to compare the parameters

of desensitization during application of these drugs to the outer and inner side of the chemosensitive post-junctional membrane.

For this reason the rate of onset and recovery was measured in several experiments in which quaternary methiodide of SKF-525A, and chlorpromazine, were applied ionophoretically into the muscle fibre.

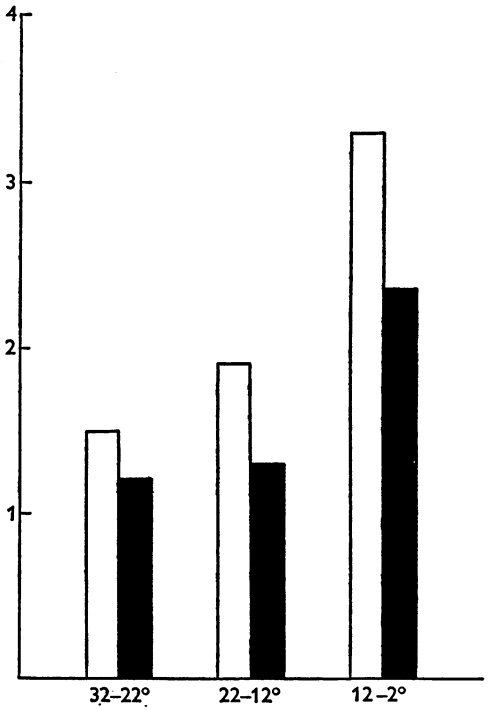

Fig. 7. Comparison of mean Q_{10} of desensitization onset (open columns) and recovery (filled columns) in three temperature ranges from 32 to 2° C. Q_{10} of the onset was calculated in each temperature range as the ratio between time taken for reduction of ACh potentials to 50 % in between the initial amplitude and final steady level of desensitization at two given temperatures. Q_{10} of recovery was similarly obtained by comparison of the half times of recovery at temperatures which differ by 10° C. Each mean value was calculated from three to twenty experiments. $5 \cdot 10^{-7}$ M chlorpromazine.

Glass micropipettes, containing one of the drugs used at a concentration of 2 M plus 1 M-KCl, were used. When the chemosensitive part of the muscle fibre was found by using the single ACh pipette, the ionophoretic pipette containing the drug was then introduced into the muscle fibre at a distance of 50–100 μm from the tip of the ACh pipette and a small positive current of about 10^{-8} A was then passed for a period of 5–15 min.

During this period and some time after it, the single ACh responses (frequency 0·3–2 Hz) started to decline, indicating desensitization.

The rate of onset of desensitization depends on the ACh dose, or on the frequency of its application, in a similar way to that seen when drugs are introduced into the muscle bath. The rate of recovery, however, remained the same, independent of the level which was reached during desensitization. For example, t_r was 5·5 sec in three experiments with intracellular injection of SKF-525A methiodide and 5·7 sec in three experiments with chloropromazine at 22° C; these values are very close to that obtained in experiments with bath-applied SKF-525A (t_r = 5·71 sec) and chlorpromazine (t_r = 5·91, see Table 3).

When the temperature of the muscle bath was lowered from 22 to 12° C, the onset of desensitization was slowed down with the Q_{10} = 1·7 (seven experiments with intracellular injection of SKF-525A methiodide) as well as the rate of recovery, the t_r being prolonged from 5·5 to 7·5 sec. The Q_{10} was found to be 1·36 in these experiments, which again fits well with the Q_{10} values obtained between 22 and 12° C when desensitization was potentiated by drugs applied extracellularly in the bath (see Fig. 7).

DISCUSSION

The rate of recovery is the least studied parameter of desensitization. Up to now, many factors are known to speed up or slow down the onset of desensitization. But little is known about factors that change the rate of recovery. Since the pioneer work of Katz & Thesleff (1957), the surprising stability of the rate of recovery has come to be generally accepted by those working with this phenomenon. The rate of recovery does not depend on the level of desensitization, which can be altered by changes of ACh concentration or the time of contact of the depolarizing drug with the membrane (Katz & Thesleff, 1957). When different cholinomimetics are used, the same rate of recovery was observed (Rang & Ritter, 1970*a*). The constant rate of recovery served, in particular, as one of the pieces of evidence for the validity of the cyclic scheme of desensitization (Katz & Thesleff, 1957; Rang & Ritter, 1970*a*). More detailed knowledge about the specific properties of the recovery kinetics is therefore very important for the understanding of desensitization. The exact measurement of the recovery parameters presents, however, serious experimental problems. Some of the generally used methods (for example, application of ACh into medium or microperfusion technique with subsequent washing off) for desensitization studies are hardly applicable for the adequate measurement of recovery. It is difficult in such cases to distinguish between the rate of recovery and the rate of dissociation and diffusion of

cholinomimetics from the sites of their fixation on the post-synaptic membrane. For this reason the methods based on the local ionophoretic micro-application of depolarizing agents are more suitable, because they minimize delays caused by diffusion of ACh to and away from the site of its action. As has been shown here, practically identical results can be obtained by the classical method of double-barrel ACh-micropipettes (Katz & Thesleff, 1957) and single-barrel pipette methods by which desensitization as well as recovery is measured, in the presence of potentiating drugs. This latter method not only proved to be more convenient, but it ensured good reproducibility and a lower scatter of values. An important feature of this method is that more precise measurements of the early stages of the onset as well as of the recovery can be made. Katz & Thesleff (1957) showed that test-responses sometimes continue to decline even when the conditioning dose of ACh from the double-barrel pipette is switched off (see Figs. 2 and 3 in their paper from 1957). This might be explained as being due to a continuing leakage of ACh from the conditioning barrel. However, another explanation is that perhaps there is overlapping of the affected membrane zones beneath the barrels bearing in mind that the conditioning dose is much larger than the test dose.

Another noteworthy effect was to be seen in experiments with single-barrel pipettes, especially when potentiating drugs are used (Magazanik, 1970, 1971; Magazanik & Vyskočil, 1973). The post-junctional membrane can be in a desensitized state even when there is no ACh present, or the concentration of ACh is near zero. As was shown in Fig. 2, there is no change in the time course of individual ACh responses during the train which leads to desensitization. This indicates that the time of activation of the post-junctional membrane by each single dose of ACh remains unchanged during the exponential decline of the amplitude of ACh responses.

When the first ACh potential is evoked there is an activation phase which is followed by a 'tail' of inactivity. When the next dose of ACh is applied after a sufficiently short time (1–3 sec) these interact with the membrane at a moment when the recovery is not yet complete and the response is therefore correspondingly lower. The summation of these inactivating events continues throughout the train and eventually a steady state is reached (e.g. Fig. 1) in which the desensitization level is exactly the same as the activation produced by the following pulse. By changing the intervals between individual ACh pulses, one can affect not only the final level of the amplitude of the response but also the rate of decline.

It is thus evident that the process of recovery not only begins when rhythmic application of ACh is terminated, but starts much earlier, immediately following the first response, and all subsequent responses in

the train. This means that the actual rate of onset of desensitization and recovery should be somewhat lower than those calculated by the above mentioned method from the experimental curves. If no partial recovery had taken place after each response, one could expect an even faster rate of the onset of desensitization.

The experiments with temperature changes showed that both processes, onset as well as recovery, did not follow a simple reaction curve stemming from the Arrhenius equation over the whole temperature range between 2 and 32° C. In both cases, the slope of the curve changed at 12° C, indicating that there is more than one reaction involved in each process. This finding is in good agreement with the cyclic scheme of desensitization (Katz & Thesleff, 1957; Magazanik & Vyskočil, 1973) which implies a complex sequence of reactions during desensitization. On the other hand, the rate of onset has higher temperature coefficients and a different slope of the curves expressing Arrhenius relationship, which might indicate that different molecular mechanisms are operating in the onset of desensitization and recovery.

It has been rather difficult, up to now, to provide a detailed explanation of the mechanism underlying both processes. The data presented here may be considered from the point of view that desensitization is accompanied by molecular changes of the ACh receptor and by an increase of receptor affinity for ACh (Katz & Thesleff, 1957). On the other hand, the effect of temperature can also be explained if one assumes that the phenomenon of desensitization is operated by changes in subsequent stages of activation, i.e. in the system controlling ionic permeability of the membrane (Magazanik, 1968, 1970, 1971; Magazanik & Vyskočil, 1970, 1973). It may be relevant that substances which speed up the onset of desensitization have no effect on the temperature dependence of the kinetic parameters of recovery from desensitization. The decline of the rate of both onset and recovery curves during lowered temperatures was found to be basically identical whether a double ACh pipette (without chlorpromazine or SKF-525A) or a single pipette (in the presence of drugs) was used. This indicates that *temperature-dependent change* of desensitization onset rate is apparently not affected by the presence of chlorpromazine or SKF-525A. It rather seems that these drugs affect one of the kinetic constants of desensitization only, namely, the rate constant for transition of the cholinergic system from the active into the inactive state (Magazanik & Vyskočil, 1973). Temperature, on the other hand, changes not only the rate of the onset desensitization, but also the rate constant for recovery of the system from the inactive into the active state.

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