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THE EFFECT OF TEMPERATURE CHANGE UPON TRANSMITTER RELEASE, FACILITATION AND POST-TETANIC POTENTIATION

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SUMMARY

1. End-plate potentials (e.p.p.s) and miniature end-plate potentials (m.e.p.p.s) were intracellularly recorded from rat diaphragm phrenic nerve preparations *in vitro* at temperatures between 7° and 40°C.

2. The quantal content of e.p.p.s and the frequency of m.e.p.p.s showed broadly similar relationships with temperature, with maxima about 20° and above 39°C.

3. Analysis of the change in e.p.p. quantal content showed that the maximum about 20°C was accompanied by a similar maximum of p, the probability of release of quanta. The maximum above 39°C was associated with a rise in n, a presynaptic store of material needed for release.

4. The rate at which transmitter could be mobilized was linear in an Arrhenius plot with an apparent activation energy of 25 kcal deg^{-1}.

5. Facilitation and post-tetanic potentiation (PTP) were shown to be entirely attributable to changes in p.

6. It is suggested that facilitation and PTP have a common basis and that the (temperature-dependent) rate of Ca removal from intracellular sites at which it exerts its action is as important a determinant of the magnitude of quantal release as is the amount of Ca combining with these sites.

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INTRODUCTION

Temperature changes are known to be a powerful tool in the unravelling of biological mechanisms. In the field of neuromuscular transmission, for instance, the demonstration that neuromuscular delay has a high temperature coefficient (Samojloff, 1925; Katz & Miledi, 1965*b*), while consonant with orthodox theories of chemical transmission (Katz, 1969; Hubbard, 1970) has discredited alternative theories of transmission such as those of Nachmansohn (1959, 1970). In the present investigation this tool has been applied to the transmitter release process.

Many investigators have found that the ambient temperature has a profound effect on spontaneous release from motor nerve terminals in the frog (Fatt & Katz, 1952; Takeuchi, 1958; Li & Gouras, 1958) and in various mammalian preparations (Boyd & Martin, 1956*a*; Liley, 1956*a*; Li, 1958; Feigen, Peterson, Hofman, Genther & Van Heyningen, 1963; Hofmann, Parsons & Feigen, 1966; Hubbard, Jones & Landau, 1967). Similarly, temperature changes affect the amount of transmitter released by nerve impulses (Boyd & Martin, 1956*b*; Thies, 1965; Hofmann *et al.* 1966), neuromuscular 'depression' and 'facilitation' (Eccles, Katz & Kuffler, 1941; Thies, 1965; Hofmann *et al.* 1966; Balnave & Gage, 1970) and the rate of transmitter 'mobilization' (Hofmann *et al.* 1966). However, no systematic study of these effects has been undertaken and no full temperature data provided for any one preparation.

METHODS

All experiments were performed *in vitro*, using the rat diaphragm phrenic nerve preparation. The mounting of the preparation, bathing solutions and intracellular recording methods have recently been fully described (Hubbard *et al.* 1968*a*, *b*). For intracellular recording of end-plate potentials (e.p.p.s.), neuromuscular transmission was partially blocked. This was done either by raising the $MgCl_2$ concentration of the bathing solution or by adding (+)-tubocurarine Cl (1–1·5 mg/l.) to it.

The quantal content (m) of e.p.p.s recorded from preparations exposed to solutions with high [Mg] was estimated from the ratio of mean e.p.p. amplitude to mean miniature end-plate potential (m.e.p.p.) amplitude or if the e.p.p.s were larger, by analysis of variance of the amplitude of successive e.p.p.s. In solutions with normal [Ca] and [Mg] the preparations were paralysed with (+)-tubocurarine Cl (Burroughs Welcome) and m was again estimated by analysis of the variance of successive e.p.p.s. When e.p.p. amplitudes exceeded 3 mV the estimates of m were corrected according to Martin (1955). The method and precautions are fully described elsewhere (Hubbard *et al.* 1968*b*). In curarized preparations when both m and quantal size (q) were followed, the phrenic nerve was stimulated with forty supramaximal stimuli at 100/sec at 1 min intervals. Usually a regression was fitted to the last 20 e.p.p.s in a train and quantal size (q) computed from the variance and mean of the regression. The estimates of q from the various tetanic trains recorded from one end-plate were usually averaged and the mean used to convert e.p.p. amplitudes into quantal units.

When a trend was found in the successive q estimates from a single junction, the regression of q on time was computed (Brooks & Thies, 1962) and the values of the regression used in the conversion. The average amplitude of the last twenty e.p.p.s in a train was taken at an index of the rate (dm) at which transmitter release could be maintained and was expressed as mV or after division by q as quanta.

The rundown of e.p.p. amplitudes in curarized preparations at the beginning of nerve stimulation can be at least partially explained by the exhaustion of a store (of amplitude, n) of some material needed for release (Elmqvist & Quastel, 1965;

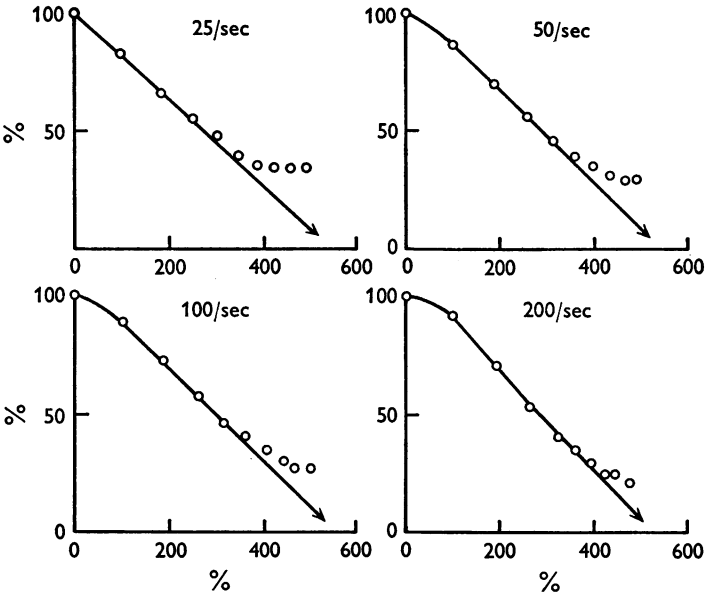

Fig. 1. The effect of varying the frequency of nerve stimulation on estimates of n, the store of transmitter readily available for release. Each graph shows the amplitude of successive e.p.p.s in response to tetanic nerve stimulation (ordinate) plotted as a function of the total amplitude of all e.p.p.s previously evoked during the tetanus (abscissa). All measurements are expressed as a percentage of the amplitude of the first e.p.p. evoked by the stimulation. Each point represents the mean of twenty experiments. The frequency of nerve stimulation is stated above the appropriate graph. The abscissal intercept of the extrapolation of the rapidly descending parts of the curves provides an estimate of n.

Christensen & Martin, 1970). The magnitude of n was computed by plotting the first five e.p.p.s in a tetanic train of 40 e.p.p.s (m_1, m_2, . . ., m_5) each against the sum of all previous e.p.p.s (Elmqvist & Quastel, 1965). A linear regression was fitted to all the five points of this plot if the size of the second e.p.p. was less than or equal to 85 % of the first. Otherwise the regression was fitted to points 2–5 of this plot. The intercept of this regression on the abscissa gives the estimate of n which is probably an underestimate since it neglects replacement during the release train. For comparative purposes n could be expressed simply as a percentage of the first e.p.p. (Fig. 1), but if the amplitude or quantal content of the e.p.p.s was measured the value then

could be expressed in mV or quanta. In the same experiments from which n was computed, it was also possible to calculate p, the probability of release from the store, using the relationship m (quantal content of e.p.p.) = np (del Castillo & Katz 1954).

Before examining the relationship of n and the ambient temperature it was necessary to find out whether measurement of n was dependent on the frequency of stimulation. Certainly the rate at which transmitter release can be maintained is frequency dependent (Elmqvist & Quastel, 1965) and it could be that the same process replenished n, in which case a large increase in n would be expected as the frequency of stimulation increased. As Fig. 1 shows, n was not significantly changed as the frequency of nerve stimulation was increased from 25 to 200/sec (30° C). It may further be noted that there was apparently no interaction between experiments, that is, no cumulation of effects.

The bathing temperature was measured by a thermistor in the solution above the preparation and recorded by an ink-writing recorder. It was altered by changing the temperature of the water circulated in a heat exchanger through which the bathing solution flowed immediately before entering the preparation bath. Usually a preparation was allowed at least 1 min to equilibrate at any given temperature before starting to record from it as preliminary experiments indicated that changes in m.e.p.p. frequency with temperature were complete well within such a period. Sometimes it was useful to record from a junction during a slow temperature change. In these cases the rate of temperature change never exceeded 1°C/min.

RESULTS

The relationship between spontaneous transmitter release measured as m.e.p.p.s and the ambient temperature is complex and shows three general sections (Fig. 2*B*); a portion at temperatures below 20°C in which m.e.p.p. frequency increases with temperature, a region between 20° and 30°C in which m.e.p.p. frequency *decreases* with temperature, and above 30°C a portion in which m.e.p.p. frequency rapidly increases as the temperature increases (Liley, 1956*a*; Li, 1958; Hubbard *et al.* 1967).

In the present investigation these observations were confirmed and amplified by recording m.e.p.p.s continuously from single junctions as the temperature of the bathing solution was increased at 1°C/min over the range 7–38°C and back to 10°C. Similar results were obtained from preparations partially blocked with an excess of $MgCl_2$ in the bathing medium (Fig. 2*B*) and in preparations bathed in solutions without blocking drugs. Three experiments with mouse phrenic nerve hemidiaphragm preparations gave very similar results while one experiment with a cat tenuissimus preparation was also suggestive of a complex relationship between m.e.p.p. frequency and ambient temperature (see Fig. 4 in Hubbard, 1970).

As Fig. 2 shows, in Mg-paralysed preparations it was possible to make concomitant determinations of quantal content (Fig. 2*A*, filled circles), m.e.p.p. frequency (Fig. 2*B*, filled circles) and m.e.p.p. amplitude (Fig. 2*C*, filled circles). Fig. 2*A*, *B* indicate that both m.e.p.p. frequency and e.p.p.

quantal content have a maximum at about 20° C. No parallel to the marked acceleration of m.e.p.p. frequency as the temperature was raised from 30° to 38° C, however, appeared in these experiments. M.e.p.p. amplitudes (Fig. 2*C*) fell over the temperature range explored. This can presumably be explained by changes in the subsynaptic response to ACh, which shows a similar decline as the temperature is raised (Harris & Leach, 1968), and was not further explored.

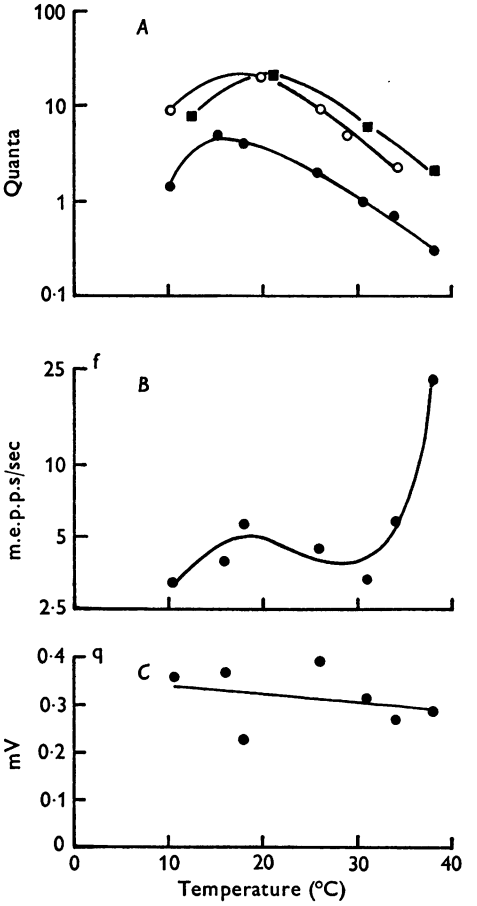

Fig. 2. The relationship between temperature and transmitter release in the presence of raised [Mg]. *A*, e.p.p. quantal content (ordinate) determined at various temperatures (abscissa) from two junctions exposed to a [Mg] of $1{\cdot}2 \times 10^{-2}$ M (open circles, filled squares) and from one junction exposed to a [Mg] of $1{\cdot}5 \times 10^{-2}$ M (filled circles). *B* shows m.e.p.p. frequency (ordinate) and *C* shows mean m.e.p.p. amplitude (ordinate) at various temperatures (abscissa) for the junction whose quantal content was plotted in *A* with filled circles.

Similar experiments with a smaller [Mg] in the bathing medium gave similar results despite the larger quantal content of the e.p.p.s (Fig. 2*A*, squares and dotted circles). Again when curarized preparations (Fig. 3*A*, dotted circles) bathed in otherwise normal solutions were examined at 10, 15, 20, 30, 34 and 38°C, e.p.p. quantal content had the same general relationship with temperature as was found in Mg-blocked preparations (Fig. 2*A*).

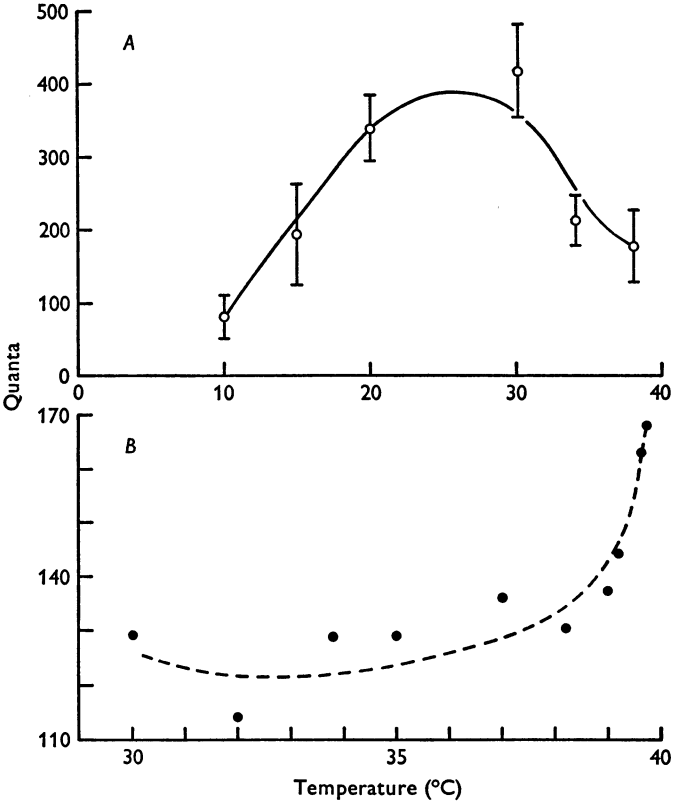

Fig. 3. The relationship between e.p.p. quantal content and temperature in curarized preparations. *A*, 10–38°C. Each point on this graph represents the mean ± 1 S.E. of 7–12 determinations of quantal content from junctions in preparations bathed at that particular temperature. *B*, 30–40°C. Each point in this graph represents a single determination of quantal content. All points are from the same junction. Abscissa: temperature in °C. Ordinate: e.p.p. quantal content.

It was not possible to keep preparations at temperatures above 38°C long enough to make observations of quantal content at several junctions. Recordings from single junctions exposed to high temperatures did show (Fig. 3*B*) that at about 39°C there was an increase in m. Indeed so steep

was the relationship between temperature and quantal content in this range that 0·2°C variations in temperature produced easily detectable changes in quantal content.

Mobilization. It is known that the rate at which quantal release by nerve impulses can be maintained (mobilization) is a function of the frequency of stimulation (Elmqvist & Quastel, 1965; Maeno & Edwards,

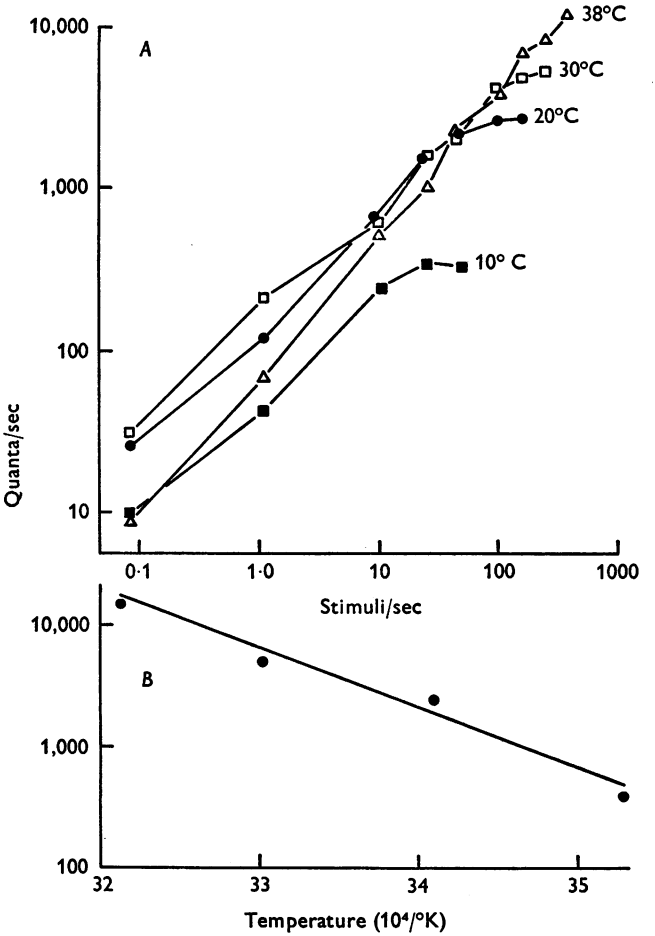

Fig. 4. The effect of temperature on transmitter mobilization. *A*, mean quantal content of e.p.p.s 21–40 of a tetanic train at the indicated frequency and temperature multiplied by the stimulus frequency (ordinate) and plotted as a function of the stimulus frequency (abscissa). Note the logarithmic co-ordinates. Each point is the mean of the results from two junctions studied at each temperature over the whole range of frequencies. The quantal size in each case was calculated from the variance of the amplitude of e.p.p.s elicited by stimuli at 0·1/sec. *B*, Arrhenius plot of maximum release rates found in *A* at each temperature as a function of 10^4/°K.

1969). In the present investigation, as Fig. 4*A* shows, the maximum mobilization at any particular junction was found by examining quantal release at several different frequencies of stimulation, at 10, 20, 30 and 38° C. Two junctions were studied at each temperature and e.p.p.s 21–40 of a 40-member train elicited by nerve stimulation at the appropriate frequency were analysed. It is clear that this maximum rate was a function of temperature. Indeed it will be noted (Fig. 4*A*, open triangles) that at 38° C mobilization was still increasing at the highest frequency of stimulation assayed (400/sec).

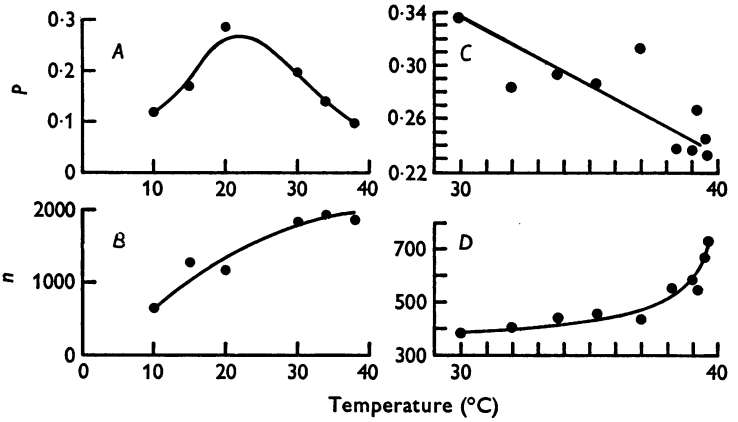

Fig. 5. The variation of p and n with temperature. *A* and *C*, the relationship between temperature and the transmitter release probability (p). *B* and *D*, the relationship between temperature and the readily available store of transmitter (n). The points in *A* and *B* are based on data from experiments similar to those illustrated in Fig. 4. The points in *C* and *D* are from the experiment illustrated in Fig. 3*B*. Note the abscissa in *A* and *B* extends from 0° to 40° C and in *C* and *D* from 30° to 40° C. The ordinate scale in *A* and *C* denotes probability and in *B* and *D* shows n expressed in quanta.

Fig. 4*B* shows the maximum mobilization for each temperature in an Arrhenius plot. The relationship appears linear and indicates that the mobilization process is highly temperature-dependent. An activation energy of 25 kcal deg^{-1} was calculated from these data.

The presynaptic store and the probability of transmitter release. Fig. 5 illustrates the average relationship between n (Fig. 5*B*), p (Fig. 5*A*) and temperature found at 8–10 junctions stimulated at 50/sec at the indicated temperature. Fig. 5*A* shows that p has a relationship with temperature which is very similar to that observed for e.p.p. quantal content (Fig. 2*A*); that is, it rises to a maximum at about 20° C and then decreases with further elevation of temperature. In contrast, n (Fig. 5*B*) increases over most of the temperature range examined. In these experiments n appeared to be

near a maximum of about 30°C. Close examination of this part of temperature range however indicated that n increased rapidly as the temperature was raised above 38°C (Fig. 5*D*), while p showed a further decline in this range (Fig. 5*C*).

Facilitation

It is well known that the amount of transmitter released from the neuromuscular junction by a testing nerve impulse can be increased by preceding this nerve impulse with another (conditioning) nerve impulse at a short (msec) interval. This process has been termed facilitation. The

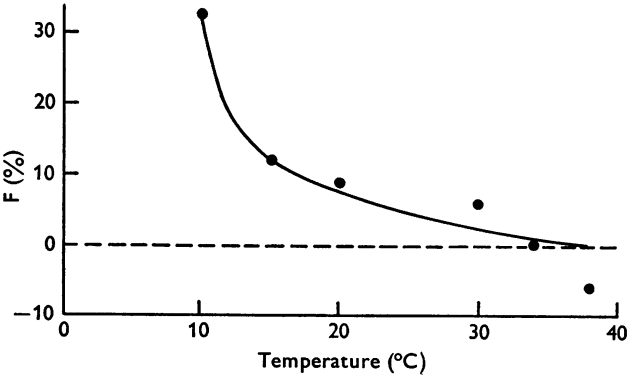

Fig. 6. The relationship between facilitation and temperature. Ordinate: $(p_2-p_1) \times 100/p_1$, where p_1 and p_2 are the release probabilities for the first and second e.p.p.s respectively in a series evoked at 50/sec. Abscissa: temperature (°C).

assumption that facilitation of transmitter release is due to an increase in p, satisfactorily explains many observations (Mallart & Martin, 1967, 1968). However, there is no direct evidence that facilitation is indeed caused by a relative increase in p rather than n; Hubbard (1963) has suggested that facilitation is due to a 'mobilization' of transmitter, presumably leading to a relative increase in n.

As Fig. 6 shows, 'p' for the e.p.p. evoked in response to the second of two nerve impulses at a 20 msec interval was markedly larger than p for the e.p.p. evoked by the first impulse at 10°C. This facilitation of p_2 became smaller as the temperature was increased and disappeared as the temperature was raised above 35°C. The records of e.p.p. amplitudes indicated that similar observations could be made of the facilitation of e.p.p. amplitude. It appeared worth while then to try and decide whether facilitation can be attributed solely to an increase in p or is also accompanied by a change in 'n'. If facilitation increases n, higher estimates of n should be obtained by an analysis of e.p.p.s resulting from tetanic stimuli as the

frequency of stimulation is increased. Fig. 1 indicates no such effect was detectable.

Clear evidence that facilitation can be attributed solely to changes in p was obtained by doing experiments at 10° or 20°C. Transmitter mobilization into the readily available store is greatly reduced at such temperatures (Fig. 4*A*) while facilitation, expressed simply at the ratio of the second to the first e.p.p. of a series, is greatest at these temperatures. Accordingly, e.p.p.s recorded from single junctions were evoked by 10–15 stimuli at 10/sec or 50/sec in preparations maintained at 10° or 20°C. Five minutes were allowed between each set of stimuli, and the order in which any one junction was stimulated was varied. The data was analysed in the usual manner for estimates of n and p.

It was found that the facilitation of evoked release induced by increasing the test stimulus frequency (Braun, Schmidt & Zimmerman, 1966) from 10/sec to 50/sec was due to an increase in the release probability, p. There was no detectable change in n. Data from two cells, representative of the results obtained, are shown in Figs. 7*A* (10°C) and 7*B* (20°C). It can be seen that in each case the nerve impulses evoked by 50/sec stimulation (Figs. 7*A*, *B*, filled circles) released more transmitter for any given degree of store depletion than those evoked by 10/sec stimulation (Figs. 7*A*, *B*, open circles); extrapolation of the curves to the abscissa showed that there was little difference between the estimates of n at 10/sec or 50/sec in either case.

Metabolism and facilitation. It has been suggested that facilitation is due to accumulation of Ca ions (Ca^{2+}) at a site where they lead to transmitter release, and that their removal from this site is an active process (Rahamimoff, 1968). Interference with metabolism which should alter the rate of active Ca removal should then affect facilitation. Two different groups of experiments were performed to examine this question. Firstly, the effect of ouabain was examined. Rahamimoff & Colomo (quoted by Rahamimoff, 1968) have found ouabain to increase facilitation. The amplitudes of the e.p.p.s evoked by phrenic nerve stimulation (10 stimuli at 100/sec) were recorded at twenty junctions in a preparation before and during exposure to a solution containing $4{\cdot}2 \times 10^{-4}$ M ouabain for 20 min at 30°C. The average responses were calculated by pooling the data and the release probability for the first and second e.p.p.s in each series was calculated. As expected (Gage, 1965) the amplitude of the first e.p.p. of the series was larger during exposure to ouabain and p for this e.p.p. was considerably higher (0·243/0·157) than in the control situation. Facilitation was however unchanged (p_2/p_1 = 1·04 in ouabain and 1·05 in the control solution).

Secondly, a similar investigation was made of preparations exposed to glucose-free solutions for 2 hr at 30°C. The release probability for the first

e.p.p. (0·258) was also higher than that for normal bathing solutions (0·194). Again, no clear change in the facilitation for the second e.p.p. was found, the value being 10·8 % in this case, compared with 11·1 % for the normal bathing solution. The same lack of effect of glucose removal upon facilitation was observed in preparations where evoked transmitter release had

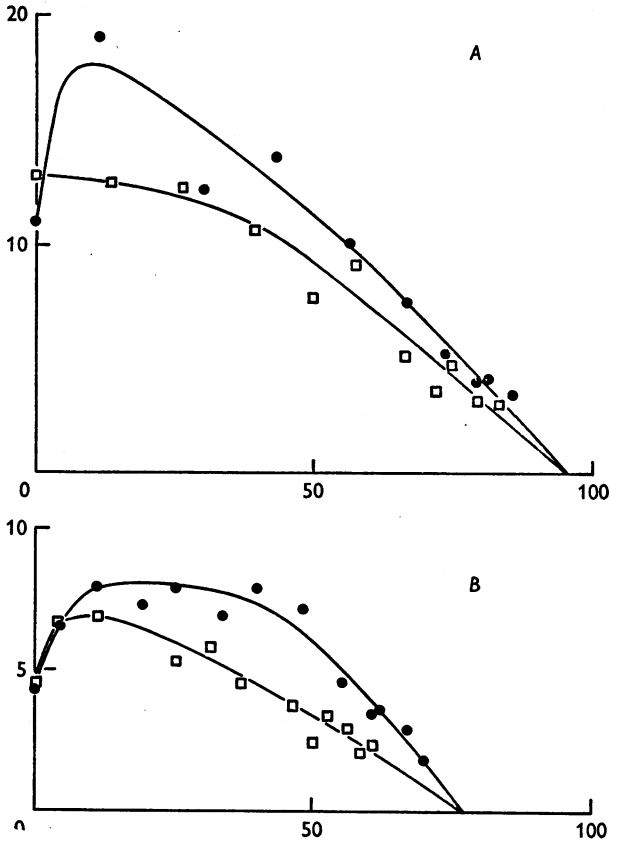

Fig. 7. The effect of increased facilitation of transmitter release on the parameters p and n. Each graph shows e.p.p. amplitudes, plotted in the manner of Fig. 1 recorded from a junction in response to nerve stimulation at 10/sec (open squares) and 50/sec (filled circles). The ordinates and abscissae are calibrated in arbitrary units of e.p.p. amplitude. *A*, 10° C. *B*, 20° C.

been markedly reduced by addition of $0{\cdot}5 \times 10^{-2}$ M-Mg to the bathing solution. At these low quantal contents, facilitation is more marked (Liley, 1956*b*). Pairs of e.p.p.s resulting from nerve stimuli 10 msec apart were recorded from junctions in two preparations bathed at 30° C. One of these preparations had been exposed for 2 hr to a $1{\cdot}5 \times 10^{-2}$ M-Mg bathing solution with no added glucose. The second preparation was bathed with a

$1{\cdot}5 \times 10^{-2}$ M-Mg glucose-containing solution. Ten to twenty pairs of e.p.p.s were recorded from each of twenty-seven junctions in the first preparation and twenty-four junctions in the second preparation. The mean increase in the amplitude of the second e.p.p. of each pair was 49 %

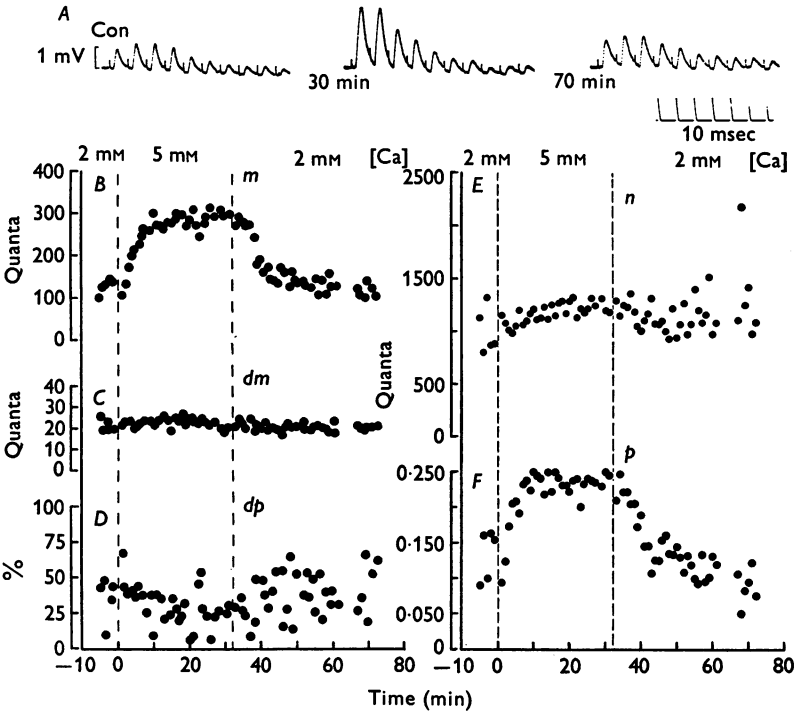

Fig. 8. The effect of increasing bathing [Ca] on the various release parameters. Bathing [Ca] was increased at the first vertical dotted line from 2 to 5 mM and reduced to 2 mM at the second vertical dashed line. Sample tetani shown in *A* were obtained in the control solution, at increased [Ca] and after return to the control situation. The ordinates indicate: *B*, quantal content of first e.p.p. in tetanic train (*m*); *C*, size of sustained tetanic release (*dm*); *D*, facilitation (*dp*) computed as $[(p_2 - p_1)/p_1] \times 100$; *E*, store of immediately available quanta of transmitter (*n*); *F*, average probability of release (*p*) calculated as m_1/n. Corresponding points in the graphs *B*–*F* were derived from the same tetanus. Tetani were obtained at 1 min intervals. The various estimates of quantal size (not shown) were averaged and used to convert *m*, *dm* and *n* into quantal units. Abscissa: time in min. Temperature of experiment 23°C. The preparation was curarized.

for the preparation exposed to a glucose-free solution. The value obtained in the control preparation, 50 %, was not significantly different.

Finally the effect of Ca was examined. Trains of 40 e.p.p. elicited by 100/sec stimuli were evoked every minute, before, during and after ex-

posure of the preparation to a rise in the [Ca^{2+}] of the bathing medium from 2–5 mM. The expected increase in e.p.p. amplitudes (Fig. 8*A*) was found and was attributed to an increase in quantal content (Fig. 8*B*). Again, as expected, the change in [Ca^{2+}] was without effect on mobilization (Fig. 8*C*) or *n* (Fig. 8*E*), but did markedly increase *p* (Fig. 8*F*). As with ouabain and glucose deprivation, facilitation (*dp*, Fig. 8*D*) was unchanged.

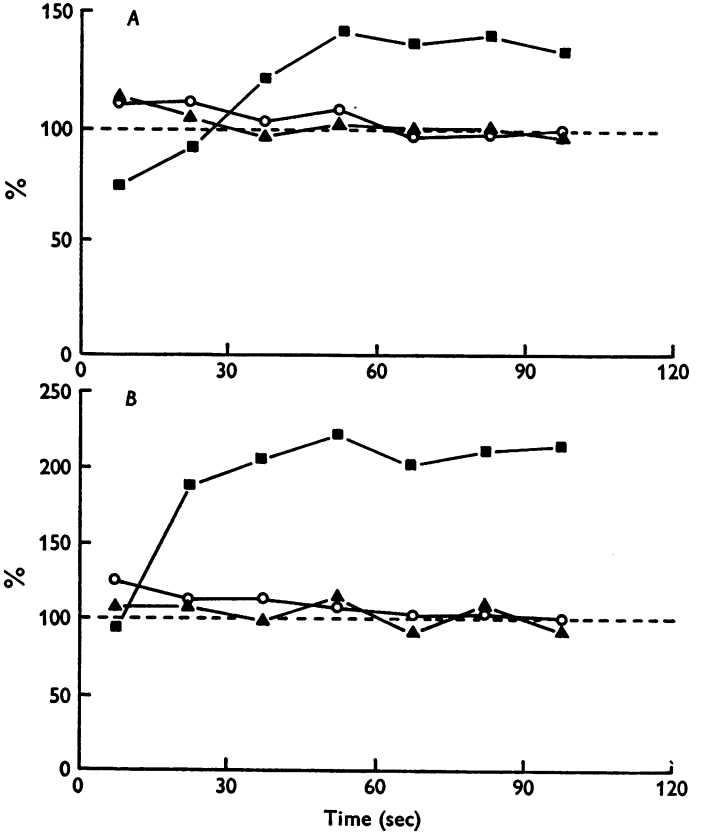

Fig. 9. The relationship between post-tetanic potentiation and temperature. *A* and *B* show results of two different experiments in each of which e.p.p.s were evoked at 1 sec intervals before and after conditioning phrenic nerve stimulation (500 stimuli at 50/sec), this procedure being performed at 10°C (filled squares), 20°C (open circles) and 30°C (filled triangles). Each point in the graphs shows the mean amplitude of 15 e.p.p.s expressed as a percentage of the mean amplitude of e.p.p.s evoked before the conditioning nerve stimulation (ordinate) and plotted in the middle of the 15 sec period in which they were recorded following the conditioning stimulation. In *A*, transmission was blocked by (+)-tubocurarine. In *B*, transmission was blocked by raising the [Mg] of the bathing solution to 15 mM.

Post-tetanic potentiation (PTP)

Experiments with curarized (Fig. 9*A*) and Mg-paralysed preparations (Fig. 9*B*) showed that PTP increased in magnitude and direction as the temperature of the bathing medium was reduced. At 10°C (filled squares, Fig. 9*A*, *B*) PTP of e.p.p. amplitudes produced by 500 stimuli at 50/sec was prolonged and of greater relative magnitude when compared with the

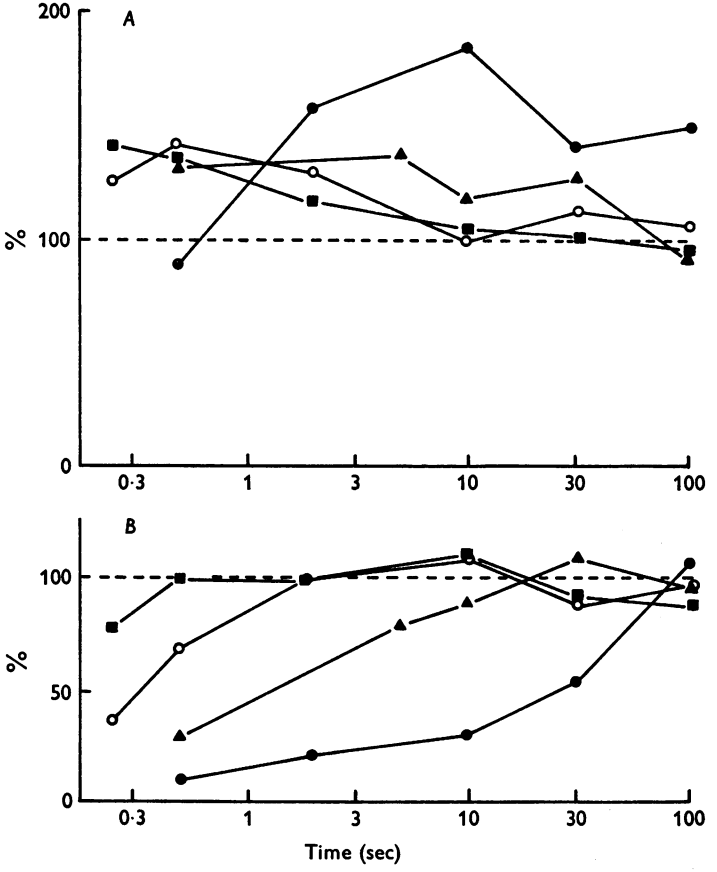

Fig. 10. The relationship between temperature and the changes in p and n following conditioning nerve stimulation (500 stimuli at 50/sec). *A* and *B* show respective estimates of p and n, expressed as a percentage of similar estimates made before the conditioning stimulation (ordinate), plotted as a function of the time, in sec, at which the estimates were made following the stimulation (abscissa). Note that the time scale is logarithmic. The filled circles, filled triangles, open circles and filled squares show results of experiments performed at 10, 20, 30 and 38°C respectively. Neuromuscular transmission was blocked by (+)-tubocurarine. For further details, see text.

PTP evoked by the same stimulation, 15–20 min later at the same junction at 20°C (Fig. 9*A*, *B*, open circles) or 30°C (Fig. 9*A*, *B*, filled triangles). The result was the same whatever the order in which the temperature changes were made.

In order to further define the nature of PTP, p and n were estimated from the quantal content of e.p.p.s evoked by 40 nerve impulses at 100/sec (or occasionally 50/sec when some nerves failed to conduct stimuli at 100/sec) before and at a variable interval after a conditioning tetanus of 500 stimuli at 50/sec. Experiments were repeated to obtain data from several cells at any one combination of temperature and post-tetanic interval. Composite curves (Fig. 10*A*, *B*) were then prepared showing the time course of changes in p and n at 10, 20, 30 and 38°C following the conditioning nerve tetanus.

As Fig. 10*A* shows, p was increased for a period after the tetanic stimulation paralleling the PTP of e.p.p. amplitudes (Fig. 9*A*, *B*). As the temperature was raised p remained above control levels for a shorter period and the magnitude of the increase above control levels was smaller. There was no evidence of any phase of absolute increase in n following nerve stimulation (Fig. 10*B*) but the approximately exponential recovery of n was greatly accelerated by raising the temperature of the preparation (Fig. 10*B*, compare the time course at 38°C shown by the filled squares and at 10°C shown by the filled circles).

DISCUSSION

The results illustrated in Figs. 2 and 3 show that for both curarized preparations and preparations where evoked transmitter release had been reduced by raising the [Mg] of the bathing solution, e.p.p. quantal content is at a maximum in the temperature range 20–30° C with a second smaller maximum beyond 38°C.

Fig. 5*A* indicates that the first maximum in this relationship is due to the temperature dependence of p, which similarly reaches a maximum between 20° and 30°C, falling with reduction or elevation of temperature beyond this range. The second maximum is attributable to the temperature dependence of n which increases markedly at temperatures above 38°C (Fig. 5*D*). It is uncertain at present what the relationship of p is to the known determinants of release – the amplitude of the terminal action potential and the ambient [Ca^{2+}]. To explain Fig. 5*A*, *C* action potential amplitudes would have to reach a maximum about 20°C and thereafter decline. An increasing nerve action potential *amplitude* up to 20°C has been reported (Gasser, 1928; Chatfield, Battista, Lyman & Garcia, 1948; South, 1961), while the decreasing *duration* of the nerve action potential

which accompanies temperature elevation (Gasser, 1928; Tasaki & Fujita, 1948; Hodgkin & Katz, 1949; Katz & Miledi, 1965*c*, 1967) could be responsible for the fall in p as temperature is raised beyond 20° C. However, m.e.p.p. frequency appears to have a similar relationship with temperature (Fig. 2*B*) over the range 10–30° C, first increasing in the range 10–20° C and then falling in the range 20–30° C. This suggests that the changes of p with temperature may be at least partly due to processes common also to spontaneous transmitter release, rather than to variations in the form of the nerve action potential. The temperature dependence of m.e.p.p. frequency (Fig. 2*B*) indicates at least two groups of reactions are concerned with the spontaneous release of transmitter. One group, dominant below 20–30° C, is influenced by depolarization of nerve terminals and the ambient [Ca]. The other group, dominant at higher temperatures, appears relatively uninfluenced by the ambient [Ca] (Hubbard *et al.* 1967). The present results indicate that e.p.p. quantal content is similarly determined. Thus an increase in p is the major factor in increasing e.p.p. quantal content as the temperature rises over the range 10–30° C (Figs. 5, 6) and p is Ca dependent (Fig. 8*F*). Above 30° C n is the major factor associated with increasing e.p.p. quantal content (Figs. 3 and 5) and n is Ca independent (Fig. 8*E*). In so far as the rate of removal of Ca from its active site may be a factor in the amount of transmitter released by a single nerve impulse (Katz & Miledi, 1968) it may be suggested that the falling phases in the p and m.e.p.p. frequency relationships with temperature have their origin in temperature enhanced removal of Ca from a site where it is active in producing transmitter release.

The demonstration that the facilitation of transmitter release produced by a conditioning nerve impulse is due to an increase in p, the release probability (Fig. 6) can similarly be explained in terms of changes in active [Ca] (Katz & Miledi, 1965*a*, 1968; Rahamimoff, 1968), and similarly a temperature enhanced decay of the active [Ca] could explain the marked dependence of the magnitude of facilitation upon temperature found in the present (Fig. 6) and other investigations (Balnave & Gage, 1970). The suggestion of Rahamimoff (1968) that Ca removal from the active site is metabolically dependent appears unfounded for the magnitude of facilitation was insensitive to the absence of glucose or presence of ouabain. Metabolic inhibitors such as sodium oxide and ouabain similarly did not affect the magnitude of facilitation at toad neuromuscular junctions (Balnave & Gage, 1970).

The lack of effect of an increase in [Ca] from 2 to 5 mM upon facilitation (Fig. 8*D*) may be plausibly explained by the residual active [Ca] already being at a maximum level in the presence of 2 mM-Ca (Rahamimoff, 1968).

The experiments on PTP (Figs. 9, 10) confirm that the magnitude and

duration of this phenomenon is markedly increased by decreasing the temperature of the preparation (Rosenthal, 1969). Analysis of the PTP changes into effects on p and n (Fig. 10) indicated that at no stage was there any significant elevation in n, any potentiation of transmitter release being mediated by an increase in p, as first suggested by Liley & North (1953). The recovery of n following nerve stimulation was delayed at low temperatures (Fig. 10*B*), and this appears an adequate explanation of the slow onset of PTP in both curarized and Mg-blocked preparations (Fig. 9*A*, *B*) at these temperatures. Slow recovery of n also appears to be the most plausible explanation for the observation of Liley & North (1953) that the delay in development of an absolute potentiation of evoked transmitter release increased with the number of stimuli in the conditioning tetanus. The estimates of p immediately following a conditioning tetanus (Fig. 10*A*) were not always the maximum values obtained for p; however, this appears to be an artifact in the measurement of p, presumably produced by the persistence for a short time of the increased mobilization induced by stimulation (Fig. 4*A*). This effect would lead to smaller 'run-down' in successive e.p.p. amplitudes in response to a brief nerve tetanus with a consequent over-estimation of n and underestimation of p. A monotonic decay in p towards control values after conditioning nerve stimulation seems most probable. It follows that facilitation and PTP may be simply quantitative variants of a common process. Both phenomena result from conditioning nerve stimulation, affect p (Figs. 6, 10*A*), and are increased in magnitude and duration by temperature reduction (Figs. 6, 9*A*, *B*). Furthermore, a raised [Ca] increases the duration of facilitation (Rahamimoff, 1968) while the time course of PTP is dependent only on the ambient [Ca] present *during* the tetanus (Rosenthal, 1969).

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