Differential Responses of Crab Neuromuscular Synapses to Cesium Ion

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ABSTRACT Excitatory postsynaptic potentials (EPSP's) generated in crab muscle fibers by a single motor axon, differ in amplitude and facilitation. Some EPSP's are large at low frequencies of stimulation and show little facilitation; others are smaller and show pronounced facilitation. When K+ is replaced by Cs+ in the physiological solution, all EPSP's increase in amplitude, but small EPSP's increase proportionately more than large ones. Quantal content of transmission, determined by external recording at single synaptic regions, undergoes a much larger increase at facilitating synapses. The increase in quantal content of transmission is attributable to prolongation of the nerve terminal action potential in Cs+. After 1-2 h of Cs+ treatment, defacilitation of synaptic potentials occurs at synapses which initially showed facilitation. This indicates that Cs+ treatment drastically increases the fraction of the "immediately available" transmitter store released by each nerve impulse, especially at terminals with facilitating synapses. It is proposed that facilitating synapses normally release less of the "immediately available" store of transmitter than poorly facilitating synapses. Possible reasons for this difference in performance are discussed.

INTRODUCTION

Synaptic diversity has been frequently observed in crustacean neuromuscular systems. The single axon which supplies the motor innervation of both stretcher and opener muscles in legs of crabs and crayfish provides synapses with different physiological properties to various fibers of these muscles (Atwood, 1965, 1967a; Bittner, 1968; Bittner and Kennedy, 1970; Atwood and Bittner, 1971; Sherman and Atwood, 1972). The excitatory postsynaptic potentials (EPSP's) generated at low frequencies of stimulation by these synapses range in size from less than 1 mV to more than 20 mV, depending on the muscle from which measurements are taken. As a rule, the larger EPSP's show less facilitation at a high frequency of stimulation than do the smaller EPSP's.

What factors are responsible for the differences in transmitter release at
different terminals? Atwood (1967 a) and Bittner (1968) suggested differences in the extent of invasion of the nerve terminals by the action potential as a possible mechanism, the terminals producing large EPSP's being more completely depolarized by the action potential than the terminals producing small EPSP's. Bittner (1968) ruled out differences in quantal effectiveness, transmitter mobilization, postsynaptic membrane properties, and the relation between depolarization and transmitter release in the terminals, as factors contributing to the generation of different EPSP's in the crayfish opener muscle. Differences in terminal depolarization by the action potential remained as one possibility which could explain the EPSP characteristics.

More recently, Sherman and Atwood (1972) found differences in synaptic size at the motor terminals in a crab stretcher muscle. Larger synapses occurred on terminals responsible for large EPSP's. Thus, differences in EPSP's at low frequencies of stimulation could be due, at least in part, to the area of synapse available for transmitter release. However, the differences of facilitation cannot be explained by this factor alone, and the question of possible electrical differences at various terminals remains unresolved.

In order to study the problem further, treatments which are known to change synaptic performance were investigated, to see whether differential effects could be obtained at terminals known to produce large and small EPSP's. Among these treatments is the cesium effect described by Gainer et al. (1967). EPSP's of crustacean muscle fibers increase markedly when they are treated with a solution containing Cs+ in place of K+; the effect is apparently presynaptic in origin, and possibly attributable to a change in configuration of the action potential in the nerve terminal (Gainer et al., 1967; Ginsborg and Hamilton, 1968).

Use was made of the cesium effect in the present study to compare the performance of high-output and low-output synapses under conditions in which the effectiveness of excitation-secretion coupling was increased. It was established that the cesium effect is associated with a prolongation of the nerve terminal potential (NTP) as predicted by Gainer et al. (1967), and that the effect is much larger at synapses with initially low quantal output. The implications for differences in synaptic performance are discussed.

**METHODS**

The shore crab *Grapsus grapsus* L., common in Bermuda, was used in the majority of the experiments. Specimens were maintained in aquaria supplied by running seawater and were kept at ambient temperature (20°-24°C). Spider crabs, *Hyas arenus* L., obtained from a supplier in New Brunswick and maintained at the University of Toronto in aerated artificial seawater at 10°C, and shore crabs, *Pachygrapsus crassipes* Randall, obtained from a supplier in California and maintained in artificial seawater at 15°C, were also used for certain experiments.

In all experiments, the outer surface of the “stretcher” muscle (reductor of the
propodite) of a walking leg was exposed for recording as described previously (Atwood et al., 1965). The single excitatory axon supplying the muscle was stimulated in the meropodite joint by short rectangular current pulses (0.08 ms) delivered through platinum wire electrodes. Excitatory postsynaptic potentials (EPSP's) were recorded intracellularly from individual muscle fibers with standard glass microelectrodes filled with 3 M KCl, of 3–15 MΩ resistance. The amplitude of the EPSP was measured at a stimulation frequency of 1 Hz in all experiments. In many experiments, the “facilitation index” (F,) was also determined by calculating the ratio of EPSP amplitude at 10 Hz stimulation to EPSP amplitude at 1 Hz stimulation, with appropriate corrections (Sherman and Atwood, 1972).

External recordings of synaptic potentials (ERSP's) and nerve terminal potentials (NTP's) at single synaptic regions were obtained with microelectrodes filled with 3 M NaCl, of 2–4 MΩ resistance. The methods for obtaining such records have been described in previous publications (Dudel and Kuffler, 1961; Takeuchi and Takeuchi, 1964; Atwood and Johnston, 1968). In some experiments a Fabri-Tek signal averager (Nicolet Instrument Corp., Madison, Wis.) was used to obtain records of small NTP's.

Preparations were kept in a standard physiological solution for marine crustaceans (of pH 7.2–7.4), which contained (in millimoles/liter): Na⁺ 470; K⁺, 8; Ca²⁺, 20; Mg²⁺, 10; Cl⁻, 536; HCO₃⁻, 2. An experimental cesium-containing solution was made up with the same constituents except for the omission of K⁺ and the addition of 8 mM Cs⁺. When the preparation was to be treated with Cs⁺, the standard physiological solution was drained from the bath and the preparation was rapidly exposed to three successive changes in the experimental solution. The preparation was then soaked in the experimental solution while EPSP's, ERSP's, and NTP's were recorded at various times after addition of the experimental solution.

Preparations of *Grapsus* were studied at room temperature (20°–22°C), whereas those of *Hyas* and *Pachygrapsus* were cooled by means of a Peltier battery to 14°C to prolong the useful life of the preparations. The development of the Cs⁺ effect is temperature dependent (Gainer et al., 1967) and changes in EPSP amplitude appeared more rapidly in *Grapsus* muscles than in *Hyas* or *Pachygrapsus* muscles. Apart from this, preparations of the three species responded similarly to Cs⁺ treatment.

Changes in postsynaptic membrane resistance of *Grapsus* muscle fibers were monitored by determining the input resistance, $R_{in}$ (Katz and Thesleff, 1957), before and after Cs⁺ treatment. For this purpose, rectangular hyperpolarizing current pulses of 200–800 ms duration were passed through an intracellular microelectrode at the tendon end of the fiber, while a second microelectrode was positioned within 50 μm of the first to record potential changes. $R_{in}$ was determined by measuring the slope of the voltage-current plot through the origin.

Quantal content of transmission was determined at individual synaptic regions from records of ERSP's, using standard procedures (reviewed in Hubbard et al., 1969, Chapter 3). At some synapses, the number of transmission failures was high, and it was possible to use ln (number of impulses/number of failures) as an approximate estimate of quantal content (cf. Atwood and Johnston, 1968; Johnson and Wernig, 1971). At other synapses there were no failures of transmission, and it was necessary to take the ratio of the mean evoked ERSP to the mean spontaneous ERSP
(see example in Fig. 6), as a measure of quantal content. Values so obtained were of use in comparing the relative extent of the Cs+ effect at different synapses.

RESULTS

Development of the Cs+ Effect

EPSP amplitudes were recorded in representative muscle fibers at various times after addition of the experimental solution. The motor axon was stimulated at 1 Hz for 5 s, and the amplitude of the last EPSP in the series was measured at each period of stimulation; thus, total stimulation was minimal.

For muscles of *Grapsus* (in which 12 fibers were studied in detail) the rate of increase in EPSP amplitude was usually greatest between 20 and 40 min after initial exposure to the experimental solution. This was true for both small and large EPSP's. Thereafter, the rate of increase slowed down, approaching zero in some fibers after 60–70 min (Fig. 1 A). However, considerable variation in rate of development of the Cs+ effect was observed both within and between preparations. This is not surprising in view of the known physiological differences among terminals of the same axon (Atwood and Bittner, 1971) and among muscles taken from different animals (Atwood et al., 1965).

Muscles of *Pachygrapsus* (15 fibers studied) and *Hyas* (6 fibers studied) treated at temperatures of 12°–15°C showed a more gradual increase in EPSP amplitude than muscles of *Grapsus* (Fig. 2).

The experimental solution produced an increase in muscle fiber input resistance (*R_in*), which attained its maximal value within a few minutes after initial exposure (Fig. 1 B). The time-course of onset and reversal of this change was much more rapid than seen for the EPSP (see also Gainer et al., 1967).

In *Grapsus* fibers, the mean value of *R_in* for 14 selected fibers increased from 724 ± 180 (SE) kΩ to 1200 ± 240 (SE) kΩ after Cs+ treatment. The increase, as judged by a *t* test for paired values, was highly significant (0.1% level).

The rate of increase in EPSP amplitude was augmented by low-frequency stimulation of the motor axon during exposure to the experimental solution (Fig. 2). Short trains of stimuli applied regularly after addition of Cs+ produced a more rapid increase in the initial growth of the EPSP than seen for comparable EPSP's in unstimulated preparations taken from the same animal. Setting the average frequency of stimulation to a value approaching 1 Hz produced more rapid initial growth of the EPSP than an average frequency of 0.4 Hz. However, at the higher frequency the rate of increase declined after 20–30 min and became more positive again when stimulation was stopped. This experiment was repeated three times, with similar results on each occasion. It is possible that defacilitation (described later) was responsible for changes in the rate of growth of the EPSP in stimulated preparations after 20–30 min.
Figure 1. (A), Time-course of EPSP growth in Cs⁺ for two fibers of a *Grapsus* stretcher muscle. EPSP amplitudes were measured periodically after 5 s. of stimulation at 1 Hz, and were plotted against time after addition of Cs⁺. (B), Time-course of change in input resistance ($R_{in}$) after addition of Cs⁺ in a representative *Grapsus* muscle fiber, for comparison with effects on the EPSP shown in (A). Cs⁺ was added at the point marked by the first arrow, and removed at the point marked by the second arrow.
Changes in Synaptic Electrical Events

The presynaptic effects of Cs+, which can be deduced from intracellular recordings of EPSP's, were studied more directly using extracellular electrodes to record synaptic potentials (ERSP's) and nerve terminal potentials (NTP's) at individual synaptic foci.

Extracellular recordings from individual synaptic regions showed clearly two changes which occurred after Cs+ treatment. First, ERSP's grew in size; this growth was brought about by an increase in quantal content of transmission associated with each nerve impulse, as will be shown in more detail later. Secondly, the externally recorded NTP increased in duration. These changes are illustrated in Fig. 3. It is likely that the first change (increase in quantal content) is related to the second change (increase in duration of NTP).

NTP's had two basic waveforms, as in the crayfish opener muscle (Dudel 1965 a, b). Some were small monophasic positive waveforms (Fig. 3 B), whereas others were larger, usually biphasic with a small positive phase followed by a prominent negative phase. (The example of Fig. 3 A shows such a waveform in which the small initial positive phase is barely seen.) The larger biphasic or predominately negative-going type of NTP was by far the more common and was almost always seen at synaptic regions of fibers with large poorly facilitating EPSP's as well as at many regions of fibers with small facilitating EPSP's. Monophasic positive waveforms were sometimes found at synaptic regions of fibers with facilitating EPSP's. Cesium treatment was
carried out while recording eight different NTP's of the first type, and three of the second type.

Both types of waveform were prolonged by Cs+ treatment, although their amplitudes were not increased. In Fig. 3, examples of both types of NTP are shown before and after Cs+ treatment. Duration of the negative-going NTP of the poorly facilitating synaptic region increased by 40% after Cs+ treatment, and amplitude decreased by 20% (Fig. 3 A). The small monophasic positive NTP at a highly facilitating synaptic region increased in duration by 35% after Cs+ treatment, with no change in amplitude (Fig. 3 B). Both rate of rise and rate of fall of the NTP's decreased after Cs+ treatment. Other experiments gave similar results. Mean increase in duration of all NTP's studied was 36%, and the mean decrease in amplitude was 15%. The former effect was statistically significant at the 1% level (t test), while the latter effect was significant only at the 10% level (t test).

Although it was not possible to impale the presynaptic nerve terminals with a microelectrode, the main trunk of the axon in the meropodite of Grapsus was penetrated and the axon spike recorded before and after Cs+ treatment. The amplitude of the spike increased, due to membrane hyperpolarization in the K+-free, Cs+-containing solution, but there was no marked increase in spike duration. After 125 min of Cs+ treatment, the time from onset to 50% decay of the spike was 0.20 ms, compared with 0.19 ms before Cs+ treatment. From onset to 90% decay, the figures were 0.8 ms before Cs+ treatment, and 1.1 ms after treatment.

These observations, taken together, indicate that prolongation of the nerve action potential occurs fairly soon in the fine synapse-bearing nerve terminals, and much later, if at all, in the main trunk of the axon.
Changes in Different EPSP's

Superficial fibers in stretcher muscles of *Pachygrapsus*, *Grapus*, and *Hyas* show diversity of EPSP types (Atwood, 1967a, b; Atwood and Bittner, 1971; Sherman and Atwood, 1972). In the present study it was found that different EPSP types responded differently to Cs⁺ treatment.

Some stretcher muscle fibers respond to a single impulse in the motor axon with a barely detectable EPSP which facilitates markedly during repetitive stimulation. Other fibers give a large EPSP of several millivolts which shows little facilitation. The range of facilitation properties and EPSP amplitudes is continuous. The ratio of EPSP amplitude at 10 Hz to EPSP amplitude at 1 Hz ($F_e$) ranges from 0.5 to 9 in *Grapus* fibers (Atwood and Bittner, 1971) and from 1 to 10 in *Hyas* fibers (Sherman and Atwood, 1972). This ratio is a useful index for classification of EPSP's.

EPSP's which initially were small and highly facilitating (high $F_e$) showed a much greater increase after Cs⁺ treatment than those which were initially large and poorly facilitating (low $F_e$). Differential growth of the two types of EPSP is shown in Figs. 4, 5, and 6. Although all fibers showed some increase in EPSP amplitude, in many of those with low $F_e$ EPSP's the increase was only 2–5 times the initial value after 1–2 h of exposure to Cs⁺. In contrast, all fibers with high $F_e$ EPSP's ($F_e \geq 3$) showed increases of 30–80 times the initial value after 1–2 h in Cs⁺. Large electrically excited membrane responses accompanied by vigorous twitches appeared in some of the latter fibers after Cs⁺ treatment (Fig. 4, B).

Changes in $R_{in}$ also occurred in all fibers, but were more pronounced in those with high $F_e$ EPSP's (Fig. 5). In fibers with low $F_e$ EPSP's the increase in $R_{in}$ often accounted for a substantial fraction of the increase in EPSP amplitude. However, fibers with EPSP's of initially high $F_e$ developed EPSP's of up to 80 times the initial value after Cs⁺ treatment, whereas the increase in $R_{in}$ was at most 5 times the initial value (Fig. 5). The results strongly suggest that a larger increase in quantal content of transmission occurs for EPSP's of initially high $F_e$.

During Cs⁺ treatment, EPSP's of all fibers tended to assume the characteristics of the initially low $F_e$ type, though in somewhat exaggerated form. The graphs of Fig. 6 show EPSP's sampled from *Grapus* fibers before and after $\frac{1}{2}$ h (A) and 1 h (B) in Cs⁺. Initial EPSP amplitude at 1 Hz is plotted against $F_e$ for each measurement. The dashed lines represent the best fit (determined by least squares) for a large sample of fibers from the same muscle obtained in previous work (Atwood and Bittner, 1971). Fibers selected for examination before and after Cs⁺ treatment had EPSP's which were representative of the entire normal spectrum of EPSP types. After $\frac{1}{2}$ h in Cs⁺ (Fig. 6 A), all EPSP's showed at least some increase in amplitude, and most
Figure 4. Growth of different EPSP's in Grapsus stretcher muscles after addition of Cs+. In A and B, the top records are of a poorly facilitating EPSP, and the bottom records of a highly facilitating EPSP, both in the same muscle. In A1 and B1, stimulation was at 1 Hz; in A2 and B2, stimulation was at 10 Hz. Records in A were taken without Cs+, while those in B were taken 35 min after addition of Cs+. In C and D, top records are of a highly facilitating EPSP and bottom records of a poorly facilitating EPSP in the same muscle. Stimulation was delivered at 1 Hz. Records in C were taken without Cs+ and those in D, 45 min after addition of Cs+. Electrically excited membrane responses are apparent in D (top). Time scale: 10 ms (A, B); 20 ms (C, D). Voltage scale: 4 mV (A bottom, B bottom, C top); 10 mV (A top, B top, C bottom); 20 mV(D).

showed a reduction of \( F_e \). In general, data points shifted upwards and to the left along the dashed line, indicating conversion of high \( F_e \) EPSP's to low \( F_e \) EPSP's. After 1 h in Cs+ (Fig. 6 B), most EPSP's were very large and poorly facilitating. Most data points fell in a cluster well above the distribution curve for normal fibers (dashed lines). On the basis of EPSP amplitude and apparent facilitation alone, there was no significant difference between initially high \( F_e \) and initially low \( F_e \) types. It should be noted, however, that postsynaptic differences between muscle fibers persisted, for the fibers with initially high \( F_e \)'s often produced large graded electrically excited membrane responses whereas fibers with initially low \( F_e \)'s did not. In addition, membrane time constants estimated from decay of the EPSP were shorter for fibers with initially high \( F_e \)'s even after treatment with Cs+ (see Fig. 4, and cf. Sherman and Atwood, 1972).

Extracellular recordings from individual synaptic regions showed clearly that quantal content of transmission increased after Cs+ treatment, and that the effect was considerably greater at synapses on fibers with facilitating EPSP's. The largest increases in quantal content were seen at synapses which
initially showed many failures of transmission at low frequencies of stimulation.

In Fig. 7, records of EPSP's and ERSP's from three different muscle fibers of different $F_e$ value are presented to show the development of the Cs$^+$ effect. Changes in the ERSP were greater for fibers in which the ERSP initially was small and of high $F_e$ (Fig. 7, A and B) than for fibers in which the EPSP was large and of low $F_e$ (Fig. 7 C).

Estimates of quantal content before and after Cs$^+$ treatment were obtained for a number of individual synaptic regions on fibers with different $F_e$'s.
Figure 6. Changes in EPSP amplitude and $F_e$ after 30 min (A) and 60 min (B) of Cs$^+$ treatment. The open circles show the condition of representative EPSP's before treatment and the filled circles show the same EPSP's after Cs$^+$ treatment. The dashed lines indicate the “best fit” for a large sample of EPSP's measured in *Grapsus* muscles without Cs$^+$. Usually it was difficult to hold the synaptic region for an hour during Cs$^+$ treatment, because single impulses often led to twitches in Cs$^+$-treated muscles. The number of successful experiments was therefore limited. Examples of calculations for fibers in which the synaptic region was held for at least 30
min are given in Table I. The increase in quantal content is much greater in fibers with high \( F_r \) values. However, the absolute value of quantal content remained higher at synaptic regions of fibers with low \( F_r \).

**Table I**

| Fiber | Initial \( F_r \) | EPSP at 1 Hz | Time of exposure | After Cs+ | Increase in \( m/V \) |
|-------|------------------|--------------|------------------|-----------|---------------------|
| 1     | 6.1              | 0.10         | 0.01             | 35        | 8.0 \( \times 0.93 \) |
| 2     | 4.3              | 0.35         | 0.07             | 50        | 14.2 \( \times 2.2 \) |
| 3     | 3.3              | 3.5          | 1.4              | 60        | 27.4 \( \times 16.8 \) |
| 4     | 1.6              | 6.0          | 4.1              | 50        | 22.1 \( \times 18.5 \) |
| 5     | 1.5              | 7.2          | 3.2              | 40        | 17.2 \( \times 11.2 \) |

**Figure 7.** Simultaneous records of EPSP's (top traces) and EPSP's (bottom traces) in *Grapsus* muscle fibers before and after Cs+ treatment. Records were made at 1 Hz stimulation, with two to four sweeps superimposed in each case. Each series (A, B, C) is from a single fiber at various times during Cs+ treatment. A1, B1, C1: before Cs+. A2, B2, C2: 10 min after addition of Cs+. A3, B3: 20 min after Cs+. A4, B4, C4: 35 min after Cs+. (Arrow in B4 shows a spontaneous ERSP). Time scale: 4 ms (A, B); 20 ms (C). Voltage scale: 4 mV (A, top); 10 mV (A2-A4 top, B1-B4 top, C1 top); 20 mV (C2-C3 top); 0.4 mV (A1-A4 bottom, B1 bottom); 1 mV (B2-B4 bottom, C1-C3 bottom).
Defacilitation

A further change in quantal output was observed in both ERSP's and EPSP's after 1–2 h of Cs+ treatment: namely, the development of defacilitation at low frequencies of stimulation. This feature is shown in the example of Fig. 8, for which records were taken from a fiber in which the EPSP at 1 Hz was initially 3.0 mV, with an F* of 3.6. The quantal content of the EPSP was initially 0.8; quantal unit mean size was 0.09 mV and remained fairly constant throughout the experiment, indicating good electrode placement. After 60 min of Cs+ treatment with only occasional stimulation, the ERSP amplitude was 26 mV and ERSP quantal content at one synaptic region had increased to 15 times its initial value, from 0.8 to 12 (Fig. 8 A). Constant stimulation at 1 Hz was then applied and mean ERSP amplitude for 20-s periods of stimulation plotted against time (Fig. 8 B). The ERSP amplitudes decreased as the stimulation progressed, but the rate of decline slowed down after 5 min of stimulation. When stimulation was stopped for a few minutes and then resumed, ERSP amplitude was initially greater than at the end of the initial 60 min of Cs+ treatment, but the subsequent decline with stimulation was more rapid. The sequence could be repeated many times. Details of the “tetanic rundown” in one sequence are shown in Fig. 8 C, in which each ERSP is plotted throughout 40 s of stimulation at 1 Hz. The early decline, and subsequent more gradual decrease in ERSP amplitudes, are clearly evident. Defacilitation after prolonged Cs+ treatment was evident at synaptic regions of both high F* and low F* types, but became more pronounced in the former.

The observations show that facilitating synapses can be converted to poorly facilitating or defacilitating synapses, as quantal content of transmission is increased far above normal by Cs+.

Discussion

The Mechanism of the Cs+ Effect

In the present study, we confirmed the Cs+-induced quantal content of synaptic transmission reported by Gainer et al (1967), using external electrodes located at individual synapses, and showed that the increase in transmitter output is coupled with lengthening of the presynaptic nerve terminal potential. Since transmitter release is known to be very sensitive to changes in duration of nerve terminal depolarization (Katz and Miledi, 1968, 1969), it is likely that most of the increase in quantal content of transmission is attributable to Cs+-induced lengthening of the presynaptic action potential.

The mechanism for the lengthening of the nerve terminal potential deserves consideration, especially since no increase in duration of the action potential in the main part of the axon was observed after prolonged exposure to the Cs+-containing solution. In squid giant axons, the surface membrane is quite im-
FIGURE 8. (A), Growth of ERSP's in a *Grapsus* muscle fiber after addition of Cs⁺ (large arrow). ERSP's were monitored by occasional brief periods of stimulation at 1 Hz (small arrows). Mean ERSP amplitude for each 20 s period of stimulation is shown by open circles. (B), Defacilitation of ERSP's in the same muscle fiber during constant stimulation at 1 Hz, starting 80 min after Cs⁺ treatment. The periods of stimulation are indicated by arrows. Mean ERSP amplitude for each 20 s period of stimulation is shown by open circles. Note recovery of ERSP amplitudes after a brief period of rest. (C), Rapid defacilitation ("tetanic rundown") of ERSP amplitude with stimulation at 1 Hz (same fiber as in A and B). Each response (filled circles) is plotted during a 40 s period of stimulation.
permeable to Cs⁺, even during the action potential (Pickard et al., 1964). When Cs⁺ is injected into the axon, the action potential is prolonged (Baker et al., 1962; Sjödin, 1967). Similar effects are seen in cat spinal motoneurones when Cs⁺ is injected (Araki et al., 1962). The prolongation is due to partial blockage of membrane K⁺ channels, thus slowing down the outward movement of K⁺ which terminates the action potential (Sjödin, 1967). A second effect, delay in sodium conductance inactivation, gives rise to a longer than normal inward sodium current (Adelman and Senft, 1967). If the effects of Cs⁺ on crustacean axons are similar to those on squid axons, prolongation of the action potential and of potential changes recorded extracellularly would be expected if Cs⁺ enters the axons.

If the above argument is accepted, the entry of Cs⁺ into the nerve terminal must be accounted for, as well as the differential effect at the synapse-bearing part of the axon. At least three possibilities can be offered:

(a) The membrane of the terminal may be different from that of the main part of the axon and may react to external Cs⁺ rather than to internal Cs⁺.

(b) Cs⁺ may slowly enter the surface membrane at the same rate everywhere by diffusion or by active transport (Sjödin and Beaugé, 1967), but since the terminals are smaller and their surface: volume ratio greater than elsewhere in the axon, the internal concentration of Cs⁺ would build up more rapidly in the terminals.
(c) The passage of Cs\(^+\) across the nerve terminal membrane may be more rapid than elsewhere.

The first possibility seems unlikely in view of the relatively slow time-course of development of the Cs\(^+\) effect. Evidence for the third possibility is provided by experiments in which trains of stimuli delivered at a low frequency to the axon during Cs\(^+\) treatment speeded up initial growth of the EPSP. Crustacean motor nerve terminals take up more extracellular material in vesicles during stimulation than at rest (Holtzman et al., 1971). Thus, it is possible that Cs\(^+\) enters the terminal more rapidly than elsewhere along the axon, and that it enters more rapidly during stimulation than at rest. Both the more rapid development of the Cs\(^+\) effect with stimulation and the differential effect at the terminal can be explained on this basis. The third possibility seems the most likely from these results.

Active transport of Cs\(^+\) into the terminals may also be involved (Sjodin and Beaugé, 1967). The present results do not permit a decision between active transport and vesicle turnover as the more important mechanism of Cs\(^+\) accumulation.

**Conversion of Synaptic Performance**

Mallart and Martin (1968) found that facilitation at frog neuromuscular synapses is inversely related to quantal content, when the latter is depressed to values less than 10 by high magnesium. Crustacean neuromuscular synapses (which normally have low quantal content of transmission even without magnesium treatment) show a similar trend, at least in singly motor-innervated leg muscles (Atwood and Bittner, 1971; Sherman and Atwood, 1972). The present study is the converse of Mallart and Martin's (1968), and has demonstrated that when quantal content is increased by Cs\(^+\), the facilitating synapses come to resemble more closely those which initially showed little facilitation. When treatment is prolonged, all synapses eventually show defacilitation, but the conversion in this direction is more striking at synapses which initially were highly facilitating.

Prolongation of the action potential in the nerve terminal would enhance excitation-secretion coupling at all synapses. However, the ability to release more than the normal amount of transmitter after Cs\(^+\) treatment is more strongly developed at high \(F_s\) synapses. After Cs\(^+\) treatment, when quantal content has increased considerably, defacilitation and "tetanic rundown" during stimulation at low frequency are seen, reminiscent of the behavior of curarized mammalian neuromuscular junctions (review in Hubbard et al., 1969). This suggests that the concept of an "immediately available store" of transmitter is applicable to crustacean as well as to frog and mammalian nerve terminals. On this basis, it can be argued that highly facilitating crustacean terminals normally release only a small fraction of the immediately available
store for each impulse. After Cs+ treatment the quantal output at some high $F_e$ synapses increases as much as 80–100 times (Table I). An output at this level would involve a larger fraction of the "immediately available store." Defacilitation may reflect the drain on the immediately available store under conditions of improved excitation-secretion coupling. Furthermore, it is likely that the difference in behavior of high $F_e$ synapses before Cs+ treatment is related to the fact that low $F_e$ synapses normally release a larger fraction of the "immediately available store" than high $F_e$ synapses. Consequently, more effective excitation-secretion coupling induced by Cs+ treatment produces a smaller increase in quantal content of transmission at low $F_e$ synapses.

These conclusions are consistent with data obtained by Johnson and Wernig (1971) in crayfish opener muscle. They found that synapses with higher quantal contents had a higher probability of release of available transmitter, and hence a smaller "reserve" of immediately available transmitter. After Cs+ treatment, the probability of release apparently increases proportionately more at synapses in which it is initially low and the "reserve" of immediately available transmitter declines accordingly.

The fact that quantal output at individual low $F_e$ synaptic regions remains larger than for high $F_e$ synaptic regions after Cs+ treatment suggests that an upper limit for transmitter release per impulse may be set by the area of synaptic contact, which in $Hyas$ at least is larger at low $F_e$ synapses (Sherman and Atwood, 1972). Since quantal content of individual synaptic release areas is correlated with size of synaptic contact, it is possible that low-frequency nerve impulses release transmitter which is proportional in amount to some function of the contact area. Since EPSP's of high $F_e$ fibers become approximately equal in size to those of low $F_e$ fibers after Cs+ treatment, it seems probable that the total release area on the fiber as a whole is not greatly different in the two types. Thus, the number of synapses is probably greater in high $F_e$ fibers than in low $F_e$ fibers, although the size of the individual synaptic contact is larger on the average for the latter type of fiber. In the crayfish, Bittner and Kennedy (1970) estimated equal numbers of synapses for the two types; but synaptic and muscle fiber differences are more dramatic in crabs (Atwood and Bittner, 1971) and one might therefore expect greater variation in synapse morphology and in the number of synapses per muscle fiber.

The Basis for Differences in Transmitter Release

Although lengthening of the nerve terminal potential occurred to similar extents at both high $F_e$ and low $F_e$ synapses, the increase in quantal content of transmission was much greater at high $F_e$ synapses, even though the total output of transmitter per stimulus remained higher for low $F_e$ synapses. The question arises: what factors determine the probability of transmitter release at different terminals of the same axon?
We can visualize at least two possibilities. The first is that the action potential invades some terminals completely and others incompletely (Dudel, 1963, 1965 a, b; Sherman and Atwood, 1972). The second is that the action potential invades all terminals completely and that probability of release at low frequencies is set by some function of synaptic contact area (Sherman and Atwood, 1972), and/or by differences in terminal membrane calcium conductance.

Although Dudel and Kuffier (1961) failed to elicit antidromic action potentials by stimulating nerve terminals at synaptic regions, Dr. R. S. Zucker (personal communication) has succeeded in doing this. Dudel (1965 a) interprets the small monophasic positive NTP of certain synaptic regions as resulting from a decrementally conducted spike, whereas Katz and Miledi (1965) have shown that similar records are obtained from closed endings of nerve terminals which support a fully propagated spike. Thus, the proposition that some terminals are incompletely invaded by the action potential is debatable at present.

The first possibility (incomplete invasion of some terminals) could account for the initially low quantal content (and low probability of release) at certain synapses, along with the greater effect of Cs+ on quantal content at the same synapses. If membrane K+ conductance is partially blocked by Cs+, as in the squid giant axon, membrane resistance and length constants of the terminals would increase. In terminals not fully invaded by the action potential electrotonic conduction would become more effective after Cs+ treatment. Thus, proportionately greater electrical activation of excitation-secretion coupling would occur at terminals in which some synapses were located in a region of electrotonic conduction.

However, the differential effects of Cs+ can also be explained fairly well even if the second situation given above (regenerative impulse conduction in all terminals) should actually apply. Since the probability of release of immediately available transmitter is much higher at synapses with initially high quantal content (Johnson and Wernig, 1971) it is likely that any factor which tends to increase the probability of release will have proportionately less effect at these synapses than at those with initially low probability of release. The probability of release in normal untreated synapses may be established in part by anatomical features such as the amount of synaptic release area available for each quantum of immediately available transmitter. Thus, on present evidence, it is not possible to distinguish with certainty between the above possibilities.

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