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EXTRACELLULAR POTASSIUM AND TRANSMITTER RELEASE AT THE GIANT SYNAPSE OF SQUID

BY S. D. ERULKAR AND F. F. WEIGHT

From the Department of Pharmacology, University of Pennsylvania Medical School, Philadelphia, Pennsylvania 19174, the Laboratory of Neuropharmacology, NIMH, St Elizabeths Hospital, Washington, D.C. 20032 and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543, U.S.A.

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SUMMARY

1. The effects of changes in extracellular K concentration, $[K]_o$, on synaptic transmission were studied at the squid giant synapse with intracellular recording from the presynaptic terminal and post-synaptic axon.

2. The amplitudes of both the presynaptic spike and the e.p.s.p. varied inversely with $[K]_o$. On the average, a 10 mV change in spike height was accompanied by a 3·1 mV change in e.p.s.p. amplitude.

3. The amplitude of the presynaptic spike after-hyperpolarization (AH) varied inversely with $[K]_o$. On the average, increasing $[K]_o$ resulted in a 20% change in e.p.s.p. amplitude per mV change in presynaptic spike AH.

4. Repetitive antidromic stimulation of the post-synaptic giant axon resulted in an exponential decline in the post-synaptic spike AH, a depolarization of the presynaptic membrane potential and a reduction in the AHs of presynaptic spikes. This suggests that the K which accumulates in the extracellular spaces around the post-synaptic axon also affects the presynaptic terminal.

5. Repetitive antidromic stimulation of the post-synaptic axon resulted in a reduction in the amplitude of e.p.s.p.s. elicted by stimulation of the presynaptic axon. The reduction in e.p.s.p. amplitude relative to the change in presynaptic spike AH was quantitatively close to the change produced by increasing $[K]_o$, suggesting that the reduction in e.p.s.p. amplitude is due to the accumulation of extracellular K at the presynaptic terminal.

6. Repetitive stimulation of the presynaptic axon reduced the amplitudes of the e.p.s.p. and the presynaptic spike AH. On the average, a 1 mV change in presynaptic spike AH was accompanied by a 204% change in

e.p.s.p. amplitude, suggesting that K accumulation may only contribute to a small extent, under these conditions, to the depression of transmitter release.

INTRODUCTION

The action potential of the squid giant axon is followed by a brief period of hyperpolarization which has been shown to be due to an increased permeability of the membrane to K ions (Hodgkin & Huxley, 1952). Frankenhaeuser & Hodgkin (1956) found that a train of impulses in the squid axon resulted in a depolarization of the membrane and a reduction in the spike after-hyperpolarization ('positive phase') that was matched by increasing extracellular K concentration, $[K]_o$. They concluded that the K ions liberated by nerve impulses do not diffuse freely but accumulate in an extracellular space on the order of 300 Å wide.

Subsequent investigations have shown that K accumulates extracellularly during repetitive action potentials in other nervous tissues. For example, Orkand, Nicholls & Kuffler (1966) found that repetitive firing of amphibian nerve fibres produced a depolarization of neighbouring glial cells that was attributed to an accumulation of extracellular K. In the central nervous system of the leech, Baylor & Nicholls (1969) used the action potential after-hyperpolarization ('undershoot') as an index of extracellular K concentration and found that with trains of impulses, K could build up to about twice the normal concentration. Several recent investigations in the mammalian central nervous system have demonstrated that an extracellular accumulation of K results from repetitive neural activity (Krnjević & Morris, 1972; Vyklický, Syková, Kříž & Ujec, 1972; Singer & Lux, 1973; Kříž, Syková, Ujec & Vyklický, 1974; Somjen & Lothman, 1974; ten Bruggencate, Lux & Liebl, 1974; Lewis & Schuette, 1975; Vyklický, Syková & Kříž, 1975).

Although K is known to accumulate with repetitive nervous activity, there is little information on the effects of such accumulation on synaptic transmission. The present investigation derived from the observation that during repetitive stimulation of the presynaptic axon at the squid giant synapse, we observed a reduction in the amplitude of both the e.p.s.p. and the presynaptic spike after-hyperpolarization (AH). This raised the question whether an accumulation of K in the extracellular space might contribute to the depression of transmitter release. Consistent with this possibility, we found that increasing $[K]_o$ reduced the amplitude of the e.p.s.p. Our results suggest, however, that the accumulation of K from repetitive action potentials in the presynaptic terminal may account for only a small portion of the depression of transmitter release. On the other hand, repetitive antidromic stimulation of the post-synaptic axon

depressed the release of transmitter and we attribute this effect to the accumulation of K in the extracellular space around the presynaptic terminal.

Some of these results have been reported in preliminary form (Erulkar & Weight, 1974; Weight & Erulkar, 1976*b*).

METHODS

The experiments were conducted on the giant synapse of the squid, *Loligo pealii*, at the Marine Biological Laboratory in Woods Hole. The procedure for dissecting the stellate ganglion was similar to that described by Bullock (1948). The ganglion was placed in a small lucite chamber (1 cm diameter) and constantly perfused with oxygenated sea water cooled by a thermo-electric device. The pre- and post-ganglionic nerves extended into side chambers containing sea water, which were sealed from the main chamber by petroleum jelly. Suction electrodes were used to stimulate the pre- and post-ganglionic nerves. Artificial sea water (ASW) of the following composition was used (mM): NaCl 424; KCl 9, $CaCl_2$ 9, $MgCl_2$ 23, $MgSO_4$ 25, $NaHCO_3$ 2. K concentration was changed by addition to or subtracting from the ASW, without adjustment of the other ions. Due to the slow diffusion equilibration of the ganglion, it was usually not possible to test more than one or two changes of potassium on a preparation. Therefore, in order to compare the effects of different extracellular potassium concentrations, the results from different preparations have been averaged.

Post-synaptic spike initiation was prevented by cooling the preparation sufficiently to reduce the e.p.s.p. below threshold (Weight & Erulkar, 1976*a*). The preparation was maintained at a constant temperature ($\pm 0{\cdot}1°$ C) in each experiment; the range of temperatures in different experiments was from 6 to 12° C.

The recording micro-electrodes were filled with 3 M-KCl. One micro-electrode was placed in the post-synaptic giant axon close to the synapse, the other electrode in the mid-region of the presynaptic terminal. The electrodes used for intracellular recording in the presynaptic terminal had resistances of 10–15 MΩ and those used for the post-synaptic axon, 3–5 MΩ. In initial experiments, a silver–silver chloride pellet was used as ground reference: however, spurious d.c. potentials were observed during ion changes so that, in later experiments, intracellular potentials were recorded differentially with reference to a 1–2 MΩ-KCl micro-electrode in the bath. Signals from the micro-electrodes were led to unity gain followers (Picometric) and thence to Tektronix 3A9 amplifiers; all recording was D.C. Two oscilloscopes were used: one at high gain (2 mV/cm) for accurate measurements of the amplitude of the e.p.s.p. and the presynaptic spike AH. The second oscilloscope was used at low gain (10 mV/cm) to monitor pre- and post-synaptic membrane potential and spike amplitude. The traces on the two oscilloscopes were photographed on separate cameras. Measurements were made from enlarged photographic images on an analog to digital converter (Oscar, Model F). Calculations were performed on a PDP 12 computer, with programmes kindly written by Dr George Siggins. Least-squares analyis of exponential functions were kindly performed by Dr Martin Pring on a PDP 10 computer.

RESULTS

The effects of changing the external potassium concentration, $[K]_o$

Fig. 1 shows that changes in $[K]_o$ influenced the amplitude of the e.p.s.p. An increase in $[K]_o$ from 9 mM in the ASW to steady levels of 10·5

and 12 mM, progressively decreased the amplitude of the e.p.s.p. (Fig. 1*A*); conversely, decreasing $[K]_o$ to 6 and 3 mM progressively increased the amplitude of the e.p.s.p. (Fig. 1*B*). Within the range of $[K]_o$ tested (3–12 mM), the relationship between $[K]_o$ and the percentage change in e.p.s.p. amplitude was linear. The slope of the line indicated that a 24·5 % change

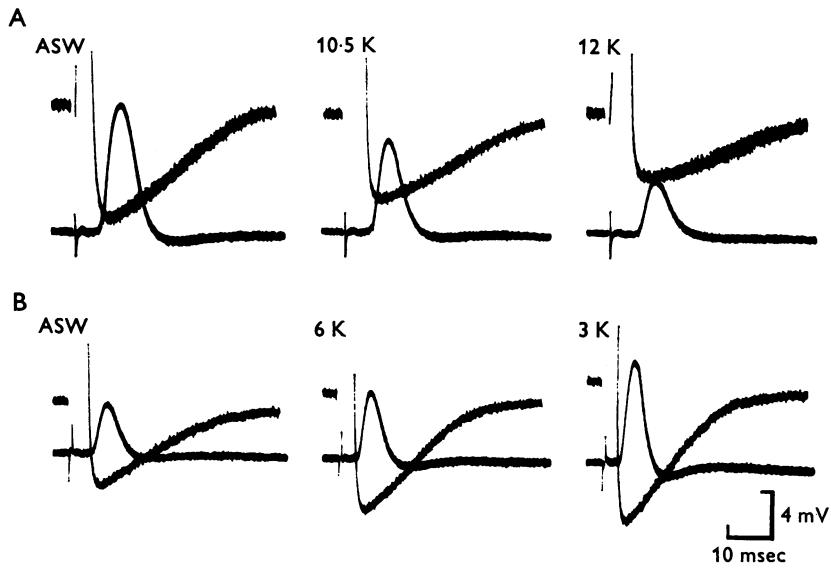

Fig. 1. Effect of external potassium concentration on the e.p.s.p. and presynaptic spike AH. A, responses in ASW (control) and in ASW with external K concentrations of 10·5 and 12 mM. B, responses in ASW (control) and in ASW with external K concentrations of 6 and 3 mM. Experiments in B from a different preparation than in A. In experiments in which $[K]_o$ was reduced, it was necessary to start with a small e.p.s.p. in ASW to prevent the firing of action potentials generated by the large e.p.s.p.s which occured in low $[K]_o$. Beams were adjusted to keep AH and e.p.s.p. on the oscilloscope so that the traces do not show changes in membrane potential (recorded on a separate oscilloscope).

in e.p.s.p. amplitude occurred per mM change in $[K]_o$. This reflects the extent of the participation of external K in transmitter release without involvement of other factors (such as transmitter depletion) associated with nerve activity at the terminal.

Changes in $[K]_o$ in the range used produced only small changes in resting membrane potential (approximately 0·2–0·4 mV per mM change in $[K]_o$) (cf. Hodgkin & Katz, 1949; Frankenhaeuser & Hodgkin, 1956). From previous experiments (Hagiwara & Tasaki, 1958; Miledi, 1969; Llinás, Joyner & Nicholson, 1974) only a small change in e.p.s.p. amplitude would be expected to result from the effect of changing $[K]_o$ on post-synaptic membrane potential.

Fig. 1 also illustrates the effect of $[K]_o$ on the presynaptic spike AH. Since K conductance is high during the AH, the membrane behaves as a potassium electrode and the amplitude of the AH can be taken as an index of $[K]_o$ (Hodgkin & Katz, 1949; Hodgkin & Keynes, 1955; Frankenhaeuser & Hodgkin, 1956). The ratio of percentage change in e.p.s.p. to change in AH can therefore be used to assess whether a change of transmitter release results from the accumulation of extracellular K. This relationship is shown in Fig. 2. The slope of the curve for an increase in $[K]_o$ in Fig. 2 gives a 20 % change in e.p.s.p. amplitude per mV change in the presynaptic spike AH.

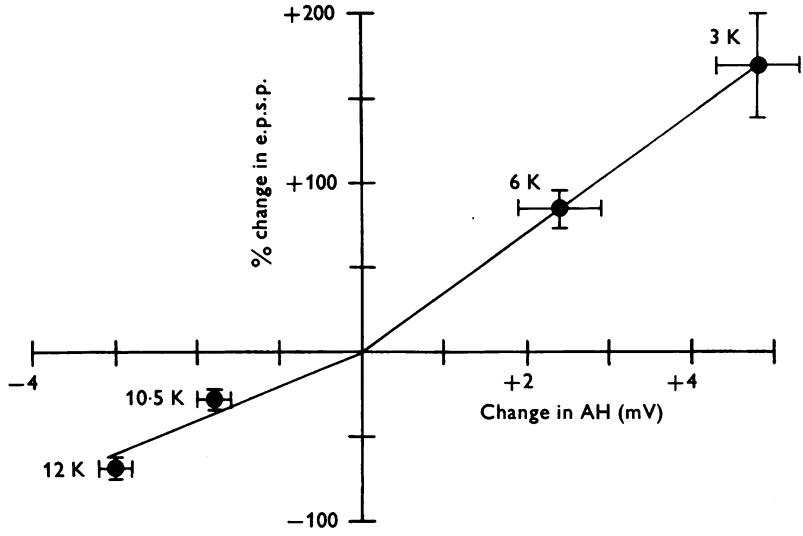

Fig. 2. Relation between the change in the presynaptic spike AH (mV) and the % change in e.p.s.p. amplitude in different external K concentrations. Each point represents the mean ± S.E. of five to nine experiments.

One mechanism that could account for the effect of $[K]_o$ on e.p.s.p. amplitude is that it may be mediated by changes in the amplitude of the presynaptic spike (Hodgkin & Katz, 1949; Frankenhaeuser & Hodgkin, 1956). Such presynaptic changes were observed, as shown in Fig. 3*B*. In Fig. 3*A* changes in e.p.s.p. amplitude are plotted against changes in presynaptic spike height. The slope of the relationship shows a 3·1 mV change in e.p.s.p. amplitude per 10 mV change in spike height. The effect of $[K]_o$ on the amplitude of the spike in the presynaptic terminal of the squid stellate ganglion is greater than the effect on the spike in the giant axon previously reported by Hodgkin & Katz (1949) and Frankenhaeuser & Hodgkin (1956). It remains to be determined whether this quantitative

difference is due to the low temperature of the present experiments or to a greater sensitivity of the terminal action potential to $[K]_o$.

Effects of repetitive post-synaptic action potentials

During repetitive antidromic stimulation of the post-synaptic giant axon at 25 Hz, the spike AH declined exponentially with an average time constant of 8·4 ± 1·5 (S.D. of mean; $n = 7$) sec. This presumably corresponds to the slow decline in AH observed previously by Frankenhaeuser & Hodgkin (1956), following a more rapid decay with a time constant of 30–100 msec.

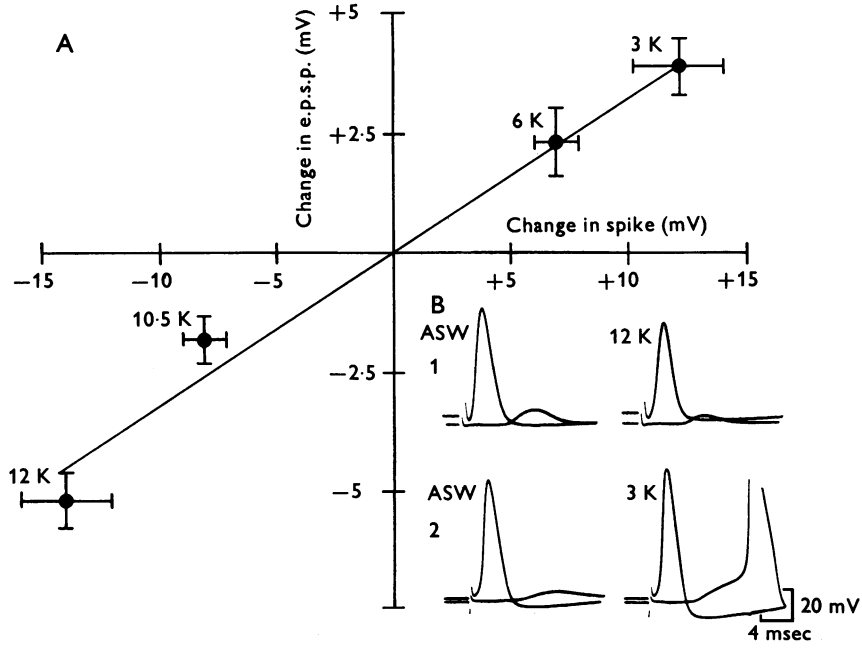

Fig. 3. A, relation between the change in presynaptic spike amplitude and the change in e.p.s.p. amplitude in different external K concentrations. Each point represents the mean ± S.E. of five to nine experiments. B (inset), Effect of external K concentration on presynaptic spike. 1 (top), response in ASW (control) and in ASW with external K concentration of 12 mM. 2 (bottom). Response in ASW (control) and in ASW with external potassium concentration of 3 mM. 2 from a different preparation than 1.

In view of the observations that increasing $[K]_o$ decreases transmitter release, the question arose whether the K accumulating around the post-synaptic axon as the result of repetitive antidromic action potentials could diffuse across the synaptic cleft and around the presynaptic terminal and thus affect the release of transmitter. To test this the following experimental paradigm was followed. The presynaptic axon was stimulated every

10 sec for several minutes which resulted in a steady state of the amplitudes of the e.p.s.p. and the presynaptic spike AH. The post-synaptic axon was then stimulated antidromically at 25 Hz for 40 sec. Stimulation of the presynaptic axon was continued every 10 sec during and for several minutes after the antidromic train. Fig. 4 illustrates one such experiment. The repetitive antidromic stimulation reduced the post-synaptic spike AH by 3–8 mV and there was a concomitant reduction in the presynaptic spike

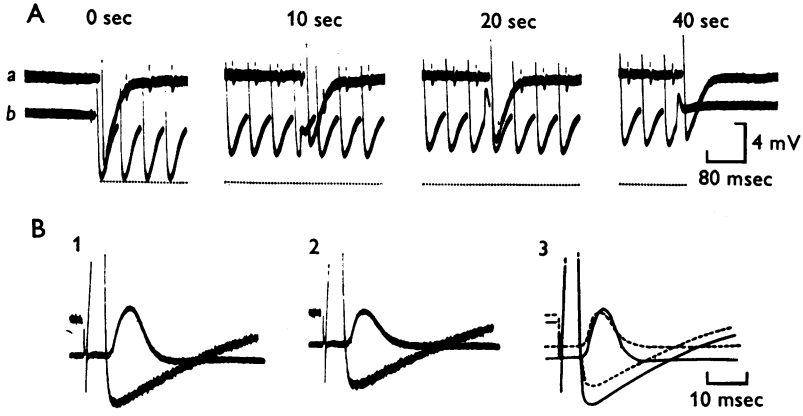

Fig. 4. Effects of repetitive antidromic stimulation of the post-synaptic axon. A, records of responses to antidromic stimulation as in Fig. 5. Specimen records show the train at the beginning (0 sec) and after 10, 20 and 40 sec. The presynaptic axon was stimulated every 10 sec before, during and following the antidromic train. The presynaptic spike AH can be seen in trace *a* and the post-synaptic spike AH in trace *b* of each specimen record. The interrupted line indicates the potential of the first post-synaptic spike AH, **B**, presynaptic spike AH and e.p.s.p. 10 sec before (1) and 10 sec after (2) the antidromic train of impulses in the experiment shown in *A*. In the tracing on the right (3), the two records are superimposed for comparison.

AH of about 60–90 % of that of the post-synaptic AH (Fig. 4*A*), at different synapses. In five such experiments, when the ratio of change in presynaptic spike AH to change in post-synaptic spike AH was compared for different stimulus durations, the ratio for a given synapse varied by less than 0·1. These data indicate that the K that accumulates in extracellular spaces around the post-synaptic axon also affects the presynaptic terminal.

The effect of repetitive antidromic stimulation of the post-synaptic axon on the release of synaptic transmitter is illustrated in Fig. 4*B*. It can be seen that after antidromic stimulation, in addition to a reduction in the presynaptic membrane potential and presynaptic spike AH, the amplitude of the e.p.s.p. was also reduced. The average ratio of % change

of e.p.s.p. amplitude to change of AH was 27·6 ± 4·3 (S.E. of mean; $n = 31$), compared to a ratio of 20 for increasing $[K]_o$. This suggests that the depression of e.p.s.p. amplitude produced by repetitive antidromic stimulation is due to the accumulation of K.

Effects of repetitive presynaptic action potentials

In view of the preceding data that increasing $[K]_o$ reduced the e.p.s.p., the question was raised whether K accumulation around the presynaptic terminal during a train of presynaptic impulses could contribute, at least partially, to the reduction in e.p.s.p. amplitude (cf. Kusano & Landau, 1975). Two approaches were taken to answer this question. Firstly, the rates of change of e.p.s.p. and presynaptic spike AH amplitudes were com-

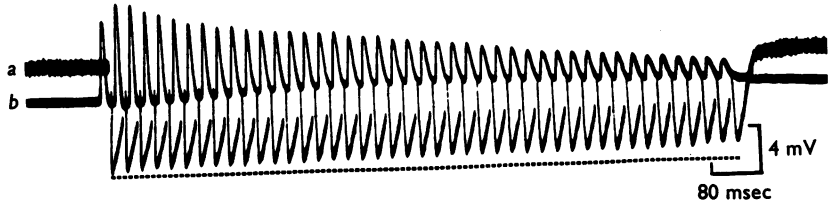

Fig. 5. Responses to repetitive stimulation of the presynaptic axon. Frequency of stimulation, 43 Hz. Trace *a* shows the recording from the presynaptic terminal and illustrates the change in amplitude of the presynaptic spike AH. The interrupted line indicates the potential of the first presynaptic spike AH. Trace *b* shows the recording from the post-synaptic axon and illustrates the change in e.p.s.p. size. The traces were recorded on moving film with standing spots on the oscilloscope; due to the lack of vertical alignment of the spots, the presynaptic spike AH follows the corresponding e.p.s.p. The high gain used for this recording precluded visualization of the full amplitude of the presynaptic spike on the oscilloscope.

pared during a train of presynaptic action potentials. The e.p.s.p. declined exponentially with an average time constant of 0·45 ± 0·09 (S.D.; $n = 5$) sec, compared with greater than 5 sec for the change in the presynaptic spike AH. Secondly, from the previous results with increasing $[K]_o$ (Fig. 2) one would expect about a 20 % decrease in a e.p.s.p. amplitude per mV decrease in AH. In experiments such as that shown in Fig. 5, the e.p.s.p. decreased an average of 204 ± 26 % (S.E. of mean; $n = 16$) per mV decrease in AH. This indicates that most of the reduction in e.p.s.p. amplitude produced by presynaptic stimulation cannot be attributed to accumulation of extracellular K. This interpretation is strengthened by the observation that during the presynaptic stimulation there was either no change or only a small reduction (< 2 mV) in the presynaptic spike amplitude, even when the e.p.s.p. had been abolished.

DISCUSSION

The results of the present experiments indicate that the change in e.p.s.p. amplitude produced by changing $[K]_o$ can be accounted for by the change in presynaptic spike height. The relationship between presynaptic spike height and e.p.s.p. amplitude agrees essentially with data from experiments by others on the same preparation using different techniques to alter the amplitude of the presynaptic spike (Hagiwara & Tasaki, 1958; Takeuchi & Takeuchi, 1962; Miledi & Slater, 1966; Katz & Miledi, 1967).

The alterations in the release of transmitter by repetitive antidromic stimulation of the post-synaptic axon are presumably due to the accumulation of K around the presynaptic terminal. It is unlikely that the antidromic stimulation activates some pathway that synapses on the terminal, for no such pathway has been shown to exist (Young, 1939; Hama, 1962; Castejon & Villegas, 1964; Young, 1973) and no synaptic potentials were recorded in the terminal associated with the antidromic stimulation. On the other hand, the reduction in presynaptic spike AH is evidence for K accumulation.

Whether a similar modulation of transmitter release by repetitive post-synaptic impulses occurs at other synapses is unclear (Singer & Lux, 1973; Kriz *et al.* 1974). If such a mechanism does exist, it could function as a negative feed-back system which could affect the input of a number of synaptic pathways converging on a neurone and in this way play an integrative role in the nervous system (cf. Weight & Erulkar, 1976*b*).

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