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CHANGES IN EXTRACELLULAR POTASSIUM CONCENTRATION PRODUCED BY NEURONAL ACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE LEECH

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SUMMARY

1. Experiments were made on single neurones and glial cells in the central nervous system of the leech to study the accumulation of K that occurs in the extracellular spaces around neurones as a result of impulse activity.

2. The resting potential of a neurone is too insensitive to be used for the estimation of small changes in K concentration. The undershoot of the action potential, however, provided a reliable indicator of the K accumulation that occurs around a neurone during activity.

3. After a single impluse the amplitude of the undershoot of a second action potential was decreased; the effect corresponded to a peak increase in K concentration of about 0·8 mM/l. immediately after the spike and declined exponentially with a time constant of about 100 msec. With trains of impulses the K concentration increased exponentially, again with a time constant of about 100 msec. The final value of K depended on the frequency and could build up to about double the normal concentration of 4 mM/l.

4. The build-up of K was markedly reduced when the extracellular space surrounding a neurone was enlarged by removing its glial investment.

5. Synchronous, repetitive activation of groups of neurones caused a slow depolarization of neighbouring glial cells in the C.N.S. of the leech, similar to that observed in amphibia and mammals. The change in glial membrane potential was also used to estimate the changes in K concentration and these values agreed with measurements derived from the undershoot.

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6. Increases of K concentration in the bathing fluid of the same order as those caused by neural firing markedly affected the frequency of 'spontaneous' neuronal discharges and synaptic potentials occurring within certain neurones in the C.N.S.

7. The possible effects of physiologically occurring increases of K concentration on integration are discussed.

INTRODUCTION

When a neurone gives an action potential, the membrane repolarizes from the peak of the impulse because K ions carry positive charge out of the cell as they move down their electrochemical gradient. The quantity of K which moves out across 1 cm^2 of membrane during one impulse has been estimated to be about 4×10^{-12} M in squid axons (Shanes, 1954; Keynes, 1951; Keynes & Lewis, 1951) and about 10^{-12} M in mammalian unmyelinated fibres (Keynes & Ritchie, 1965). Two lines of physiological evidence indicate that the small amount of liberated K does not immediately diffuse away from the neurone; instead, it appears to accumulate in a restricted space just outside the membrane, where it gives rise to a temporary increase in K concentration. First, Frankenhaeuser & Hodgkin (1956) found in squid axons that the membrane potential underwent changes after impulses which could be quantitatively explained by a rise in the K concentration in a cleft about 300 Å wide adjacent to the membrane. Secondly, Orkand, Nicholls & Kuffler (1966) observed that impulses in amphibian optic nerves produced a depolarization in the associated glial cells which could be attributed to an increase in the extracellular K.

Numerous electronmicroscopical studies of both the peripheral and central nervous systems (see, for example, Horstmann & Meves, 1959) have shown that the clefts making up the extracellular spaces are narrow enough (about 150 Å) to allow accumulation of K. The membranes of neurones are often in close apposition to those of glial cells which may enclose a number of neural processes within a common compartment.

The close proximity of these membranes raises the possibility that K liberated by one neurone might not only depolarize the glia but also influence the behaviour of a neighbouring neurone. In this paper we have measured the K accumulation that occurs in the vicinity of an individual neurone within the C.N.S. It will be shown that a train of impulses causes the K concentration to rise by 3–4 mM/l. and that changes of this order can have consequences on integrative mechanisms. Brief reports of some of these findings have already been published (Nicholls & Baylor, 1968*a*, *b*).

METHODS

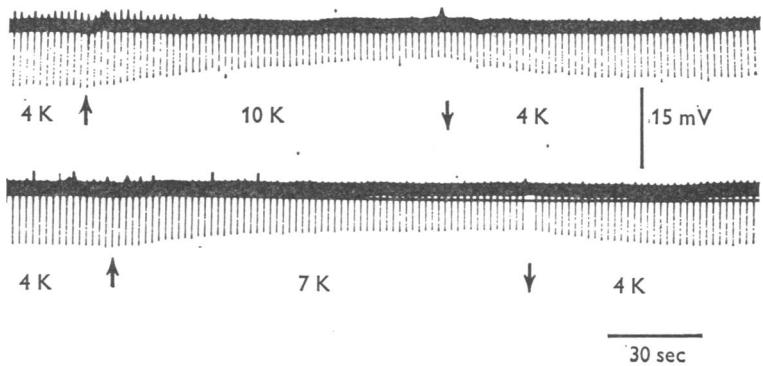
Experiments were made at room temperature on isolated ganglia from medicinal leeches using techniques which have been fully described elsewhere (Nicholls & Baylor, 1968*c*). The preparation was immersed in a stream of flowing Ringer solution which entered the bath from elevated reservoirs and was continuously removed by a suction tube. The composition of the fluid in the bath could be changed within about 10 sec by turning a tap. The normal Ringer fluid contained (mM/l.): Na^+ 115, K^+ 4, Ca^{2+} 1·8, Cl^- 122, Tris maleate buffered to pH 7·4 with NaOH 10 and glucose 11. Solutions containing higher concentrations of K were made by substituting KCl for NaCl. Recordings were made between a micropipette filled with 3 M-KCl and a reference electrode made of polyethylene tubing pulled to a fine tip and filled with 3 M-KCl in agar. Connexions with the electrodes were made by chlorided silver wires. Electrode pairs were selected to give less than 2 mV potential change when solutions were changed. The stimuli used in most cases were electrical shocks delivered to a root of the ganglion with a suction electrode. The glial covering of neurones was removed by the technique described by Kuffler & Potter (1964). For descriptions of the fine structure and morphology of leech ganglia see Coggeshall & Fawcett (1964); the sensory cells responding to touch (T), pressure (P) and noxious (N) mechanical stimuli applied to the skin are described in Nicholls & Baylor (1968*c*) and briefly in the third paper in this series (Baylor & Nicholls, 1969*b*).

RESULTS

Effect of increased K concentration on the undershoot of the action potential. The principle of our experiments has been to use the membrane properties of a neurone to estimate the accumulation of K in its vicinity following impulse activity. Since the resting potential of a quiescent neurone is relatively insensitive, the undershoot of the action potential was chosen as an indicator for changes in K concentration (see Frankenhaeuser & Hodgkin, 1956); at this point in the action potential cycle the relative K conductance of the neuronal membrane is higher than at rest or at the peak of the action potential (Hodgkin & Huxley, 1952). Curves relating the amplitude of the undershoot to external K were constructed by varying the K concentration in the bathing fluid while stimulating a cell at 0·5–1/sec. We used sensory neurones responding to pressure (P cells) for most of the experiments since their action potentials have large undershoots (see Nicholls & Baylor, 1968*c*) and continue to invade the soma during prolonged trains. Quantitative studies were not made on sensory neurones responding to touch or noxious stimuli but it was clear that they behaved in a similar manner to P cells with respect to changes in external K and repetitive activity.

Records from a typical experiment are shown at the top of Fig. 1. Action potentials were elicited by stimulating the neurone's axon in the dorsal branch of the posterior root. At the arrow, the K concentration in the Ringer fluid flowing into the bath was increased from 4 to 10 mM/l. The undershoots of the action potentials underwent a progressive decline as the composition of the solution surrounding the cell changed, while at the same time the resting potential showed little change. Below this record

is another test made on the same cell in which the K concentration was changed from 4 to 7 mM/l. The effect on the undershoot was similar but less marked. At the bottom of the figure is a calibration curve showing the pooled results of similar experiments on sixteen cells. With K increases of less than 3 mM/l. the relation between undershoot amplitude and K change was approximately linear, although at higher K concentrations the slope progressively decreased.



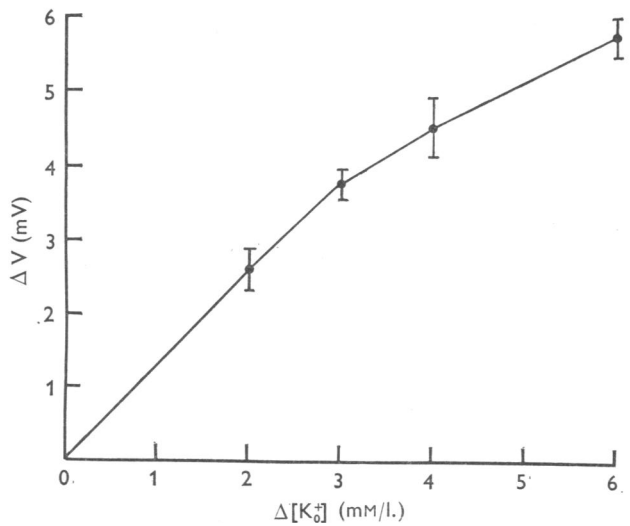


Fig. 1. Effect of varying external K on the undershoots of action potentials. Above: high gain recordings of undershoots (upstrokes of action potentials not seen) in a pressure cell activated by stimulation of its axon in the posterior root. At arrows the K concentration in the Ringer fluid was changed to 10 mM/l. and 7 mM/l. from the normal value of 4 mM/l. Below: change in size of undershoot plotted against the increase in external K concentration. Each point mean ± S.E. of measurements made on at least six cells.

Knowing the relation between the amplitude of the undershoot and the external K concentration, it was possible to match undershoot changes produced by activity with equivalent K concentrations.

Undershoot decrements produced by activity. During trains of action potentials, leech sensory neurones showed a decrement in the undershoot of the impulse similar to that observed in squid axons by Frankenhaeuser & Hodgkin (1956). The final decrement was more pronounced with higher

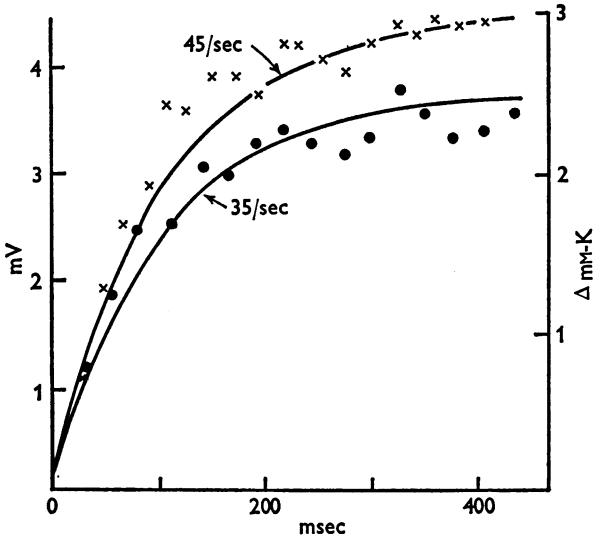


Fig. 2. Time course of the decrease in amplitude of undershoots during trains of impulses at 35/sec and 45/sec. The ordinate on the left is the amplitude of the undershoot in mV and the abscissa is the time in msec after the first impulse of the train. See Fig. 4 for the type of record used for these measurements. The ordinate at the right converts undershoot changes to increases in the K concentration around the cell; it was constructed from a calibration curve for this cell made as described in the text. The fact that the time constant of the undershoot decrement is similar at the two frequencies suggests that the build-up of K is occurring exponentially (see also Fig. 3). Continuous curves are drawn according to the equation in the text.

frequencies of stimulation. The effect in leech neurones, as in squid axons, grew exponentially with a time constant which, within limits, was independent of frequency. Figure 2 shows an example from an experiment in which a pressure neurone was activated by electrical stimulation of its endings through a suction electrode applied to the skin of its receptive field (Nicholls & Baylor, 1968*c*). The points show the decline in the size of the undershoot at frequencies of 35/sec and 45/sec (see Fig. 4 for the type of record from which such measurements were made). The smooth curves were drawn according to the equation $y = A\{1-\exp(-t/T)\}$ (Franken-

haeuser & Hodgkin, 1956), where y is the change in size of undershoot, A is the final value of the decrement at the particular frequency of stimulation, t is the time after the first impulse, and T is a time constant chosen to fit the points, being 100 msec for both curves. The K scale on the right side of the graph was constructed from a calibration curve measured in the same cell, as described in the previous section. At 45/sec the undershoot changed as if a K concentration of 3 mM/l. had built up around the cell 400 msec after the beginning of the train. This does not represent the maximum frequency or K build-up that can occur. P cells can fire at over 60/sec with stimulation of the skin, resulting in a K increase of 4 mM/l. or more.

The increase in K concentration produced by a single impulse was estimated from the decrement in the undershoot of the second action potential in twin shock experiments of the type shown in Fig. 3. On the left are superimposed recordings of a series of trials in which a second impulse followed the first at various intervals. The amplitude of the second undershoot showed a progressive decrease in size as the inter-shock interval was shortened. On the right side of the Fig. the amplitude of the second undershoot has been plotted as a function of the intershock interval. The points are the measured values, while the smooth curve is an exponential of the type described above. In this experiment the time constant for the decline in K concentration (or the recovery of the undershoot) was 100 msec, in agreement with the value obtained for the build-up of K during trains in the cell shown in Fig. 2. On the right side of the graph the decrease of successive undershoots has been converted to K concentrations outside the neurone as in Figs. 1 and 2; it shows that at the shortest interval of 40 msec the K concentration was greater by 0·5 mM/l. while at the peak of the effect, at zero time, about 0·75 mM/l. had accumulated above the normal 4 mM/l.

If summation of the effects of single impulses were responsible for the decrement observed during trains of impulses, it should be possible to predict the results obtained during trains by the summing of the effects of individual impulses. Such tests were made and for the first few impulses in the train there was good agreement between the predicted decrements of the undershoots and those observed experimentally. With prolonged trains discrepancies appeared, possibly as the result of hyperpolarization (see Baylor & Nicholls, 1969*a* and Fig. 6) and/or changes in the quantity of K liberated per impulse.

In the experiments described so far, the P cells were activated by stimulating their axons in the periphery. This procedure must also have excited the touch cells and caused synaptic excitation in other pathways (see Nicholls & Baylor, 1968*c*). In order to avoid possible complications from

activity of other cells and make certain that the analysis was confined to single neurones, P cells were also stimulated by currents passed through the micro-electrode in the presence of Mg (10–20 mM/l.) in the bathing fluid. This concentration of Mg effectively blocks chemical synaptic transmission, raises the threshold and prevents stimulation of other cells (see Baylor & Nicholls, 1969*b*). Under these conditions, the decrement in the undershoot was similar to that observed when receptors were activated in the skin as in Figs. 2 and 3.

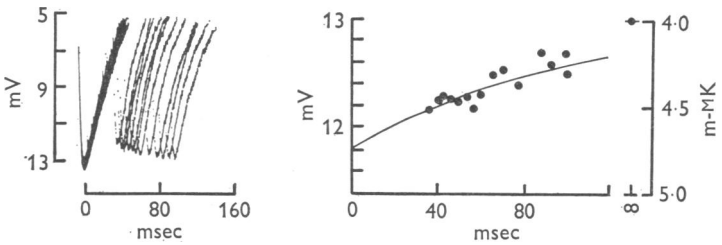


Fig. 3. Effect of one impulse on undershoot of a second action potential (upstrokes not seen). Left: superimposed frames of undershoots produced by twin shocks to the axon of the cell at various intervals. As the interval between action potentials became shorter the amplitude of the second undershoot decreased. Right: plot from same experiment to show the amplitude of second undershoot as a function of the inter-shock interval. The continuous curve is an exponential with a time constant of 100 msec. The scale on the right converts undershoot changes to K concentration changes. The exponential extrapolated to a K concentration of 4·75 mM/l. at 0 time (i.e. 0·75 mM/l. more than normal). Note that the time constant for the decline of K after a single impulse is similar to that of the build-up during a train (see Frankenhaeuser & Hodgkin (1956), and Fig. 2).

Undershoot decrement and K sensitivity of the undershoot after removal of glial surround. Although decrements in the undershoot could be matched by equivalent increases in K concentration, the possibility remained that changes observed in leech neurones were not produced by K accumulation. For example, an undershoot decrement without K build-up might occur if the K conductance of the neuronal membrane changed exponentially with time as a result of impulses or if the invasion of the cell body became less complete. Additional evidence that K accumulation was responsible for changes in the undershoot was provided by removing the glia surrounding a neurone (Kuffler & Potter, 1964). This procedure, by exposing more broadly much of the neuronal surface directly to the bathing solution and by disrupting the pericellular clefts, would be expected to reduce the build-up in K concentration. It should, however, not change the properties of the neuronal membrane (Nicholls & Kuffler, 1964).

In electron micrographs made by Dr David Wolfe (personal communication; see also his micrograph of Fig. 14 in Kuffler & Nicholls, 1966) of

neurones from which the glia has been removed, many patches of neuronal membrane can be seen to face the outside medium without the interposition of glial membranes. Figure 4 shows records made from a neurone

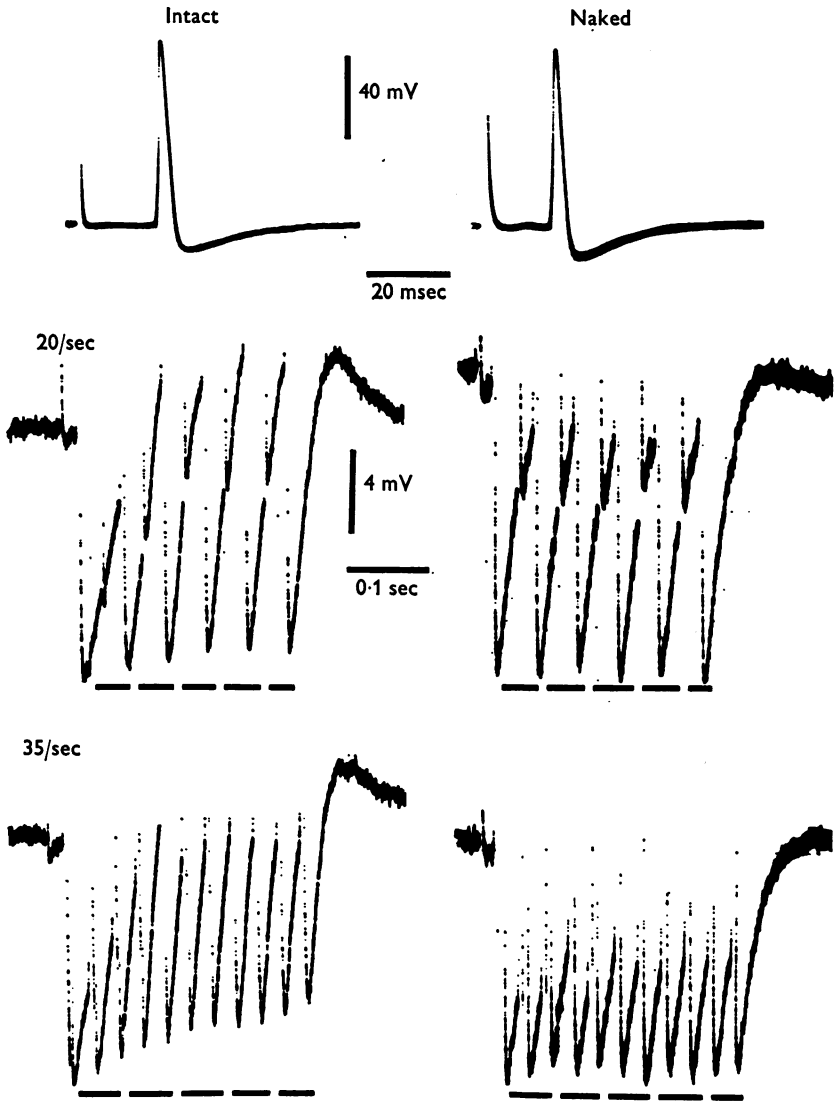


Fig. 4. Effect of removal of the surrounding glia on the undershoot decrement of action potentials in a train. The same pressure cell was impaled before and after removing the glia (see Kuffler & Potter, 1964). Stimuli were applied to its axon in the posterior root. After removal of the glia the undershoots no longer showed a decrement during trains, although the action potential and the sensitivity of the undershoot to K (not shown) were unchanged. In five other cells the K build-up was reduced, though not always so dramatically, by the operation.

before and after its glial surround was removed. On the left (above) is a record of the action potential of the intact cell, and below this are records at higher gain of the undershoots of impulses at stimulation frequencies of 20/sec and 35/sec. A characteristic reduction in the size of the undershoot occurred during the trains. On the right are records taken after the glial covering of the cell had been removed. The action potential appeared similar to that of the intact cell. During trains of impulses, however, the

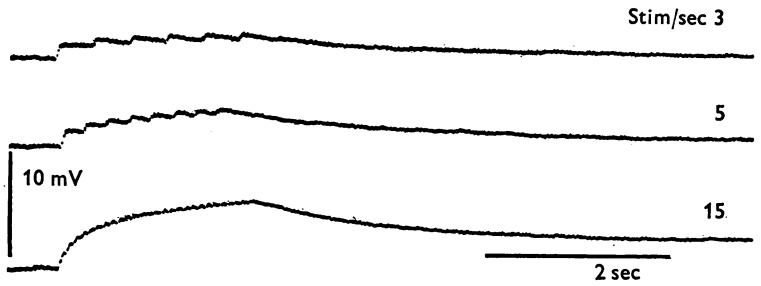


Fig. 5. Glial depolarization produced by neuronal activity. Intracellular recordings were made from the glial cell deep to the giant Retzius cell while maximal stimuli were applied to the posterior root through a suction electrode at the frequencies indicated on the right. Each stimulus to the axons was followed by a slow depolarization; these potentials summed during trains to produce a larger depolarization, the size of which varied with the frequency. This result indicates that a build-up of K occurs in the restricted spaces around the glial cell as a result of neuronal activity.

undershoot no longer showed an appreciable decrement. Measurements of the K sensitivity of the undershoot of this cell before and after the operation showed no change. This result once more confirms the idea that K accumulation produced the decrements in the undershoot. It also shows that glial cells act as spatial barriers around neurones (see Discussion). Five other experiments were made in which the undershoot and K sensitivity were studied in the same cell before and after removing the glia. All these cells also showed a marked decrease in the K accumulation after the operation; there was however some scatter so that the effects were not always as marked as those shown in Fig. 4. This is not surprising in view of the fact that a variable proportion of the neuronal membrane is still covered by glial membranes (see Fig. 14 in Kuffler & Nicholls, 1966).

Glial depolarization produced by neuronal activity. Another test of the idea that K accumulates around active leech neurones has made been by recording the membrane potential of a glial cell in the C.N.S. while stimulating neurones. The membranes of glial cells in the leech nervous system are intimately associated with those of neurones (Coggeshall & Fawcett, 1964) and their resting potentials obey the Nernst equation for changes in

K concentration over a wide range (Nicholls & Kuffler, 1964). If K accumulated in the extracellular spaces as a result of activity, synchronous activation of a group of neurones should produce a glial depolarization similar to that observed in the amphibian optic nerve by Orkand *et al.* (1966) and in the mammalian brain by Goldring & Karahashi (1965). Figure 5 shows such a depolarization in a glial cell, situated deep to the giant Retzius cell in the middle of the ganglion; it was identified as glia by its 65 mV resting potential (20 mV higher than that of the neurones in this preparation) and by its inability to give regenerative responses (Kuffler & Potter, 1964). It was presumed to be the neuropile glial cell, although marking experiments were not made. Stimuli were delivered to the posterior root with a suction electrode. Each individual shock was followed by a slowly declining depolarization. These unitary depolarizations summed during trains of stimuli to give a maintained plateau, the size of which varied with the frequency of stimulation. Depolarizations of over 10 mV were obtained at 15/sec corresponding to an average increase in the K concentration of 10 mM/l. or more over the entire surface of the glial cell (see Nicholls & Kuffler, 1964). This figure must represent a lower limit to the build-up which accumulated near some regions of the glial membrane, since portions of the cell and others to which it is electrically coupled (see Kuffler & Potter, 1964) face extracellular space in which the K build-up would inevitably be smaller. This experiment provides an independent confirmation that the K concentration can increase by several mM/l. as a result of trains of action potentials.

Figure 6 shows simultaneous recordings from a pressure neurone and a glial cell during prolonged repetitive stimulation of the posterior root with maximal shocks. The undershoots of the neuronal action potentials (lower trace) decreased in size with a time course similar to the glial depolarization (upper trace). It can be seen that after cessation of neuronal stimulation the two cells underwent slow potential changes of opposite polarity. The glial cell remained depolarized for some time; as in the optic nerves of amphibia, the repolarization of glial cells was considerably slower than the depolarization (Nicholls & Kuffler, 1964; Orkand *et al.* 1966). At the same time the neuronal resting potential exhibited a long lasting hyperpolarization. The properties and mechanism of the neuronal hyperpolarization will be discussed in the following paper (Baylor & Nicholls, 1969*a*).

Effects of K increases on neuronal activity. The results presented so far have shown that K increases of about 3–4 mM/l. can occur in the C.N.S. as a result of neuronal activity; the records in Fig. 1 indicate that these concentrations have only a small effect on the resting potential of quiescent neurones. It therefore seemed natural to ask whether K concentration changes of this order are sufficiently large to affect neuronal performance.

The effects of K on activity were studied by recording from a neurone while increasing the K concentration in the bathing fluid. The upper trace of Fig. 7 shows a recording made from a large neurone situated just laterally to the most anterior touch cell. The discharge of 'spontaneous' action potentials in the absence of stimulation is shown in the initial portion of the record; the small size of the impulses is due to the failure of invasion of the cell body. At higher gain a series of EPSPs can be seen to build up

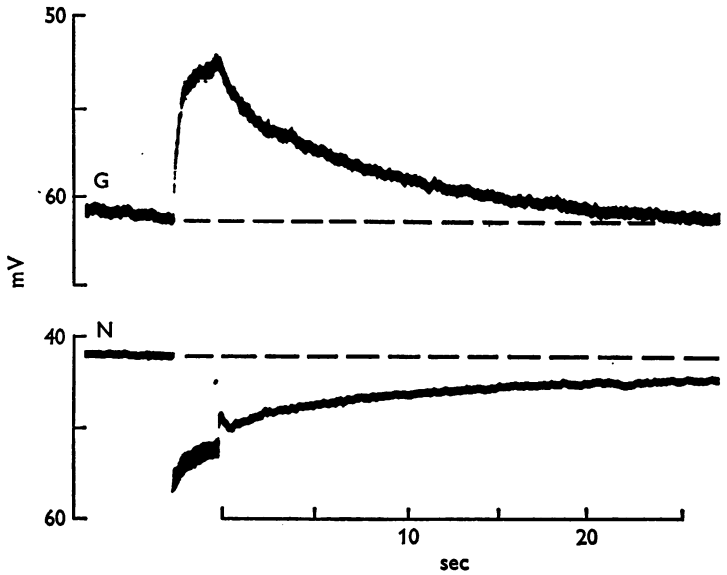


Fig. 6. Simultaneous glial depolarization and neuronal hyperpolarization produced by repetitive activity. G and N denote the records from the glial cell and the neurone. Note that the resting potential of the glial cell was 20 mV greater than that of the neurone. The glial cell was impaled deep to the soma of the giant Retzius cell, presumably in the neuropile (Coggeshall & Fawcett, 1964), while the neurone was a pressure cell about 75 μ away. Maximal stimuli were delivered at 35/sec to a posterior root, activating the axon of the pressure neurone, as well as many other fibres entering and leaving the ganglion. During the train of stimuli, the glial cell became depolarized rapidly at first and then more slowly; over the same period of time the undershoots of the neuronal action potentials (upstrokes not seen) progressively decreased in size. Both these effects can be attributed to K accumulation. After the train the glial cell remained depolarized, but the neurone underwent a hyperpolarization (see Baylor & Nicholls, 1969*a*).

to threshold before each action potential. At the arrow the K concentration in the bathing fluid was increased from 4 to 7 mM/l.; the firing slowed down and eventually stopped. On returning to the original solution, firing resumed and gradually returned to its original frequency. This effect of increased K was observed in five other tests in this same cell and was consistently observed when recordings were made from the corresponding cell

in other ganglia. The result shows that an increase of 3 mM/l. in the K concentration of the bathing fluid is large enough to influence the firing patterns of neurones in leech ganglia. In this experiment the cell being recorded from serves as an indicator of activity in other interconnected cells impinging on it.

The lower trace of Fig. 7 shows another experiment made on a different cell. This neurone is the most anterior and medial cell in the ganglion and typically shows large, spontaneous IPSPs (see left side of Fig. 7). Because

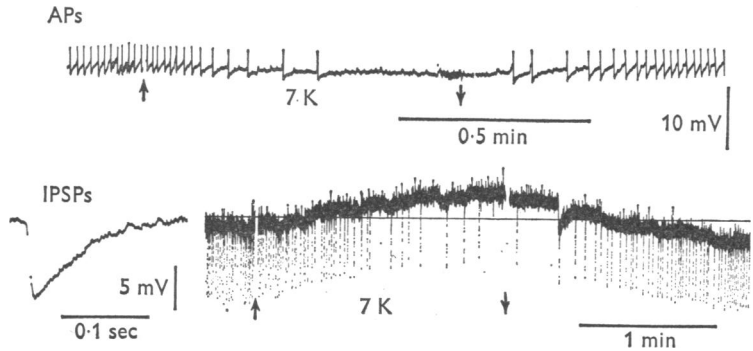


Fig. 7. Effect of increase in K concentration on spontaneous discharges and synaptic potentials. Above: low gain record from a spontaneously active cell adjacent to the touch neurones in the anterior packet. At the first arrow the K concentration in the Ringer fluid was increased from 4 to 7 mM/l. Below intracellular recording from another neurone in the anterior part of the ganglion with large spontaneous unitary IPSPs, an example of which is shown on left. When the K concentration was raised from 4 to 7 mM/l. (right) the IPSP frequency slowed markedly. In both instances the original activity resumed when the normal solution was restored. These results indicate that small increases in external K can change neural signalling.

of their large amplitude they are presumably due to impulse activity in inhibitory cells. The right-hand record, taken on a slow time base, was made from the neurone while the K concentration was increased to 7 mM/l. (at the arrow). The IPSPs appear as dense downstrokes below the base line in the initial part of the recording. In the high-K fluid the IPSP's decreased in frequency but not in amplitude and eventually stopped, while the resting potential showed a slight depolarization. On returning to the original solution, at the second arrow, the IPSP frequency and the resting potential returned to their original values. This observation suggests that the concentration changes affected the firing patterns of presynaptic cells. As in the example in the upper part of Fig. 7, the situation is too complex for a complete analysis; the simplest explanation would be that the inhibitory cell stopped firing as a result of an altered input. In other neurones (e.g. touch cells) the effects of single stimuli, applied to a presynaptic pathway, were potentiated by increased K.

DISCUSSION

The results show that the undershoot of the action potential of sensory neurones is sensitive to small changes in the external K concentration. Each action potential produced a reduction in the amplitude of the undershoot of successive impulses similar to that observed by Frankenhaeuser & Hodgkin (1956) and these changes could be matched by equivalent K concentrations. From these measurements it was clear that the concentration around a pressure neurone could be increased by 3 mM/l. (Fig. 2) when it fired at 45/sec. These cells are capable of conducting impulses at more than 60/sec and the touch cells can fire at over 100/sec, so this estimate of K build-up does not represent the maximum that can be achieved. For example, the concentration was more than doubled around some neurones firing at 50/sec. If the undershoot changes during activity were due to build-up of K in a restricted space around the neurone, then removal of the glial surround, by enlarging the spaces available for diffusion of K, should reduce the accumulation of K. This was shown to occur (Fig. 4). The result also indicates that the glial cell membrane acts as a barrier that effectively prevents K from diffusing away.

A check of the estimate of the amount of K that accumulates can be made by considering some of the quantities involved in the results of the twin shock experiment (Fig. 3) in which the increase in K produced by one impulse was estimated. The initial increment in K was about 0·75 mM/l. Assuming the extracellular space around the neurone is about 150 Å wide (see Coggeshall & Fawcett, 1964) one can calculate the amount of K which would have to be liberated by one impulse across 1 cm^2 of neuronal membrane to achieve a concentration of 0·75 mM/l. Although there is no independent measure of K fluxes in leech neurones, the answer can be compared to values obtained in other systems to see if it is of a reasonable magnitude. Making the calculation

K liberated per impulse/cm^2 membrane = (cleft width) (K concentration increase produced by one impulse)

= (150 Å) (0·75 mM/l.) = $1{\cdot}1 \times 10^{-12}$ M/cm^2.

This figure of 1·1 pM/cm^2 agrees well with Keynes & Ritchie's (1965) value of 1 pM/cm^2 obtained in mammalian unmyelinated fibres.

The glial depolarization produced by neuronal firing provides an independent confirmation that K accumulates in the extracellular space of the leech C.N.S. during activity. This is because it has been shown that glial cells in leech ganglia (like those in the optic nerves of amphibia) are sensitive to small changes in extracellular K, their membrane potential behaving like a K electrode (Nicholls & Kuffler, 1964; Orkand *et al.* 1966).

We have, however, no explanation at present for the slow time course of the repolarization (see Figs. 5 and 6); a similar asymmetry, which cannot be accounted for in terms of simple diffusion, is observed when K is applied to the bathing fluid and it has also been seen in glial cells of *Necturus*. In contrast to the glia the membrane potential of neurones is relatively so insensitive to K around the physiological range of 3–6 mM/l. that such changes give rise to a depolarization of only 1–2 mV; a fivefold increase (i.e. to 20 mM/l.) would be required for 5 mV of depolarization (see Kuffler & Nicholls, 1966). It is of interest to note that the neurones can actually be hyperpolarized after activity at the same time as the glial cells are depolarized by K. This observation which is discussed more fully in the next paper (Baylor & Nicholls, 1969*a*) is relevant to the interpretation of evoked potentials or recordings made from the surface of the C.N.S.

Effects of K on signalling. Although the changes in membrane potential produced by K increases of 3–6 mM/l. are small, it is to be expected that they will influence the release of transmitter and the threshold of postsynaptic membranes. Furthermore, it will be shown in the following paper (Baylor & Nicholls, 1969*a*) that the membrane potential change produced by a small increment in K concentration can be amplified by previous activity. Our experiments made with spontaneously active neurones do in fact show that changes in K concentration of the same order as those produced by activity can influence the frequency of firing. Although the effects were clear, the mechanism was obscure in the many instances where the frequency of impulses or IPSPs were reduced in high K (Fig. 7). These effects are paradoxical in that one might at first expect increased K to decrease membrane potential and accelerate firing. The real situation is undoubtedly more complex, however, because the cells recorded from are elements in a long and complex chain of neurones that interact with one another to integrate the performance of a segmental ganglion. One cannot therefore guess at the site at which K is acting. Whatever the mechanism of the effect it is clear that small changes in external K can dramatically affect the firing of spontaneously active neurones in the ganglia.

One of the questions that arises from these experiments is whether a K signalling mechanism could operate between neurones. Although our results are consistent with the idea of such a mechanism, it remains to be shown directly that the K released by one neurone can affect the integrative action of another. If such interactions do occur, it follows that the pattern in which neurones are gathered into fascicles by glial cell processes would be of crucial significance in determining the nature of the interactions. Two neuronal processes enclosed in a common glial wrapping would share a small extracellular space, so that K liberated by one neurone would affect its neighbour. Neurones separated by fingers of glial

cytoplasm would not be expected to interact as strongly, since a glial cell membrane rather than that of a neurone, would be closest to the region where the K concentration was highest. Thus glial cells would perform a function as spatial barriers allowing K to accumulate around specific groups of neurones within the C.N.S. In the C.N.S. of fish (Furukawa & Furshpan, 1963) and mammals (Peters & Palay, 1965) there are regions where glial cells have been found to have a precise anatomical relationship to neurones. It will be of interest to determine whether the glial cell processes are distributed in the neuropile of leech ganglia in an orderly and stereotyped manner, like the neurones.

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