EXTRACELLULAR POTASSIUM CHANGES
IN THE SPINAL CORD OF THE CAT AND THEIR RELATION TO
SLOW POTENTIALS, ACTIVE TRANSPORT AND
IMPULSE TRANSMISSION

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SUMMARY

1. By means of K-specific double-barrelled micro-electrodes the time course of changes in K^+ concentration in the extracellular space of the lumbar spinal cord was examined after peripheral tetanic stimulation and after a single volley in a mixed peripheral nerve in non-anaesthetized, intercollicularly decerebrated and spinalized cats.

2. Tetanic stimulation (100 Hz) which increases the $[K]_e$ from 3 to 9 mM is followed by a phase of reduced $[K]_e$ during which $[K]_e$ decreases by 0·5 mM below resting level, lasting 1–2 min before returning to its original resting level. Evidence is presented that this subnormal phase of $[K]_e$ reflects active processes redistributing accumulated K^+ from extracellular space.

3. The subnormal phase of $[K]_e$ can be registered only when the micro-electrode is located in very close vicinity of discharging neurones and is not primarily dependent on the absolute level of increased $[K]_e$. This can be considered as evidence that the neurones and not the glial cells are responsible for active reabsorption of K^+ from the extracellular space.

4. Increased $[K]_e$ is reflected in focally recorded potentials as a negativity and decreased $[K]_e$ as a positivity. The latency of focally recorded positivity is, however, shorter than the latency of reduced $[K]_e$. This makes it likely that the positivity reflects not only passive hyperpolarization of glial elements, but also an active, electrogenic ion transport across neuronal membrane.

5. The shortest latency of increased $[K]_e$ induced by a single volley in a mixed peripheral nerve was found to be 9 msec; the peak, representing 0·5 mM, was attained after 40 msec and the total duration was 200 msec. A theoretical consideration is put forward that the time course of transient increase in $[K]_e$ is consistent with the suggestion that K^+ which

accumulates in the spinal cord after neuronal discharge is responsible for primary afferent depolarization.

6. Evidence is presented that increased $[K]_e$, induced by a long-lasting peripheral stimulation, is accompanied by decreased efficacy of impulse transmission.

INTRODUCTION

Potentials arising in the spinal cord as a result of peripheral stimulation were analysed in studies by Gasser & Graham (1933), Barron & Matthews (1938) and Bernhardt (1952). Later studies in which intracellular techniques were employed have shown great specificity in synaptic actions that contributes to their generation (see Eccles, 1964). Much less attention was, however, paid to ionic changes in the extracellular space that accompany neuronal discharges which can contribute to the generation of potentials in the spinal cord and can also play a role in modifying impulse transmission as suggested by Barron & Matthews (1938).

The finding that membrane resting potential of glial cells precisely follows changes of extracellular K concentration $[K]_e$ (Orkand, Nicholls & Kuffler, 1966) enabled the studies in which slow potentials, arising in the spinal cord in response to peripheral stimulation, could be related to changes of $[K]_e$ (see Somjen, 1973).

Direct measurements of transient changes in $[K]_e$ in the nervous system became feasible by means of K-specific micro-electrodes (Walker, 1971; Vyskočil & Kříž, 1972; Neher & Lux, 1973). It was shown that tetanic stimulation of peripheral nerves can increase $[K]_e$ in the spinal cord from the resting 3 mM level to 6 mM. Such an increase in $[K]_e$ can account for appreciable depolarization of the cells, the membrane potential of which follows the prediction given by the Nernst equation. It was suggested that a transient increase in $[K]_e$ may account for primary afferent depolarization (PAD) (Vyklický, Syková, Kříž & Ujec, 1972; Krnjević & Morris, 1972; Bruggencate, Lux & Liebl, 1974; Kříž, Syková, Ujec & Vyklický, 1974). This possibility was, however, doubted for several reasons, one being the difference between the time course of PAD and the transient changes in $[K]_e$ (Somjen & Lothman, 1974).

In this study the time course of changes in $[K]_e$, arising in the lumbar spinal cord in response to peripheral stimulation, was related to slow potentials recorded focally or from the cord dorsum. Evidence will be presented of an active process that removes accumulated K^+ from the extracellular space and of the close positive correlation between the time course of the transients in $[K]_e$ and the PAD. A preliminary note has already been published (Vyklický, Syková & Kříž, 1975).

METHODS

Results were obtained on seventeen non-anaesthetized, spinalized and decere-brated cats. The dissection and decerebration were made under halothane anaes-thesia (Narcotan, SPOFA). The lumbosacral spinal cord was exposed by laminectomy at the L_6–S_1 segments. The posterior tibial nerve, the common peroneal nerve, the nerve to gastrocnemius and soleus muscles and the sural nerve were dissected in the left hind limb, cut, and the central stumps were placed on bipolar electrodes. The spinal cord and the nerves were covered with liquid paraffin. After dissection was completed, the animal was decerebrated at the intercollicular level, the spinal cord was transected at Th_{11}–Th_{12} level and anaesthesia was discontinued. All animals were immobilized by gallamine triethiodide (Tricuran, Germed) and ventilated artificially with air. Expired CO_2 was monitored continuously and adjusted to 3·8–4·3 %. The arterial blood pressure, unless specifically stated, was kept over 100 torr; if necessary, noradrenaline in saline solution was given by slow i.v. infusion.
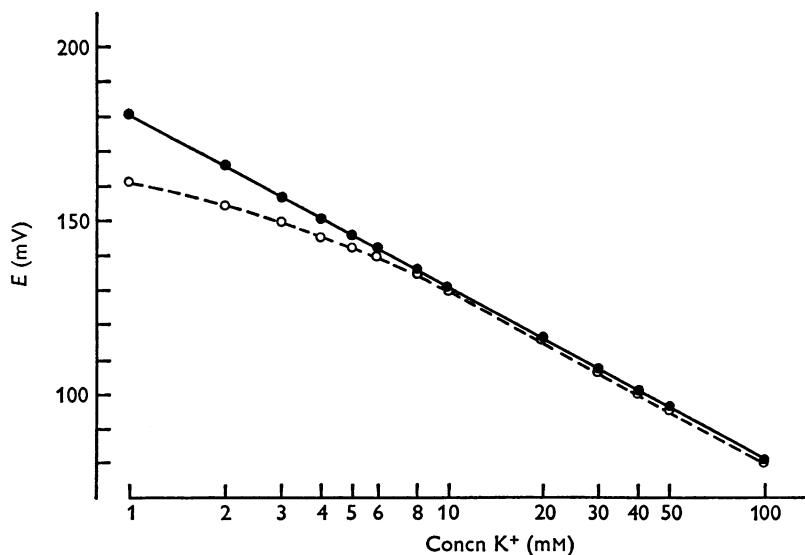

Fig. 1. Calibration curve of a double-barrelled potassium specific micro-electrode in solutions of various K^+ concentrations. The potential changes in mV were plotted against concentration of K^+ in mM (continuous line). The dashed line represents the same micro-electrode calibration in K^+ solutions with 150 mM-NaCl background.

K specific electrodes

The technique of preparing K-specific micro-electrodes was described in detail by Vyskočil & Kříž (1972) and summarized by Kříž et al. (1974). In principle the tip of one channel of a double-barrelled micro-electrode was filled with liquid K ion exchanger (Corning 477317) up to a height of 200–500 μm and the rest of this channel was filled with 0·5 M-KCl. The other channel was injected with 0·15 M-NaCl. The recording technique was as described by Kříž et al. (1974). The changes of [K]$_e$ were measured differentially between the two channels of a double-barrelled microelectrode

by means of a DC differential, high-impedance preamplifier ($R_{imp}10^{14}$ Ω) with crossed feed-back negative capacity (Ujec & Beránek, 1967) and focal potentials were registered between the NaCl-filled channel and ground. Recordings were made on a Tektronix 502A oscilloscope and a highly sensitive linear ink recorder (Labora EZ 5) with a rise time 0·3 sec for 10 mV. The potentials from cord dorsum were recorded by means of a RC-coupled preamplifier (time constant, 1·5 sec). The sensitivity of electrodes was tested in solutions containing various concentrations of KCl with a 150 mM-NaCl background. It is important to use this background which represents the highest concentration that can be expected in extracellular fluid as there is a deviation from linearity on the semilogarithmic scale at low K+ concentrations (see Fig. 1). This deviation is caused by the limited selectivity of K-specific micro-electrodes which is about 50 to 1 for K+ and Na+ respectively (Walker, 1971). A concentration change of 1 mM within the range 3–6 mM K+ at room temperature reliably produced 2·0–5·0 mV potential shifts. Special calibration curves were drawn for each electrode and the actual measurements were fitted to these.

RESULTS

Changes in $[K]_e$ and slow potentials in the spinal cord produced by tetanic stimulation of peripheral nerves

It was frequently observed that the level of increased $[K]_e$ was not sustained during the whole period of stimulation, but declined after its maximum was reached (Kříž et al. 1974). It could not be decided whether this decline in $[K]_e$ was induced by a decreased frequency of neuronal discharges or whether the transport mechanism for active reabsorption of K+ from the extracellular space became more effective after a certain period of high frequency stimulation.

Fig. 2 and the subsequent Figs. 3–6 present evidence that an active process is triggered which contributes to replacement of K+ from extracellular space when $[K]_e$ significantly increases. The upper record in Fig. 2 represents the time course of the change in $[K]_e$ induced by 100 Hz stimulation of the posterior tibial nerve as recorded differentially with a double-barrelled K-specific micro-electrode inserted in the region of the intermediate nucleus in the L7 segment. It can be seen that the maximum, 9 mM-$[K]_e$, was reached after about 17 sec of stimulation. The level of $[K]_e$ decreased, after attaining the maximum, although the stimulation continued at the same intensity and frequency. When the stimulation was discontinued, accumulated K+ dissipated from extracellular space with a half-time of 5–6 sec. The processes responsible for redistribution did not stop their action after the original resting level was reached, but continued, producing a long-lasting subnormal phase of $[K]_e$. Such a transient reduction in $[K]_e$ was found to be 0·5 mM and it took about 2 min before it returned to its original resting level.

The negative potential shifts, accompanying high-frequency neuronal discharges, induced by peripheral stimulation, were found to be closely
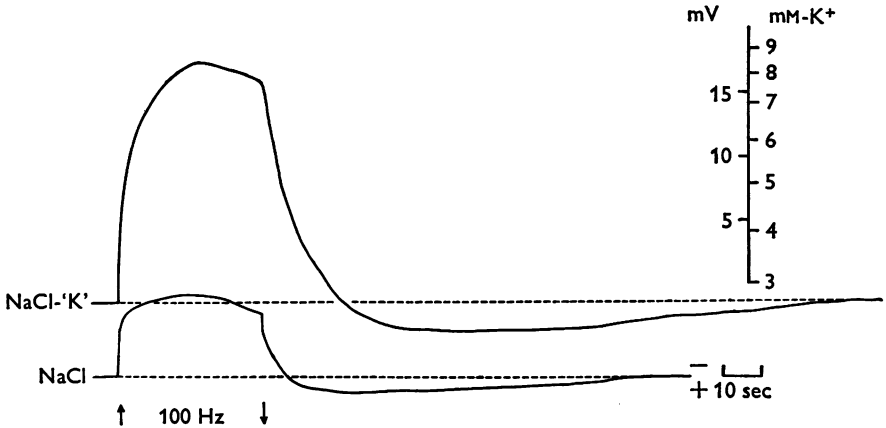
Fig. 2. Changes in [K]$_e$ produced by tetanic stimulation of posterior tibial nerve. Upper record (NaCl–'K') represents the time course of the changes in [K]$_e$ evoked by 100 Hz stimulation lasting 35 sec. Increase of K+ activity from 3 to 9 mM is followed by long-lasting subnormal level of [K]$_e$. The lower record (NaCl) represents the corresponding negative potential shift obtained by NaCl channel against ground followed by a long-lasting positivity.

correlated with the time course of [K]$_e$ (Somjen & Lothman, 1974). This is also demonstrated in the lower record in Fig. 2, that shows the slow negative potential, recorded with a NaCl-filled channel against ground. The indentation in the declining phase of the potential shift was apparently produced by sudden cessation of neuronal discharge, while the gradual more or less exponential decline exhibited to a certain extent a similar time course as the dissipation of accumulated K+ from extracellular space. The period after which the potential shifted from negativity to positivity was, however, 12 sec shorter than the time of appearance of the subnormal level of [K]$_e$. A possible explanation for the discrepancy in the time course of [K]$_e$ and slow potentials will be dealt with in the Discussion.

The effect of the duration of 100 Hz peripheral stimulation on the subnormal phase of [K]$_e$

The duration and the amplitude of the subnormal phase of [K]$_e$ after peripheral stimulation was found to be correlated with the total duration of stimulation. This is shown in Fig. 3 which demonstrates records of changes in [K]$_e$ produced by 2, 3, 10, 18 and 35 sec of 100 Hz stimulation of the posterior tibial nerve. Stimulation lasting 35 sec resulted in a subnormal phase of [K]$_e$ about 0·5 mM below the resting level. Only slight difference in the total duration of the subnormal [K]$_e$ was found after

18 sec peripheral stimulation. Shorter periods of stimulation were, however, accompanied by a much less expressed subnormal phase of $[K]_e$ and no decrease below the resting level was detected after the 2 sec stimulation.
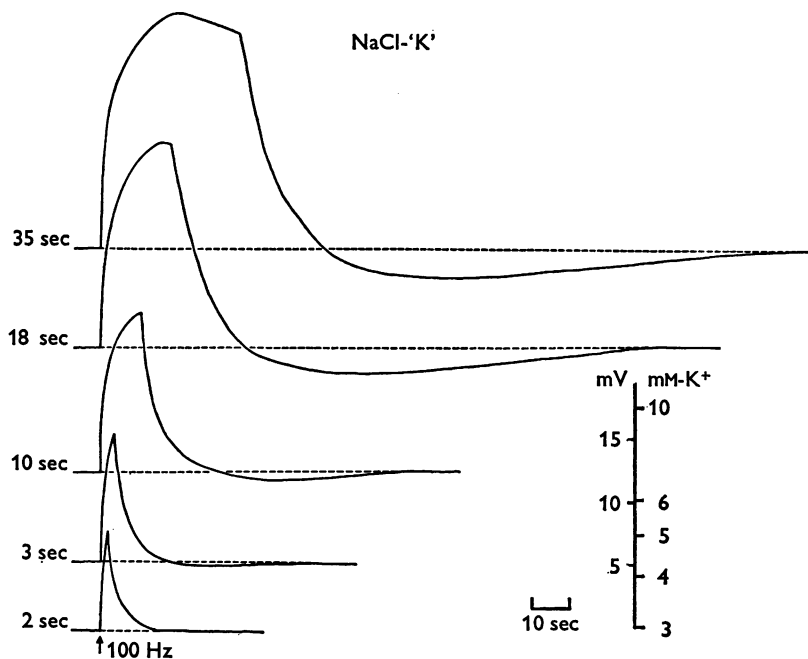

Fig. 3. *Changes of* $[K]_e$ in L7 segment induced by various durations of peripheral stimulation at 100 Hz. The posterior tibial nerve was stimulated for 35, 18, 10, 3 and 2 sec at 100 Hz. Pronounced reduction in $[K]_e$ followed the accumulation of K^+ after 35 and 18 sec stimulation. No detectable decrease of $[K]_e$ below resting level was observed after 2 sec stimulation.

The dependence of the magnitude of the subnormal phase of $[K]_e$ *on the frequency of peripheral stimulation*

The effect of frequency of peripheral stimulation on the increase of $[K]_e$ in the spinal cord was demonstrated by Kříž et al. (1974). Fig. 4 confirms this finding and shows further that the magnitude and the total duration of the subnormal phase of $[K]_e$ that follows the accumulation of K^+ in the extracellular space correlates positively with the level of its increase. In the most active region of the spinal cord stimulation of the posterior tibial nerve at 100 Hz produced neuronal discharges that raised the $[K]_e$ to about 9 mM. Such an accumulation of K^+ in the extracellular space was followed by a delayed reduction below resting level by as much as 0·5 mM. Other recordings in Fig. 4 show that the reduction of $[K]_e$ which follows its increase could also be observed at lower frequencies of peripheral stimula-

tion, but it was much less pronounced. However, even after stimulation at 3 Hz when [K]$_e$ increased by only 1 mM, the transient reduction of [K]$_e$ was still present.
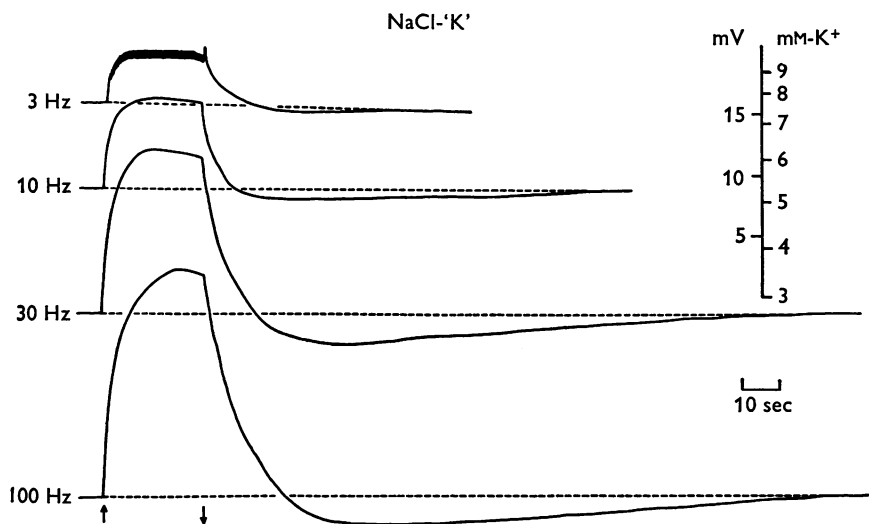

Fig. 4. The effect of frequency of stimulation on the subnormal [K]$_e$. Tetanic stimulation of posterior tibial nerve at 3, 10, 30 and 100 Hz shows that the magnitude of increased [K]$_e$ positively correlated with the amplitude and total duration of the subnormal phase of [K]$_e$. Even after stimulation at 3 Hz which increased [K]$_e$ by only 1 mM the transient reduction of [K]$_e$ was still present.

The effect of electrode position on the subnormal phase of [K]$_e$ after peripheral stimulation

The spatial distribution of accumulated K+ in the extracellular space of the spinal cord after peripheral stimulation was demonstrated by Kříž et al. (1974) and Bruggencate et al. (1974). It was shown that maximal accumulation of [K]$_e$ in the extracellular space occurs around the intermediate nucleus. The records in Fig. 5 show changes in [K]$_e$ in the L6 segment, produced by stimulation of the common peroneal nerve at 100 Hz at various depths. The late subnormal phase of [K]$_e$ was not recorded until the micro-electrode reached a depth of 1 mm, although the accumulated K+ already exceeded the resting level by 1 mM at a depth of 0·7 mm and by 2 mM at a depth of 0·9 mm. It can therefore be concluded that the subnormal phase of [K]$_e$ can apparently be detected only in the closest vicinity of elements that are responsible not only for accumulation of K+, but also for its subsequent active reabsorption from the extracellular space.

Effects of lowered blood pressure induced by hypoxia on processes leading to accumulation and reabsorption of K^+ in the extracellular space

The resting level of $[K]_e$ in the cerebral cortex of the rat was shown to be very sensitive to anoxia (Vyskočil, Kříž & Bureš, 1972). Fig. 6 presents evidence that the process most sensitive to anoxia is the one that removes the accumulated K^+ from the extracellular space. The uppermost record shows the time course of the changes in $[K]_e$ when the blood pressure was
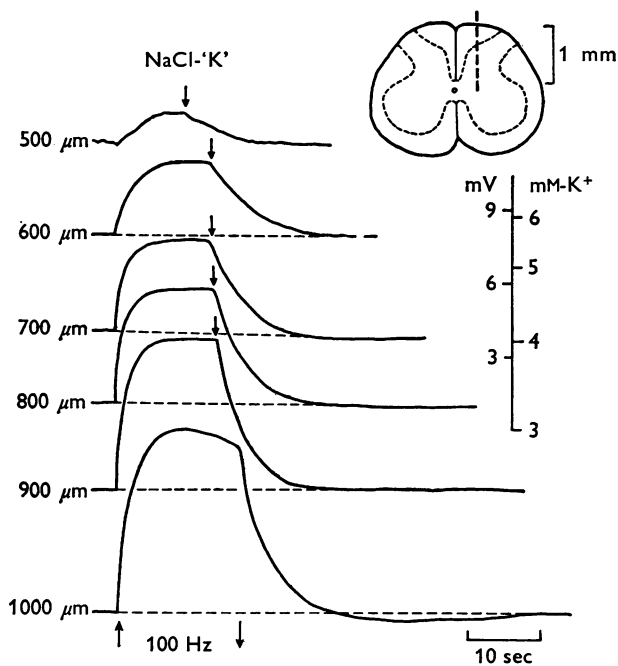

Fig. 5. The distribution of changes in $[K]_e$ in L6 segment produced by peripheral stimulation of the common peroneal nerve at 100 Hz. No reduction in $[K]_e$ was observed down to a depth of 900 μm although the $[K]_e$ increased by 2 mM. Subnormal phase of $[K]_e$ appeared at a depth of 1000 μm (the direction of the micro-electrode track is shown schematically in the drawing).

120 torr. Stimulation of the posterior tibial nerve at 100 Hz for 28 sec resulted in an increase of $[K]_e$ to 8·5 mM. When stimulation was discontinued, accumulated K^+ dissipated with a half-time of 5 sec and was followed by a long-lasting reduction of $[K]_e$ by about 0·3 mM which lasted more than 1 min before returning to the original resting level. When artificial respiration was reduced, impaired oxygenation resulted in a gradual decrease of blood pressure. The other records show changes of
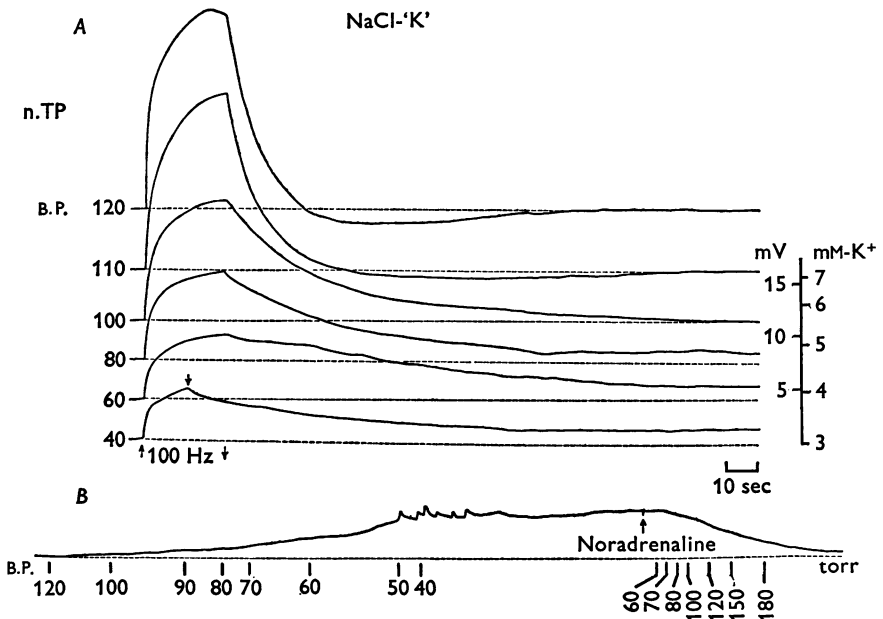
Fig. 6. The effect of hypoxia on the changes in [K]ₑ produced by peripheral stimulation. *A*, the time course of increased [K]ₑ and its subnormal phase induced by stimulation of the posterior tibial nerve at 100 Hz were registered at various levels of blood pressure during development of hypoxia produced by reduced ventilation. The posterior tibial nerve was stimulated for 28 sec, but at blood pressure of 40 torr the period of stimulation was 15 sec. *B*, spontaneous increase of the resting level of [K]ₑ produced by hypoxia and its recovery after an injection of noradrenaline. n.TP, posterior tibial nerve.

[K]ₑ induced by the same peripheral stimulation at different levels of blood pressure. It can be seen that when the blood pressure was lowered by 100 torr the subnormal phase of [K]ₑ completely disappeared, although tetanic stimulation still produced an elevation of [K]ₑ up to 5·5 mM. At this level of blood pressure the processes participating in the replacement of accumulated K+ were still active enough to return the [K]ₑ to its original resting level, but the half-time of replacement was 20 sec. At a blood pressure of 80 torr or lower peripheral stimulation resulted in a much smaller increase of the [K]ₑ and the processes of its equilibration were extremely slow and incomplete. The record in Fig. 6*B* demonstrates that the resting level of [K]ₑ increased gradually during deterioration of the preparation in the animal even without peripheral stimulation. The original resting level of the [K]ₑ increased by 1 mM when the blood pressure was lowered to 40–50 torr. But when the blood pressure was raised by an i.v. infusion of noradrenaline, the [K]ₑ returned gradually after a while to the original resting level.

Changes in $[K]_e$ produced in the spinal cord by a single volley in peripheral nerve and their relation to the focal and surface potentials

The time course of transient changes in $[K]_e$ produced in the spinal cord by a single volley in a peripheral nerve differed considerably. The latency varied between 9–20 msec, the peak was reached between 40 and 200 msec and the total duration was 200 msec–3 sec. Fig. 7A–C demonstrates a transient increase in $[K]_e$ by 0·5 mM induced in the L7 segment by a single
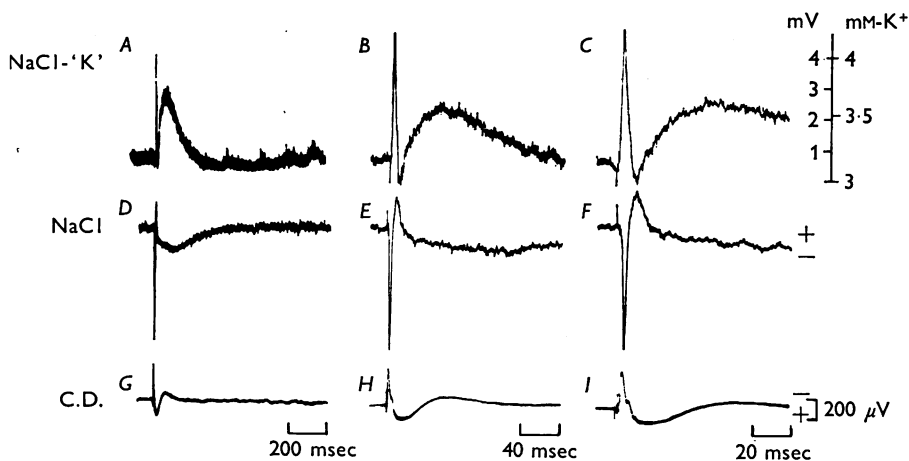

Fig. 7. Changes in $[K]_e$ produced by a single volley of impulses in the posterior tibial nerve.

A–C, slow transient changes in $[K]_e$ at three different recording speeds. The sharp component (< 10 msec) reflects potential changes produced by neuronal discharge which we were unable to compensate, apparently because of a large difference in the resistance of the two channels of the double-barrelled micro-electrode. The resistance of the channel filled with NaCl solution was 3 MΩ while the channel filled with ion exchanger exhibited 100 MΩ.

D–F, are local potentials recorded with NaCl channel against ground.

G–I, corresponding cord dorsum potentials at L7 segment.

volley in the posterior tibial nerve. The early sharp component (< 10 msec) reflects potential changes produced by neuronal discharge which we were unable to compensate, apparently because of the large difference in the resistance of the specific ion and the reference channel of the electrode. These changes, however, did not interfere significantly with the transients in $[K]_e$. The records at three different speeds represent $[K]_e$ transients which were the fastest we have observed. The latency was about 9 msec, the peak was reached in about 40 msec and the total duration did not exceed 200 msec. The corresponding records D, E, F which are focal

potentials measured with the NaCl-filled channel of the electrode against ground demonstrate that the time course of the slow component of the potential correlates well with the change in $[K]_e$. Records *G–I* show the cord dorsum potentials recorded simultaneously. The latency of the P wave, arising as a result of depolarization of primary afferents, corresponded to the start of the transient change in $[K]_e$, but its peak was reached in about 20 msec which was 20 msec less than the latency of the peak of transient increase in $[K]_e$. The question whether the changes in $[K]_e$ and PAD are causally related will be dealt with in the Discussion.
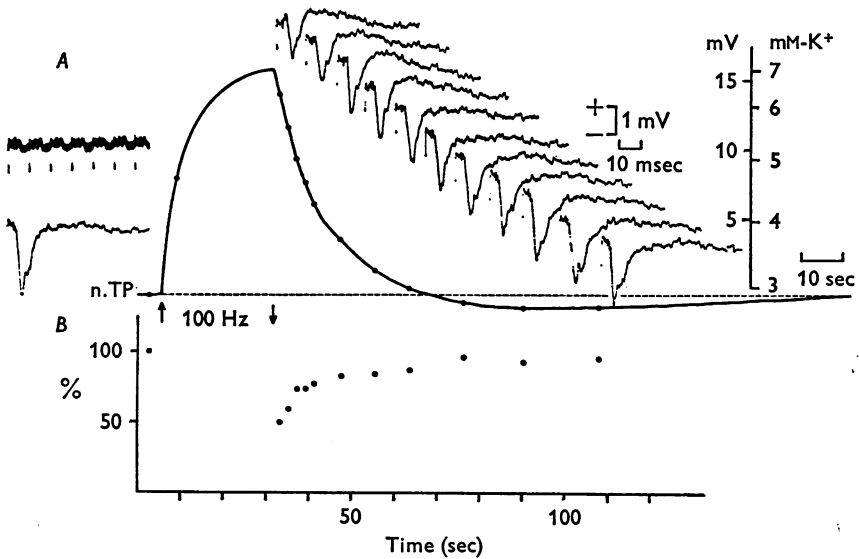

Fig. 8. The effect of accumulated $[K]_e$ on the amplitude of the focal potentials. *A*, record of $[K]_e$ change induced by stimulation of the posterior tibial nerve at 100 Hz. The focal potentials were evoked by a single volley in the same nerve at intervals shown by points on the curve of $[K]_e$. In *B* the amplitudes of focal potentials were plotted as percentages of the control against time.

The effect of increased $[K]_e$ on impulse transmission in the spinal cord

The efficacy of synaptic transmission has been shown to be decreased during depolarization of presynaptic terminals; this forms the essence on which the theory of presynaptic inhibition is based (see Eccles, 1964). The sudden increase in the $[K]_e$ can on the other hand also lower the membrane potential of the post-synaptic elements and in this way increase the probability of their firing. To gain information on whether there are changes in impulse transmission in the spinal cord that correlate with the

accumulation of K^+ in extracellular space we examined the changes in the amplitude of the field potentials and ventral root discharges at various levels of $[K]_e$.

Fig. 8A demonstrates an increase in $[K]_e$ produced by stimulation of the posterior tibial nerve at 100 Hz and examples of the field potentials evoked by single volley in the same nerve, recorded with the NaCl channel at intervals, indicated by dots on the record of $[K]_e$. In B the amplitudes of the field potentials were expressed as percentages of the control and plotted against time during the $[K]_e$ elevation. Two seconds after tetanic stimulation was discontinued, the amplitude of the field potential was reduced to about 50 % of the control and the recovery of the amplitude closely correlated with the time course of the $[K]_e$ changes. The amplitude of the field potentials did not recover completely after the $[K]_e$ reached its original level and was still diminished by about 5 % during the phase of subnormal $[K]_e$.
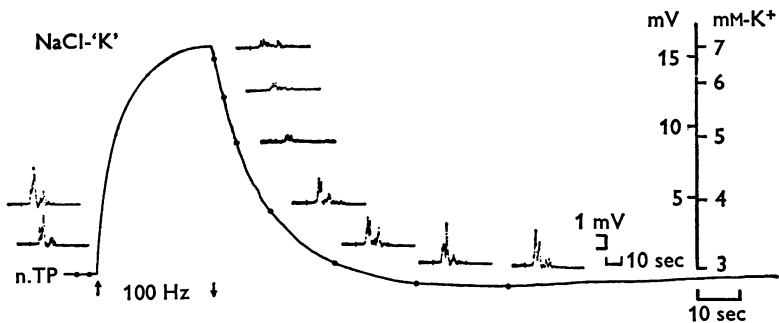

Fig. 9. The effect of accumulated K^+ on the ventral root discharge. The curve represents the time course of $[K]_e$ change induced by 100 Hz stimulation of the posterior tibial nerve (n.TP). The ventral root discharges were evoked by single volley in the sural nerve at intervals pointed at the record of $[K]_e$.

Fig. 9 demonstrates L7 VR discharges, elicited by single stimuli applied to the sural nerve at various levels of increased $[K]_e$, induced by stimulation of the posterior tibial nerve at 100 Hz. It can be seen that the VR discharges were much depressed during the phase of $[K]_e$ elevation.

DISCUSSION

The present results demonstrate that $[K]_e$ as measured with a K-specific micro-electrode in the spinal cord can increase during neuronal activity from the 3 mM resting level up to 9 mM. This value is higher than that which was found in the previous studies (Vyklický et al. 1972; Krnjević & Morris, 1972; Somjen & Lothman, 1973; Kříž et al. 1974;

Bruggencate *et al.* 1974). The most probable reason for this finding is the improved technique of preparing the double-barrelled electrodes which allow recording from a closer vicinity of discharging neurones and the fact that non-anaesthetized preparations were used in this study.

The results further demonstrate a delayed subnormal phase of $[K]_e$ which follows after accumulation of K^+ in the extracellular space of the spinal cord. This phase of reduced $[K]_e$ can be considered as direct evidence for the presence of active processes, contributing to the redistribution of K^+ from extracellular space. The extreme sensitivity to oxygen supply undoubtedly reflects their metabolic character.

Indirect evidence for transient reduction in $[K]_e$ was presented in the cat's cerebral cortex by the finding of hyperpolarization of glial cells. The hyperpolarization followed the depolarization elicited by intense cortical excitation. It was suggested that the subnormal phase of $[K]_e$ reflected either the activation of a K^+ sensitive electrogenic pump, located within the glial cell membrane (Grossman & Rosman, 1971; Sypert & Ward, 1971), or the operation of a neuronal pump which resulted in a transient decrease in $[K]_e$ and passive hyperpolarization of glial elements (Ransom & Goldring, 1973).

The experiments in which we tried to show whether the subnormal phase of $[K]_e$ depends on absolute level of $[K]_e$ or on the particular position of the micro-electrode in the pool of discharging neurones could help to decide between these two possibilities. From the records in Figs. 4 and 5 it can be seen that the late subnormal phase in $[K]_e$ could accompany an increase by as little as 1 mM when the micro-electrode was introduced in a location where accumulation of K^+, produced by high frequency stimulation, was followed by pronounced subnormal phase in $[K]_e$ (Fig. 4). When on the other hand we changed the electrode position by 100 μm, no late reduction of $[K]_e$ below the resting level occurred although its preceding increase was more than 2 mM (Fig. 5). Assuming that glial cells possess similar properties irrespective of their locality this finding implies that the discharging neurones and not the glial cells are the main source for the active reabsorption of accumulated K^+.

The negative potentials recorded focally during repetitive peripheral stimulation are apparently produced by neuronal discharges, post-synaptic potentials and by depolarization of local elements induced by increased $[K]_e$. The particular contribution of these two components can be distinguished in the declining phase of the negative potential that exhibits both fast and slow declining phases (lower record in Fig. 2). The fast component obviously reflects the sudden cessation of neuronal discharge and the slow one corresponds to the depolarization of cellular elements produced by accumulated K^+ in the extracellular space.

The transient reduction in $[K]_e$ is reflected in focally recorded potentials as a positivity. The latency of the late positivity and also its total duration was, however, shorter than that of the transient reduction of $[K]_e$ (Fig. 2). Although no direct evidence can be presented, it is likely that this positivity does not only reflect passive hyperpolarization of glial elements, but that it also involves electrogenic processes of active ion transport located in neurones.

The original suggestion that the ionic changes in extracellular space induced by neuronal discharging can account for depolarization of primary afferents in the spinal cord (Barron & Matthews, 1938) was revived when it was discovered that the K^+ accumulates in the extracellular space in amounts high enough to produce their depolarization (Vyklický *et al.* 1972; Krnjević & Morris, 1972; Kříž *et al.* 1974; Bruggencate *et al.* 1974). The difference between the time course of PAD and the transient increase of $[K]_e$ as measured with K-specific micro-electrodes was, however, difficult to reconcile with this suggestion (Somjen & Lothman, 1974). Fig. 7 presents evidence that when the micro-electrode is located closely to discharging neurones, the difference in time course between the $[K]_e$ transients and the P wave is not so great as to exclude the possibility that the PAD is produced by transient increase in $[K]_e$. In this context it has to be realized that there is a distinct difference in the distance of primary afferent terminals and the K-specific micro-electrode from the source of increased K^+, i.e. the neurones. Primary afferents are separated from neurones by a narrow cleft of about 150–200 Å and these neurones can be discharged from various afferent inputs.

Allowing for approximations and considering a neurone as a point source which releases K^+ instantaneously, the time at which maximum concentration is reached (t_{max}) at a distance r, can be calculated from the following equation (Curtis, 1964; Jaeger, 1965):

$$t_{max} = \frac{r^2}{6D \times 10^8}$$

(t_{max} is in sec, r in μm, D is the diffusion coefficient, its value for K^+ at 37° C is 2·3 cm²/sec). The calculated time needed for K^+ diffusion to reach its peak from a point source at a 200 Å distance would be only 0·03 μsec. This means that the latency of PAD would be practically the same as the latency of discharging neurones.

On the other hand a transient increase in $[K]_e$ as measured with the micro-electrode is produced by many sources of K^+ spread over a relatively large region. When neglecting a possible delay produced by narrow pathways in extracellular space and active processes of reabsorption the time needed to reach the peak of $[K]_e$ transient can be understood as a

function of the mean distance of discharging neurones from the tip of the micro-electrode. For a peak latency equal to 40 msec such a mean distance would be about 24 μm. These calculations explain thus the discrepancies between the time course of the transient increase in $[K]_e$ and the PAD and allow for the suggestion that PAD can be produced by a transient increase of $[K]_e$. The discrepancies in the action of various drugs and the specific interaction between the transient increase of $[K]_e$ and the PAD remain, however, to be clarified (Bergmans, Burke, Fedina & Lundberg, 1974; Bruggencate *et al.* 1974).

The effect of long-lasting peripheral stimulation on synaptic transmission in the spinal cord is not a phenomenon which could be explained in simple terms. Both the facilitatory effects, produced by post-tetanic potentiation as well as the depressing effects due to habituation of reflexes in addition to complex synaptic interaction can be expected. The correlation between the time course of $[K]_e$ and the depression of field potentials and the ventral root discharges suggests that a long-lasting increase in $[K]_e$ results in decreased efficacy of synaptic transmission. Whether the depression occurs only at the presynaptic level or also at the post-synaptic elements needs a further study.

It would be farfetched to speculate on the possible participation of increased $[K]_e$ in the processes resulting in long-term plastic changes in the C.N.S. The well known activation of metabolic processes combined with increased O_2 consumption that is induced by increased $[K]_e$ do not, however, exclude this possibility.

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