Potassium and the Recovery of Arterial Smooth Muscle after Cold Storage

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ABSTRACT The influence of K on the performance of vascular smooth muscle was studied by observing the mechanical performance of the muscle under conditions in which the magnitudes of $[K_i]$ and of the $[K_i]:[K_o]$ ratio varied in opposite directions. During prolonged storage at 4°C the artery strips lost K and their ability to respond to stimuli. Subsequently they were transferred to recovery solutions of various $[K_o]$ at 38°C. The initial rate of K_i reaccumulation and steady state $[K_i]$ were greater in solutions of higher $[K_o]$. Conversely for any time during recovery, the greater $[K_o]$, the smaller the $[K_i]:[K_o]$ ratio. When the strip was placed in the warm recovery solution it first contracted and then relaxed. The initial contraction was not relatable to $[K_o]$ of the recovery solution but the subsequent relaxation was greater in rate and magnitude as $[K_o]$ was greater. As the muscles recovered further they went into tonic contracture. As the $[K_o]$ in the recovery solutions was greater these contractures occurred after shorter recovery times, and attained greater amplitude at a faster rate. Solution-switching experiments indicated a dependence of responses to electrical shocks on both the $[K_i]:[K_o]$ ratio and $[K_i]$. Conclusions drawn were: (*a*) increased $[K_i]$ increases contractility, (*b*) increased $[K_i]$ increases the rate of relaxation, (*c*) excitability is decreased by too high or low a $[K_i]:[K_o]$ ratio, and (*d*) the extent of tonic shortening depends on the $[K_i]:[K_o]$ ratio.

Smooth muscle preparations are often stored for hours or days in physiologic salt solutions at low temperatures. Subsequently, when these preparations are incubated at body temperature, they gradually regain the responsiveness which they lost during cold storage. Since this recovery seems to parallel the reaccumulation of lost intracellular K (K_i), it was thought that a comparison of the time courses of these two parameters during recovery might afford some insight into the role K plays in the behavior of arterial smooth muscle.

There are at least four sites at which K ions may influence tension develop-

ment by arterial smooth muscle cells: (*a*) the membrane, (*b*) the coupling mechanism between the membrane and the contractile apparatus, (*c*) the contractile apparatus, and (*d*) the metabolic processes which provide energy to the contractile apparatus.

Previous studies indicate that the influences of electrolytes on the behavior of vascular smooth muscle are similar to those on the more extensively studied tissues. Arterial smooth muscle cells maintain transmembrane gradients of K, Na, and Cl qualitatively comparable to those maintained by other muscle cells (1–3). Resting and action potentials have been recorded from arterial smooth muscle preparations (4–6). Small increases in the external K concentration ($[K_o]$) increase the excitability of frog sartorius muscle by partially depolarizing the membrane, thus bringing the resting potential closer to a critical firing level (7); similar results have been obtained from uterine smooth muscle (8). Since arterial smooth muscles contract tonically it is possible that the $[K_i]:[K_o]$ ratio may be a factor controlling tone by changing the resting potential. The resting potential may influence tension directly, or indirectly by changing the frequency or shape of the action potentials.

$[K_i]$, *per se*, may affect the mechanical behavior of muscle directly. Evidence for the dependence of both twitch and resting tension on $[K_i]$ has been obtained from skeletal (9) and heart muscle (10). It has been reported (11) that K-free solutions, cardiac glycosides, and high stimulation rates cause increased resting tension of arterial strips and greater responses to electrical stimulation. Increasing $[K_o]$ causes increased responsiveness of arterial strips to epinephrine stimulation (12).

In the present study carotid artery strips were first depleted of K_i by cold storage. The strips were then allowed to reaccumulate K_i by incubating them at 38°C in sets of solutions, differing in K concentration. Tonic length of the strips and isotonic shortening in response to electrical stimuli were recorded during recovery. The rate and final level of reaccumulation of K_i were found to be monotonically increasing functions of $[K_o]$. On the other hand the increase in $[K_i]:[K_o]$ ratio was a monotonically decreasing function of the $[K_o]$. Thus it was possible, to some extent, to separate the influences of these two parameters, $[K_i]$ and the $[K_i]:[K_o]$ ratio, on the responses and tonic shortening of the arterial strips.

METHODS

Carotid arteries were removed from adult mongrel dogs 2 days before they were to be studied. Most of the adventitial layer was dissected away and helical strips 2 × 25 mm were cut from the remaining muscular tubes. Each end of a strip was tied to a platinum wire, one of the wires being wound on a glass supporting rod. The wires later served as stimulating electrodes. Prepared strips were stored in this condition

to minimize the time required to mount them in the muscle warming chamber for study. All incubation solutions had the following complement of salts in mM: NaCl, 88; $NaHCO_3$, 25; Na_2HPO_4, 0.5; $CaCl_2$, 2.5; $MgSO_4$, 1.2. In addition each incubation solution contained either 0, 0.5, 1.5, 3, 6, 12, or 24 mM KCl and accordingly, 73, 72, 70, 67, 61, 49, or 25 mM sucrose, to maintain the osmolarity nearly constant at 310 mOsM. All solutions were equilibrated before and during incubations with a gas mixture of 95 per cent O_2, 5 per cent CO_2.

The strips were kept in the 6 mM K incubation solution at 4°C for 36 to 50 hours before an experiment. In order to allow the sucrose to preequilibrate in the extracellular space, the strips were placed in a solution containing 6 mM K and the same concentration of sucrose as the later incubation solution, at least 2 hours before the beginning of an incubation (1). The total osmolarity was kept at 310 mOsM by adjusting the concentration of NaCl. At the time of study the glass rod supporting the preparation was fixed in position and the free platinum wire was hooked to an isotonic recording lever while the strip was still in the 4°C solution. The cold bath was then removed and the 38°C recovery bath was raised into position, immersing the muscle. The change of baths required only a few seconds. The strips were stimulated electrically at 15 minute intervals by 5 second trains of 60 cycles/sec. sine waves. The voltage gradient was always less than 5 volts/cm.

In order to obtain paired mechanical and electrolyte data the parts of the carotids not used for strips were cut into rings and used for electrolyte analyses. These rings of recovering carotid were placed under the same tension and stimulated at the same frequency with the same strength stimuli as were the strips used for mechanical recording. Water content was taken as the difference between the wet weight and weight after drying overnight at 105°C. Na and K were extracted in water and assayed by flame photometry performed as previously described (1). All amounts of water and K are referred to as amounts per kilogram of tissue wet weight.

Since sucrose had been added to the incubation media in known amounts, it was used to estimate extracellular space. Water extracts of tissue rings were analyzed colorimetrically for sucrose, using Dreywood's anthrone reaction (13). The extracellular water of the preparations was calculated as the amount of water in which the determined amounts of tissue sucrose would have to be dissolved to give a solution of the same concentration as that of the bathing medium. The differences in extracellular water per kilogram between tissues sampled at different times during recovery or between tissues recovering in different solutions were not statistically significant. However, the differences between tissues from different dogs were larger and usually significant. The extracellular water of carotid artery preparations from sixteen mongrel dogs in milliliters per kilogram was 251 $\pm$ 28 (SD). The values for extracellular space obtained using various "non-penetrating" molecules are generally larger as the molecules are smaller (13). Sucrose being a relatively small molecule gives relatively large values. Even with the preequilibration of the sucrose, quick transient movements of water across the cell membranes might be undetected using the above methods. However, any lasting or increasing changes occurring during the recovery process would have been detected. There were no measurable changes in the water content of the tissues during recovery.

The value for extracellular water was subtracted from total tissue water to obtain the amount of intracellular water. K_i concentrations are calculated as milliequivalents of K_i/liter of intracellular water. The extracellular K was calculated assuming that it would be present in the extracellular water at the same concentration as in the bathing solution. The amount of intracellular K was taken as the difference between the total tissue K and the amount calculated to be extracellular.

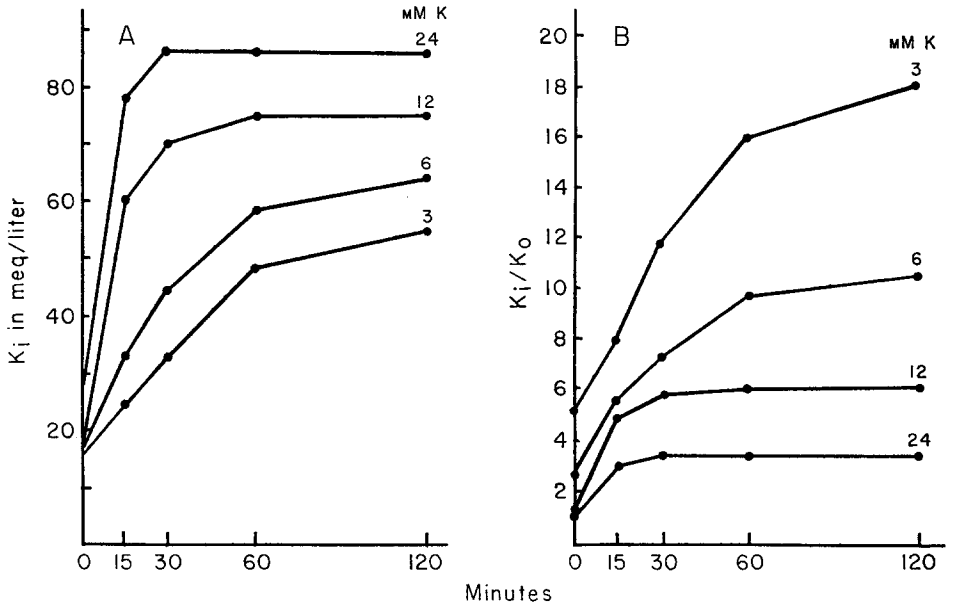

FIGURE 1. Time courses of $[K_i]$ (A) and the $[K_i]:[K_o]$ ratio (B) of a set of muscles recovering from cold storage in solutions of various K concentrations. The values for K_i are calculated as milliequivalents per liter of intracellular water. Each point represents the average of two muscles.

RESULTS

A. Ion Movements during Recovery

When arterial smooth muscles are kept at low temperatures they lose K_i and gain Na_i; they also lose K_i and gain Na_i as a result of dissection (14). In our experiments the tissues were put directly into the cold after removal from the dogs; therefore, the decrease of ion gradients caused by the cold was enhanced by that resulting from the dissection. The $[K_i]$ of the stored tissue did not fall to Gibbs-Donnan levels; this might be expected since the washout of K_i to a new level would be a very slow process even without the complication that probably a small amount of metabolism continues at 4°C. In any case, the ability of the muscle cells to maintain electrochemical gradients of Na and K is crippled by decreasing the temperature to 4°C.

The total amount of K lost by the muscles during dissection and storage always exceeded the amount of Na gained. The reverse was true during recovery. The amount of K moving in was between 1.2 and 2.2 times as great as that of Na moving out. The total Na movement, however, may include movement to extracellular Na binding sites as metabolism is restored (1). The cells did not change volume significantly, therefore the cellular exchange ratio may be closer to unity. Daniel and Robinson (15) have reported similarly related movements of K and Na in isolated uterine segments recovering from cold storage. Steinbach (16) and Stephenson (17) found that the exchange of K for Na by frog sartorii during recovery from cold storage was about one to one.

Rings of dog carotid muscle were allowed to recover in solutions containing different concentrations of K. Figure 1A shows the time courses of K_i reaccumulation by rings from one dog. It is evident that the steady state K concentration attained by the cells increases with increasing $[K_o]$. Although the K_i concentrations are higher as the K_o concentrations are higher, the $[K_i]:[K_o]$ ratios are lower; therefore, when the time courses of the $[K_i]:[K_o]$ ratios during recovery are plotted the order of the curves is reversed (Fig. 1B).

B. Tonic Length Changes

Fig. 2 shows the time courses of changes in tonic length and responsiveness of the muscle strips as they were allowed to recover in solutions differing in K content. When the 4°C storage solution was replaced by a 38°C solution, the change of temperature caused a contraction of unpredictable amplitude. After 2 to 5 minutes the muscles began a protracted relaxation which was dependent on the $[K_o]$ and lasted as long as 60 minutes. The long relaxation phase was then followed by a tonic contracture which occurred sooner and had a greater amplitude as $[K_o]$ was greater. The current study gives some insight into the mechanism of the relaxation and also into that of the observed contractures.

RELAXATION AND $[K_i]$ The rate and degree of the tonic relaxation which characterizes early recovery are functions of the [K] in the recovery solution (Fig. 2). The speed of relaxation varies directly with the rate of reaccumulation of K_i (compare Figs. 1 and 2). The $[K_i]$, rather than the $[K_i]:[K_o]$ ratio, appears to be the determinant of the speed of relaxation since in the high $[K_o]$ solutions where relaxation is most rapid, the increase in the $[K_i]:[K_o]$ ratio is slowest. Conversely, any given ratio is reached sooner in a low $[K_o]$ recovery solution but any given degree of relaxation occurs later. The dependence of relaxation on $[K_i]$ was demonstrated strikingly when no K was added to the recovery solution; K_i could not reaccumulate and no relaxation

occurred (Fig. 2). Therefore, whatever the factor is that develops during recovery and effects relaxation, it is related to the reaccumulation of K_i. It would seem that K_i itself is the most likely factor of this sort. However, it must be noted that other possibilities exist. The membrane potential for example may not follow the $[K_i]:[K_o]$ ratio in these experiments. Thus direct measurements of membrane potential are needed to examine its role in the relaxation process.

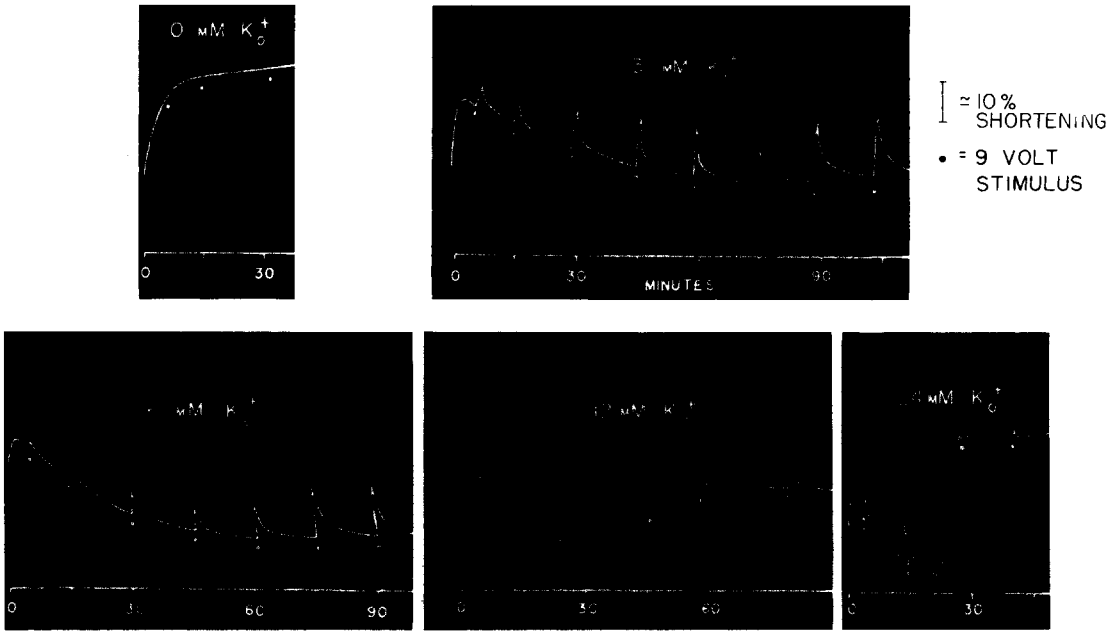

FIGURE 2. Isotonic recordings of arterial strips (2 cm long) during their recovery in solutions containing various K concentrations. Dots below records indicate the delivery of 5 sec. trains of 60 cycles/sec. 9 volt stimuli.

LOW K_i CONTRACTURE The reverse aspect of the dependence of relaxation on $[K_i]$ is the maintained shortening or contracture that develops in the absence of K. When recovery from cold storage is carried out in a K-free solution such a contracture occurs (Fig. 2). A similar contracture develops when a muscle which has recovered in a solution of 6 mM K is presented with a K-free solution (Fig. 4, B and C). A 5 to 10 minute latent period precedes the onset of this contracture. Since the value of the $[K_i]:[K_o]$ ratio was greatest just after the K-free solution was introduced and before the $[K_i]$ had fallen appreciably, the long latent period is evidence that the contracture is not due to the change in the ratio but to the decrease in $[K_i]$. In other experiments in which the change was from 6 mM K to solutions containing 0.5 and 1.5 mM K the contracture was also seen, but its onset was more delayed and

its magnitude was not as great. It is concluded that this type of contracture is caused by low $[K_i]$.

HIGH K_o CONTRACTURE A quite different type of contracture develops when $[K_o]$ is high. This contracture is obviously different in origin and character from the contracture occurring in low $[K_o]$ solutions. The

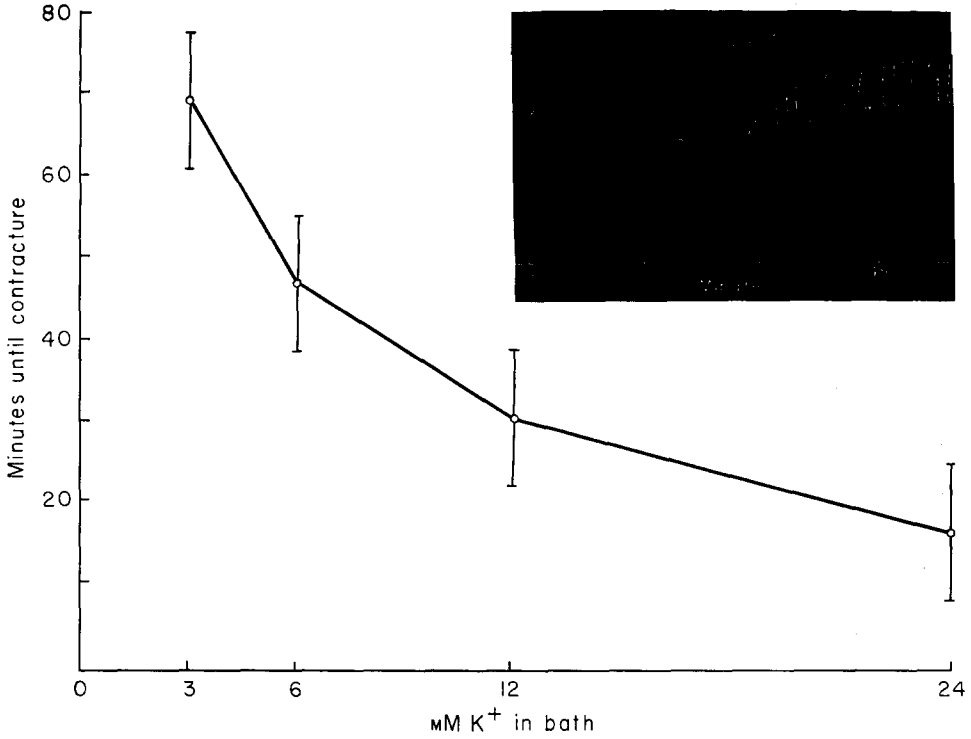

FIGURE 3. Relation between the time of onset of "contracture" and $[K_o]$. Vertical bar indicate standard deviations of the means (n = 5). Inset shows an example of strip becoming spontaneously rhythmic at the time the contracture occurred in most preparations. Note this preparation has not been electrically stimulated. Disturbances in the tracing at 10 and 42 minutes were caused by changing bath.

relaxation process already described is eventually interrupted by such a contracture. In 12 and 24 mM K solutions the onset of the contracture was abrupt as if excitation occurred in one part of the muscle and spread to the rest of the strip. When strips from one animal were allowed to recover, some in 12 mM and some in 24 mM solutions, the onset of contracture in each case was accompanied by rhythmic contractions (Fig. 3). This again suggests that the contracture occurred when one part of the preparation became active and this activity spread throughout the muscle mass. In contrast to the abrupt contractures occurring in 12 and 24 mM K_o, those in solutions

containing 3 or 6 mM K were so gradual that it was difficult to tell when they started. In the presence of periodic electrical stimulation these small contractures appeared as incomplete relaxation from phasic responses.

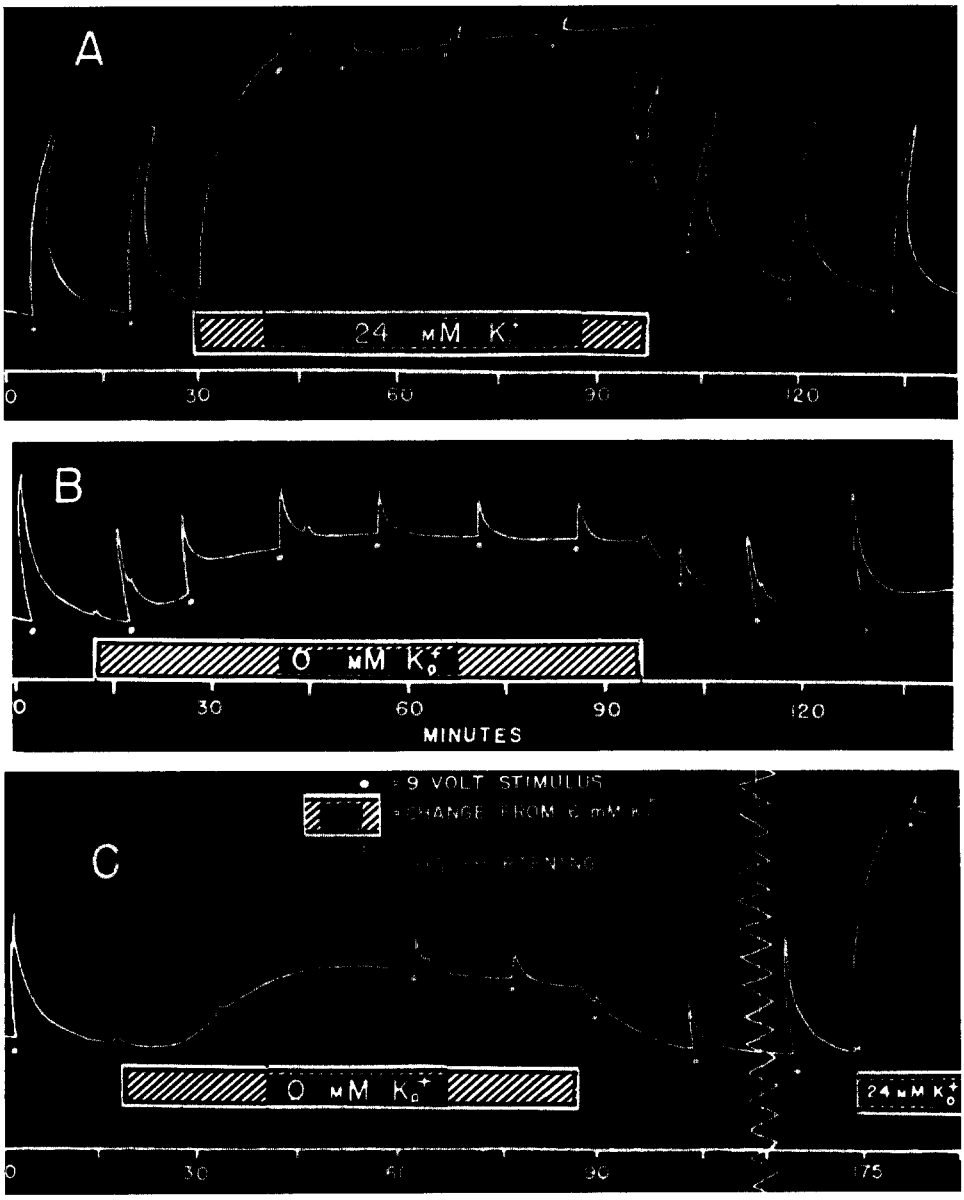

FIGURE 4. Isotonically recorded responses to quick changes of $[K_o]$ of muscles which had fully recovered in 6 mM K solutions. The K_o concentration was 6 mM except where noted. Each dot indicates stimulation by 5 second train of 60 cycles/sec. at 9 volts.

However, the amplitude of the contracture was greater as $[K_o]$ was greater for all values of $[K_o]$ greater than 3 mM (Fig. 5).

The high $[K_o]$ contractures may be elicited in their most predictable and dramatic form when, after a muscle has recovered in 6 mM K_o, the bath is replaced by one containing 24 mM K (Fig. 4). The difference in the $[K_i]$: $[K_o]$ ratio rather than in the $[K_i]$ appears to be the important initiating factor since this contracture began within seconds after the switch was made.

Evidence presented up to this point emphasizes a dependence of the high $[K_o]$ contracture on the $[K_i]$:$[K_o]$ ratio. The contracture is greater when the ratio is lower which suggests that it is a manifestation of increased membrane excitability. The following observations, however, suggest that the high $[K_o]$ contracture cannot be due simply to the $[K_i]$:$[K_o]$ ratio. The time required for the onset of contracture in the muscle recovering from cold storage was greater in solutions containing less K (Fig. 3), yet any given ratio of $[K_i]$:$[K_o]$ is attained more quickly as the recovery solution contains less K (Fig. 1). Thus the time of onset of the high $[K_o]$ contracture is correlated with the reaccumulation of K_i not the attainment of some critical $[K_i]$:$[K_o]$ ratio. Apparently as muscles recover in the low $[K_o]$ solution the cells attain $[K_i]$:$[K_o]$ ratios capable of initiating contracture at a time when the $[K_i]$'s are too low to permit it. In solution-switching experiments in which the tissue had come to equilibrium with 3, 4.5, 6, or 12 mM K, the latent periods for contractures induced by substituting 24 mM K were less than 5 seconds. In these cases the stimulus is the change of the $[K_i]$:$[K_o]$ ratio but since the $[K_i]$ is past the limiting level the amplitudes of contraction are the same. Muscles which have recovered in solutions containing 1.5 mM K or less do not immediately contract when exposed to 24 mM K solutions. Of course, after a long time all muscles will come to the same length in 24 mM K solutions regardless of their history. These observations are in accord with the hypothesis that $[K_i]$ must reach a certain threshold value before high $[K_o]$ contracture can occur. It may be fruitful to speculate that the same function of $[K_i]$ which is responsible for accelerating relaxation is also operative in this requirement for high $[K_o]$ contracture. This could be a plasticizing action of K_i on the contractile protein. The low $[K_i]$ contracture could be due to the inability of the muscle to relax. Other experiments are needed to explore this speculation.

COMPARISON OF HIGH AND LOW K CONTRACTURES The dual influences of external potassium on tonic contracture are emphasized in Fig. 5 which demonstrates that the tonic shortening curve has a minimum between 1.5 and 3 mM K_o. The following observations indicate that the mechanism by which contracture is produced by low $[K_o]$ is different from that by which high $[K_o]$

contracture is produced: (*a*) In recovery experiments the onset of high [K_o] contracture is delayed while in zero [K_o] the contracture commences upon immersion of the muscle in the 38°C bath. (*b*) When muscles which were incubated in zero [K_o] and had fully developed low K contractures were exposed to 24 mM K they first relaxed to low levels before the high K contracture occurred. (*c*) In contractures resulting from recovery in 24 mM K the muscle continues to respond to electrical stimuli, while in the zero [K_o] excitability decreases to zero. (*d*) In solution-switching experiments from 6 mM K_o, con-

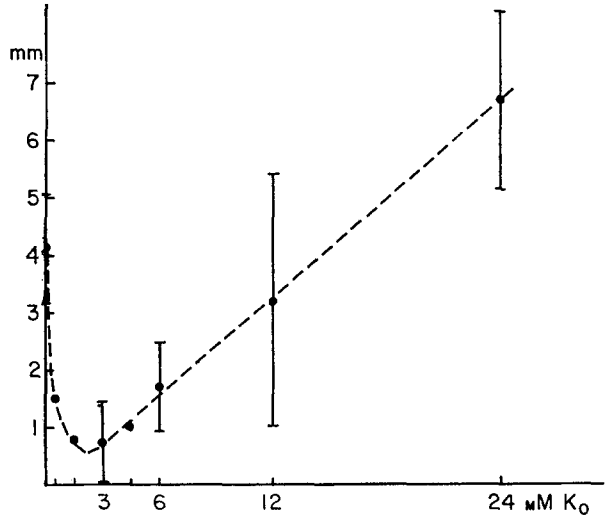

FIGURE 5. Tonic shortening as a function of [K_o] is plotted as millimeters of shortening from the maximum length after 90 minutes of recovery in solutions of various K_o concentrations. The superimposition of phasic responses (Fig. 2) had no effect on the final amplitude of tonic contractures. The vertical bars indicate standard deviations (n = 5); the points without bars are from single observations.

tracture in 24 mM K appears immediately while the contracture in zero [K_o] is delayed. Finally (*e*) zero [K_o] contractures are unaffected by relaxing agents such as isoproterenol (10^{-7} w/w) or para-hydromercuriobenzoate (2×10^{-5} w/w); high K_o contractures are reversibly relaxed by these agents.

C. Phasic Shortening

Phasic responses to electrical stimuli were obtained in all experimental circumstances except when strips were incubated in zero [K] solutions. They usually consisted of an initial rapid shortening period of perhaps 10 seconds, a period of slow change of length near maximum shortening (the isometric period), and finally, an exponential relaxation. In all recovery solutions containing K the phasic responses increased in amplitude as the muscles recovered (Fig. 2).

The increased responses therefore correlate with increased $[K_i]$ and $[K_i]:[K_o]$ ratio, presumably also with the increased membrane potential. Separation of the influences of these parameters on the phasic responses was not possible here. No consistent relation between the maximum amplitude of the responses and $[K_o]$ was found. This failure is explicable in terms of antagonistic influences of K_i, first, increasing the ability to shorten and second, increasing the

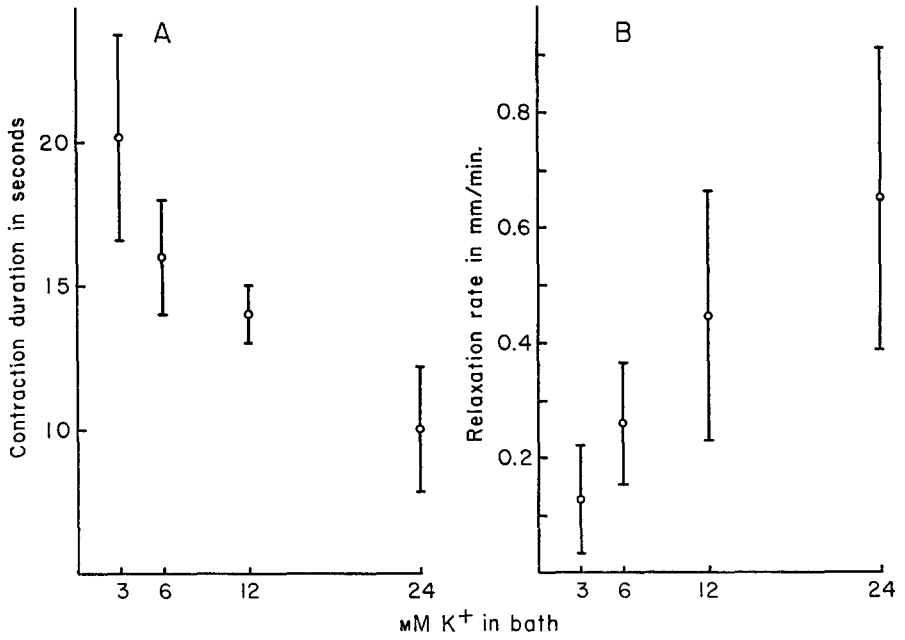

FIGURE 6. Relation between the duration of the isometric period of phasic responses to electric stimuli and $[K_o]$. B shows the maximum rate of relaxation (lengthening) after the isometric period of the phasic responses in various K_o concentrations. Only responses after recovery to the steady state of tone used. Vertical bars indicate the standard deviations of the means in both A and B (n = 10).

ability to relax. Thus, the duration of the isometric period of the phasic responses in the steady state was an inverse function of $[K_o]$ (Fig. 6A). Presumably, the duration of the phasic response may be considered as the time required for the relaxation process to develop far enough to interrupt the contraction. As would be expected from this hypothesis, the maximum rate of relaxation after electrical stimulation increases with increasing $[K_o]$ and thus with $[K_i]$ (Fig. 6B).

Another difficulty in interpreting the variation of phasic responses in different $[K_o]$'s is that these responses arise from correspondingly different tonic contractures. In the extreme the tonic contracture in 24 mM K_o comes so near the maximum response of the muscle that small phasic responses in

this case probably reflect inability of the contractile machinery to shorten further.

When a muscle which had recovered in 6 mM K_o was exposed to a bath of zero $[K_o]$, an informative series of events ensued. The phasic responses were depressed before any change of tone occurred. As shown in Fig. 4B, when such strips were stimulated electrically before the plateau of the zero $[K_o]$ contracture was reached, they did not relax completely. Perhaps the stimulation accelerates the loss of K_i. Phasic responses to electrical stimulation progressively decreased in amplitude as long as the muscle was in a K-free solution. This progressive attenuation of response continued even after the muscle had reached a plateau in tonic shortening. This decrease in responsiveness is correlated with the continuous decline of $[K_i]$ as long as the muscle is in a K-free solution. This decline of $[K_i]$ is relatively slow. Moreover the rate of loss decreases with time. When eight muscles preincubated in 6 mM K_o without storage were transferred to a K-free solution, the $[K_i]$ in milliequivalents per liter H_2O fell from 82.5 ± 2.6 SD at zero time to 67.3 ± 4.2 at 30 minutes, 60.7 ± 3.5 at 60 minutes, and 55.8 ± 8.1 at 120 minutes. The first response after restoring $[K_o]$ to 6 mM was always further attenuated (Figs. 4B and 4C); perhaps this is due to relative depolarization.

DISCUSSION

The decline and restitution of K and Na transmembrane gradients as a function of temperature in arterial smooth muscle are similar to these phenomena in striated muscle (16, 17) and uterine smooth muscle (18), at least within the limits of the data reported here. It would appear that generation of metabolic energy is necessary for the accumulation and maintenance of a high $[K_i]$. Daniel and Robinson (18) found anaerobic metabolism was sufficient for similar recovery of uterine smooth muscles. On the basis of Stephenson's (17) work on the recovery of frog sartorii from the cold, it seems probable that the reaccumulation of K_i occurs against an electrochemical gradient. The existence of a coupling between the extrusion of Na and the accumulation of K has been demonstrated for various nerves, striated muscles and red blood cells (19), and uterine smooth muscle (18). Below 3 mM concentration the rate of extrusion of Na by red blood cells is dependent on $[K_o]$. The accumulation of K_i by larger arterial smooth muscle cells is somewhat similarly dependent but over a greater range, up to 24 mM K_o. Although the fraction of the K accumulation which is passive is unknown here, the active fraction might well be the major determinant of the total uptake. In this regard it should be remembered that these cells have perhaps fifty times more surface area than an equivalent volume of heart cells, and a strong variable capacity ion pump would have adaptive value.

The loci of effects of K on shortening or tension development will be ap-

parent only when the processes at the loci are rate-limiting. The time courses of the variation of $[K_i]$, the $[K_i]:[K_o]$ ratio, and tonic shortening during recovery in various K_o concentrations above 3 mM indicate clearly that shortening is a positive function of $[K_i]$. However, if muscles are allowed to recover in 6 mM K_o and are then exposed to 12 or 24 mM K_o, they shorten rapidly, presumably because of the change of the transmembrane K gradient since sufficient time for change of $[K_i]$ level has not elapsed. This is to be expected if the processes dependent on $[K_i]$ are rate-limiting during the recovery period and the processes controlled by membrane polarization are rate-limiting during the steady state. It seems that a certain level of $[K_i]$ is necessary before shortening can take place since when triggered by sudden exposure to 24 mM K_o, muscles previously equilibrated in 3, 6, or 12 mM K_o quickly contract, all to the same extent. Muscles equilibrated in 1.5 mM K_o or less do not contract immediately when exposed to 24 mM K_o.

Preparations from only a few dogs have shown rhythmic activity. Whether this is due to muscle damage sustained in dissection, to differences between the muscles of different dogs, or simply to lack of requisite components in the bath is not known. We consider it an important observation, in any case, since it is presumptive evidence for the propagation of activity, presumably by action potentials. The best electrical recordings to date in this laboratory have shown only decrementing propagation of action potentials in carotid preparations (4). Burnstock and Prosser (20) have also recorded non-propagating action potentials from arterial smooth muscle.

Increased $[K_i]$ has a plasticizing effect on the contractile apparatus. This is seen in increased rates of relaxation during the first phase of recovery, the onset of relaxation in a phasic response, and the rate of relaxation from tonically shortened states; all are positive functions of the $[K_i]$. Conversely it would appear that if $[K_i]$ is very low, the rate of relaxation becomes rate-limiting and the muscle goes into contracture. Hajdu (10) found that the relaxation rate of frog heart also was a positive function of the $[K_i]$. There is a minimum in the tonic shortening curve between 1.5 and 3.0 mM K_o which may represent a point where the effect on relaxation is overtaken by the effect on contraction.

The extent to which heart twitch tension can be increased by a slower relaxation rate may be important relative to the mechanism of positive treppe phenomena and potentiation by cardiac glycosides. Similar opposing effects of ATP, increased contractility and plasticization, were cited by Benson and Hallaway (21) to explain an optimal concentration for the development of tension in glycerol-extracted heart preparations.

The question may be raised whether it is, in fact, $[K_i]$ which is limiting for the development of tonic shortening, or some other factor related to the recovery process. Since the ability to shorten both tonically and phasically is

correlated with increased $[K_i]$ (when the $[K_o]$ is increased), either $[K_i]$ is responsible or, during recovery from cold storage and in solution-switching experiments, available metabolic energy and $[K_o]$ have parallel effects on $[K_i]$ and the hypothetical responsible factor. The latter alternative seems quite strained unless the $[K_i]$ and the hypothetical factor are causally related. Speculation of this sort is suggested by the large amount of evidence relating Ca to the initiation and regulation of muscle contraction (22–25). Perhaps variation of the $[K_i]:[K_o]$ ratio influences the transmembrane Ca movements by affecting the membrane potential, whereas K_i or Na_i directly competes with Ca for intracellular anionic sites.

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