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The role of sodium pump in the inhibition of smooth muscle responsiveness to agonists during potassium restoration

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Summary

1. Isometric contractions of cat splenic capsular smooth muscle in response to noradrenaline and histamine were recorded.

2. Removal of potassium from the bathing medium did not change the resting tension or the responsiveness to noradrenaline. Restoration of potassium inhibited responses to noradrenaline or histamine only if the muscles were stimulated with an agonist while in the K-free medium.

3. This inhibition of responses to the agonists due to potassium was reversed rapidly by removing the ion or reversed slowly by prolonged exposure to the ion. The inhibition was also blocked by procedures or agents which block the sodium pump (ouabain, substitution of NaCl by LiCl), inhibit active processes (low ambient temperature) or prevent intracellular accumulation of sodium (substitution of choline for sodium).

4. It is proposed that under special circumstances such as when there is an increase in internal sodium concentration, the sodium pump is probably electrogenic and causes relaxation when activated by external potassium. In the normal muscle the pump is probably electrically neutral.

Introduction

Reports on the influence of a low potassium environment on the responses of smooth muscle preparations do not show a consistent effect. For example, absence of potassium reduces the response of guinea-pig taenia coli to barium (Karaki, Ikeda & Urakawa, 1967), while it enhances the responses of rabbit aorta to various agonists (Dodd & Daniel, 1960). Little is known about the effect of restoration of potassium after exposure to potassium-free medium. During recovery from prolonged exposure to a cold potassium-free medium, rat uterus and guinea-pig taenia coli show hyperpolarization (Taylor, Paton & Daniel, 1970; Casteels, Droogmans & Hendrickx, 1971). We have therefore studied the effects of removal of potassium and its subsequent restoration on the responses of smooth muscle of cat spleen capsule to agonists. This is a multi-unit smooth muscle, thus differing from other smooth muscles, e.g. uterus and taenia coli, in which the effects of low potassium have mostly been studied.

Methods

Splenic capsular smooth muscle strips were obtained from cats (1·5–2 kg) of either sex. Cats were stunned and bled; the spleen was removed and a sheet of muscle (0·5 mm thick) shaved off the surface opposite the hilum with a Stadie-Rigg microtome. Strips (15 mm×2 mm) were cut along the longitudinal axis of this sheet and mounted in individual 10 ml organ baths, bathed in Krebs-Henseleit solution (pH 7·4) at 37° C and equilibrated with a 95% O_2: 5% CO_2 gas mixture. The composition of this solution was (mM): NaCl 118; KCl 4·7; KH_2PO_4 1·1; $MgSO_4$ 1·2; $CaCl_2$ 2·5; $NaHCO_3$ 25; glucose 11; this solution is referred to as normal medium.

Isometric tension of the muscle strips was recorded with a Grass FT-03 C force displacement transducer on a Grass polygraph. The preparations were initially stretched to exert a steady resting tension of 1 gram. Before the experiment began the tissues were made to contract submaximally with (−)-noradrenaline bitartrate (Calbiochem) (1 μg base/ml) or histamine diphosphate (Nutritional Biochemical Co.) (3 μg base/ml) at 15 min intervals until responses became stable. These concentrations of noradrenaline and histamine were used throughout the experiments. The contraction was allowed to reach a plateau in each case, usually taking 1·5 to 3 minutes. The agonist was then washed out, except when KCl was to be added; in such experiments strips were exposed to the agonist for 5–7 minutes.

For potassium-free medium (K-free medium) KCl and KH_2PO_4 were replaced by equiosmotic amounts of the appropriate sodium salts. Low potassium solutions were made by introduction of known additional amounts of KCl to the K-free medium to obtain the bath concentrations indicated. The amounts added were small and therefore correction for increase in osmotic pressure was considered unnecessary. Chloride deficient K-free medium was prepared by substitution of sodium isethionate for NaCl. This left only a small (7·2 mM) concentration of chloride in the solution. Where the effects of low concentrations of sodium were to be studied, the standard K-free medium was modified by replacement of different amounts of NaCl by equiosmolar amounts of choline chloride; sodium bicarbonate was omitted and instead *N*-2-hydroxyethyl piperazine-N^1-2-ethane-sulphonic acid (HEPES, 7·4 mM) was used. The pH of the solution was brought to 7·4 by the addition of 3 mM NaOH and the O_2: CO_2 gas mixture was replaced by pure oxygen. This solution is referred to as K-free HEPE medium.

The effect of lithium was studied with the help of a modified solution in which NaCl was replaced by equimolar amounts of LiCl. This procedure was expected to result in intracellular accumulation of Li which is not transported out of the cell by the sodium pump (Keynes & Swan, 1959). Ouabain (Nutritional Biochemical Co.) was used to inhibit the sodium pump (Skou, 1965). Spleen strips depleted of endogenous catecholamines were used for experiments with lithium or ouabain. These were obtained from cats given reserpine (Serpasil, Ciba) 1 mg/kg intraperitoneally 24 h before the experiment.

Differences in results obtained from different groups of tissues subjected to various experimental procedures were tested for statistical significance by Duncan's new multiple range test (Steel & Torrie, 1960).

Results

Effect of potassium deprivation and restoration

In spleen strips from 6 cats stimulation with noradrenaline (1 μg/ml) in normal medium resulted in an isometric contraction having an initial fast phase followed by a slower phase which reached a plateau (Figure 1A). After the preparation

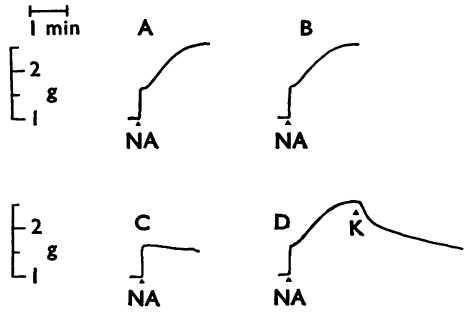

FIG. 1. Effect of exposure to K-free medium and of subsequent restoration to normal potassium medium on the responses of spleen to noradrenaline (NA ; 1 μg/ml). A, in normal medium ; B, second response after 30 min in K-free medium ; C, response 2 min after restoration to normal medium and 15 min after B ; D, 5 min after replacement with K-free medium and 15 min after C ; note relaxant effect of KCl (5 mM) added at the peak of contraction.

gave reproducible responses to noradrenaline the normal medium was replaced by the K-free medium. The medium was first changed every minute for 5 min and thereafter every 5 minutes. Omission of potassium from the bathing medium did not alter resting tension over a 3 h period. Responses to noradrenaline changed little during the first 60 min (Fig. 1B) but decreased by about 25% after 3 hours.

When the muscle strips were returned to normal medium after being stimulated with noradrenaline twice at 15 min intervals in the K-free medium no change in resting tension occurred, but the amplitude of the response to noradrenaline, tested 2–5 min later was decreased, mainly due to inhibition of the slow phase (Figure 1C). Removal of potassium from the bathing medium 5 min before the next addition of noradrenaline restored the normal response. With the agonist still in the bath and the muscle still contracted, addition of KCl (5 mM) caused a prompt decrease in tension (Figure 1D). Smaller relaxations were seen with potassium concentrations as low as 0·3 mM.

In three experiments spleen strips were stimulated with an agonist other than noradrenaline acting on different receptors. These strips were stimulated twice at 30 min intervals with histamine (3 μg/ml) in the K-free medium. The longer interval between doses was required to avoid desensitization of the strips to the agonist. KCl (5 mM) added to the bath when the muscle was fully contracted to the second dose of histamine produced a prompt relaxation as in the experiments with noradrenaline.

Potassium sulphate, 2·5 mM, was added instead of KCl, 5 mM, in 2 experiments where noradrenaline had contracted the smooth muscle in K-free medium.

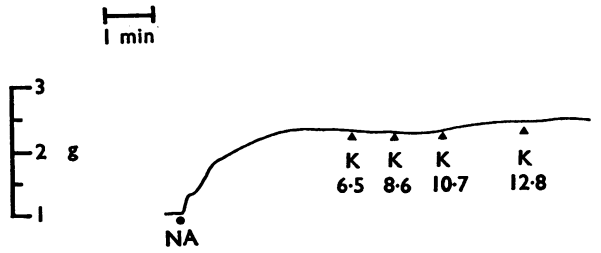

FIG. 2. Effect of KCl (K) on response due to noradrenaline (NA) (1 μg/ml) in normal medium. Bath concentration of potassium (mM) as indicated.

Relaxation occurred in each case, indicating the involvement of the potassium ion. In 3 experiments KCl was added in increasing amounts (6·5, 8·6, 10·7 and 12·8 mM) to strips contracted by noradrenaline in normal medium. In contrast to strips stimulated in K-free medium, no relaxation occurred. Instead a slight additional contraction was seen when the concentration of KCl reached 10·7 mM (Figure 2).

Inhibition of smooth muscle responses to agonists on restoration of potassium, seen in the previous experiments, required a period of exposure to K-free medium as well as repeated stimulation with an agonist while in that medium. Three strips were kept in K-free medium for 30 min without stimulation with noradrenaline. Restoration of potassium did not decrease the response to noradrenaline tested 2 min later (Figure 3).

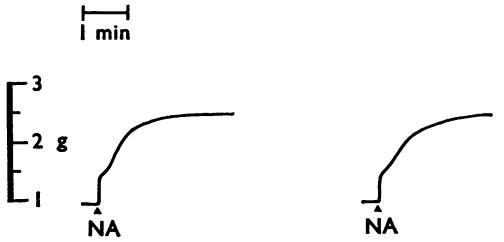

FIG. 3. A, B, contractions due to noradrenaline in normal medium. Between A and B the strip was kept in K-free medium for 30 min without stimulation by noradrenaline. Note contrast to Fig. 1C, which was preceded by two contractions due to noradrenaline in K-free medium.

Recovery from inhibition due to potassium

As already described, the response which had been depressed by restoration of external potassium concentration was restored within 5 min by removal of external potassium. However, where the restored external potassium level was maintained continuously, a slower but nearly complete recovery of sensitivity of the smooth muscle to noradrenaline occurred. Six spleen strips were stimulated

twice with noradrenaline in the K-free medium, returned to normal medium and tested with noradrenaline after 5, 15, 30 and 45 minutes. The response to noradrenaline 5 min after restoration of potassium was only 64% of the control response in the K-free medium. The responses progressively increased with continued exposure to potassium and after 45 min almost complete recovery (98% of control) occurred (Figure 4). Continued presence of noradrenaline during potassium restoration prevented the recovery of sensitivity of smooth muscle

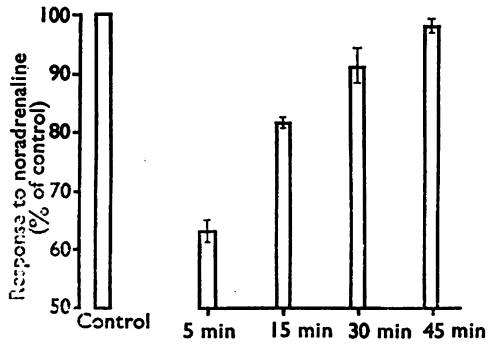

FIG. 4. Time course of loss of inhibitory effect of KCl (5 mM) on the response of spleen to intermittent stimulation with noradrenaline (1 μg/ml) in the continued presence of KCl. The strips had been previously stimulated twice with noradrenaline in K-free medium.

strips that occurred when the responses were tested by intermittent stimulation after restoration to normal medium. In 3 experiments, 2 strips were taken from the same spleen. One strip (Fig. 5A) was stimulated with noradrenaline in the K-free medium twice. Two min after raising the potassium concentration of the bath to 5 mM a single contraction in response to noradrenaline was obtained. This response was markedly smaller than the control response (mean 61%). The strip was then left in the solution containing 5 mM KCl for 30 min and a second response to noradrenaline obtained. This response showed a considerable recovery of sensitivity (mean 86% of control). The second strip from the same spleen (Fig. 5B) was stimulated with noradrenaline in K-free medium twice. At the peak of the second contraction 5 mM KCl was added, causing relaxation. The concentrations of KCl and noradrenaline in the bath were maintained over the next 30 min by repeated replacement, to prevent the decrease of noradrenaline concentration by oxidation. The inhibition of the response to noradrenaline did not disappear; the tension of the muscle diminished progressively to a mean value of 12% of the control level after 30 minutes. The need for KCl in this continuing reduction in response to noradrenaline was shown in 2 additional experiments where strips were treated in the same way as the second strip (shown in Fig. 5B) except that no KCl was added at the peak of the second response to noradrenaline. After a similar prolonged exposure to noradrenaline the response was reduced only to 80% of the initial response. This showed that desensitization could not explain the marked and prolonged inhibition of tension in Figure 5B.

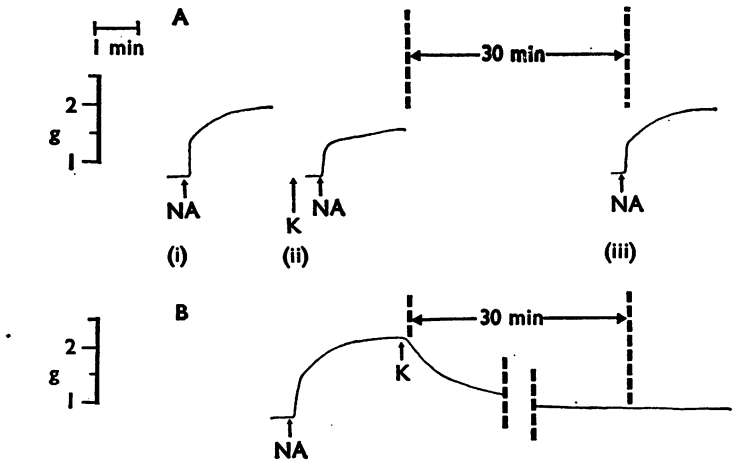

FIG. 5. Prevention by noradrenaline of recovery of spleen capsular smooth muscle from inhibition caused by potassium. A. (i) Second of two responses to noradrenaline (1 μg/ml) in K-free medium; (ii) 2 min after raising potassium concentration to 5 mM; (iii) 30 min after exposure to 5 mM potassium. B. Second of two responses to noradrenaline (1 μg/ml) in K-free medium. KCl (5 mM) and noradrenaline maintained in the bath for 30 min after the contractile response reached its peak.

Effect of potassium in the presence of low external concentrations of sodium

It seemed possible that the conditions of potassium lack and influence of agonist which predisposed the strips to the relaxant action of potassium might have favoured downhill movements of potassium out of and sodium into the cell by inhibiting the sodium pump and increasing membrane permeability to these ions respectively. The effects of various concentrations of sodium in K-free HEPE medium were therefore tested, to determine the importance of raised internal sodium versus decreased internal potassium concentrations in producing the relaxant effect of an increase in external potassium. Since low external sodium has been shown to increase release of intraneural noradrenaline (Bogdanski & Brodie, 1969), this complication was prevented by pretreatment of cats with reserpine to deplete stores of endogenous catecholamines.

Twelve strips from 3 cats were kept in K-free HEPE medium containing 140 mM sodium and stimulated twice with noradrenaline at 15 min intervals. KCl added at the peak of the second contraction caused relaxation. The strips were then exposed to K-free HEPE medium containing 71 mM, 20 mM and 3 mM sodium in different orders. They were then stimulated twice with noradrenaline in each solution and 5 mM KCl was added only at the peak of the second response (Figure 6). Inhibition of response to noradrenaline was greatest in the presence of 140 mM Na (52% of control) and decreased to 49·2 and 12% of control with external Na concentrations of 71 and 20 mM respectively. No relaxation was seen in the presence of 3 mM sodium. The difference in effect of potassium in the presence of 71 mM sodium from that in the presence of 140 mM sodium was not statistically significant. The reduction in the effect of potassium with

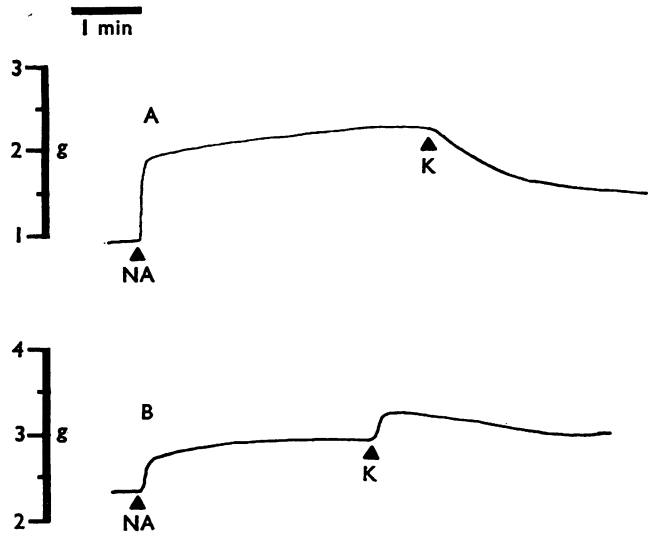

FIG. 6. Effect of low sodium concentrations on potassium-induced relaxation of the response to noradrenaline. Contractions to noradrenaline (NA; 1 μg/ml) in K-free HEPE medium containing 140 mM (A) or 3 mM sodium (B). At ▲ in each contraction KCl (5 mM) was added.

lower concentrations of sodium was highly significant ($P<0.01$). In the presence of 20 or 3 mM sodium, KCl caused a transient contraction (45·6 and 51·5% of control respectively). Additionally, the resting tension of the spleen strips increased at these two low sodium concentrations and the contractile response to noradrenaline decreased.

Effects of inhibitors of the sodium pump

As already indicated downhill sodium and potassium movements were suspected to be predisposing factors in causing inhibition of the response to noradrenaline on restoring external potassium. If the restoration of external potassium acted by restarting the sodium pump then inhibition of the pump should prevent the usual effect of potassium restoration. We therefore tested the effects of impairment of the activity of the sodium pump by ouabain, by substitution of lithium for sodium, and by lowering the temperature, which slows active processes involved in ion pumping (Rall & Gilman, 1970). For the ouabain and lithium substitution experiments, spleens were obtained from reserpine-treated cats to prevent complications due to possible release of endogenous catecholamines by these procedures (Tuttle, 1966).

In 6 experiments spleen strips were stimulated in the K-free medium with noradrenaline 4 times at 15 min intervals. KCl (2 mM) applied at the peak of the fourth contraction caused a marked relaxation (48% of initial contraction due to noradrenaline) (Figure 7A). The muscles were successively exposed to ouabain (3, 10, 30, 100 and 300 ng/ml) and stimulated twice with noradrenaline in the presence of each concentration of ouabain in K-free medium. The effect of KCl (2 mM) was tested only on the second response in each concentration (Figure 7B–F). Low concentrations of ouabain (3 and 10 ng/ml) slightly increased the relaxation

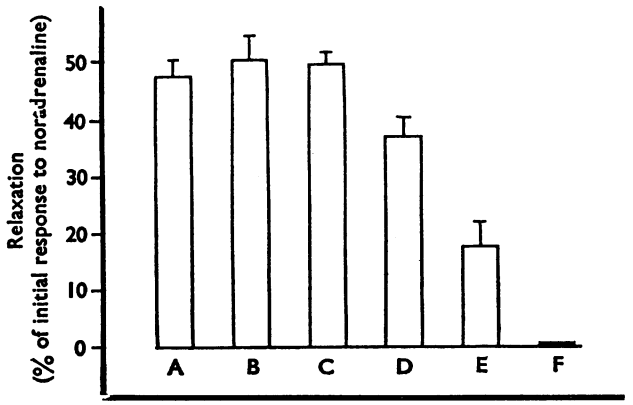

FIG. 7. Effect of ouabain on the relaxation of spleen due to potassium. Strips were stimulated with noradrenaline (1 μg/ml) in K-free medium. KCl (2 mM) was added to the bath at the peak of contraction and the ensuing relaxation was expressed as a percentage of the initial response to noradrenaline in the absence of potassium. A, control relaxation; B, C, D, E and F relaxation in the presence of 3 ng, 10 ng, 30 ng, 100 ng and 300 ng/ml ouabain respectively.

due to KCl. This difference was not statistically significant. Higher concentrations of ouabain (30, 100 and 300 ng/ml) reduced the relaxant effect of KCl to 38, 17·5 and 0% of the initial response to noradrenaline. The decrease in relaxation at each of these doses of ouabain was statistically significant ($P<0{\cdot}05$). The complete blocking action of ouabain is shown in Figure 8A. The blocking effect of ouabain on the relaxation to KCl disappeared after the glycoside was washed out. Time for recovery after washout varied between 30–45 min for the low concentrations of ouabain and was more than 90 min for the largest concentrations (300 ng/ml) used.

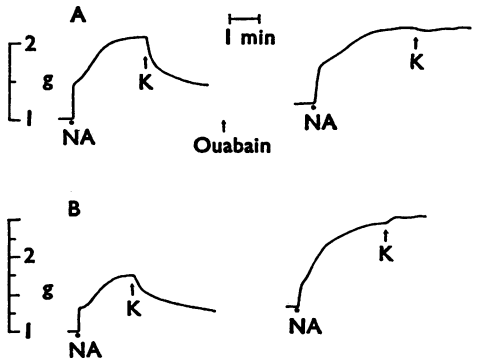

FIG. 8. Effects of ouabain and lithium chloride on relaxation due to potassium chloride. A. Response to noradrenaline 1 μg/ml (NA) in K-free medium before and after addition of ouabain 300 ng/ml. KCl (2 mM) added as shown. B. First response to noradrenaline with relaxation due to KCl was obtained in K-free normal sodium containing medium. Second response 60 min after exposure to a medium in which NaCl had been replaced by LiCl.

Lithium enters the intracellular space when lithium chloride is substituted for sodium chloride; it accumulates intracellularly because the sodium pump cannot pump it out (Keynes & Swan, 1959). Effects such as hyperpolarization when mediated by the sodium pump have been blocked in several tissues by lithium substitution (Tamai & Kagiyama, 1968; Thomas, 1969). To test the effect of lithium, spleen strips from 5 cats were stimulated with noradrenaline twice in the standard K-free medium. The relaxation due to cumulative addition of KCl resulting in bath concentrations of 0·3, 0·6, 0·9 and 1·2 mM were determined on each strip. Each increase in concentration of KCl was made after the relaxation

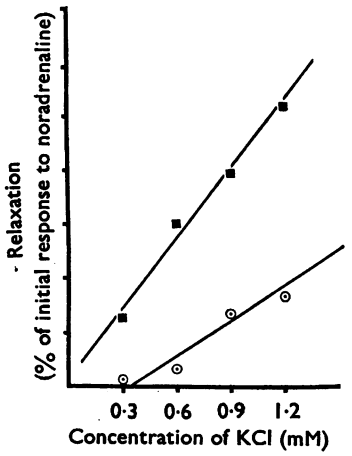

FIG. 9. Relaxation of contraction due to noradrenaline, 1 μg/ml, by potassium in the presence of K-free medium containing normal amounts of sodium (closed squares) or K-free medium with the sodium chloride entirely replaced by LiCl (circles with a dot).

due to the previous lower concentration was complete. The relaxation of the strip due to each concentration of KCl was expressed as a percentage of the initial tension due to noradrenaline (Figure 9). Noradrenaline was then washed out with the modified K-free medium in which LiCl substituted for NaCl. Resting tension increased by 0·25–0·4 g during the next 30 minutes. The strips were stimulated with noradrenaline twice during this period and the effect of cumulative increase in potassium concentration was determined at the peak of the second response (Figure 9). Relaxation due to each concentration of KCl was less in the LiCl medium and the difference was statistically significant ($P<0·05$). In 3 other experiments a longer exposure (60 min) to the lithium-containing K-free medium completely blocked the relaxant effect of higher concentrations of KCl (2–5 mM) (Figure 8B).

Effect of reduction of temperature

The effect of reduced temperature on relaxation due to KCl was determined in 4 experiments. After the usual treatment by exposure to K-free medium and noradrenaline, the relaxant effects of KCl, 4·2 mM, were tested at 37, 33

and 23° C. The relaxations produced by KCl at these temperatures were respectively 66, 50 and 0% of the initial contraction due to noradrenaline (Figure 10). Plotting the inhibitions due to potassium as a function of temperature yielded a Q_{10} of 3·3 over the temperature range of 27–37° C. This suggested the involvement of active processes in the relaxation due to KCl.

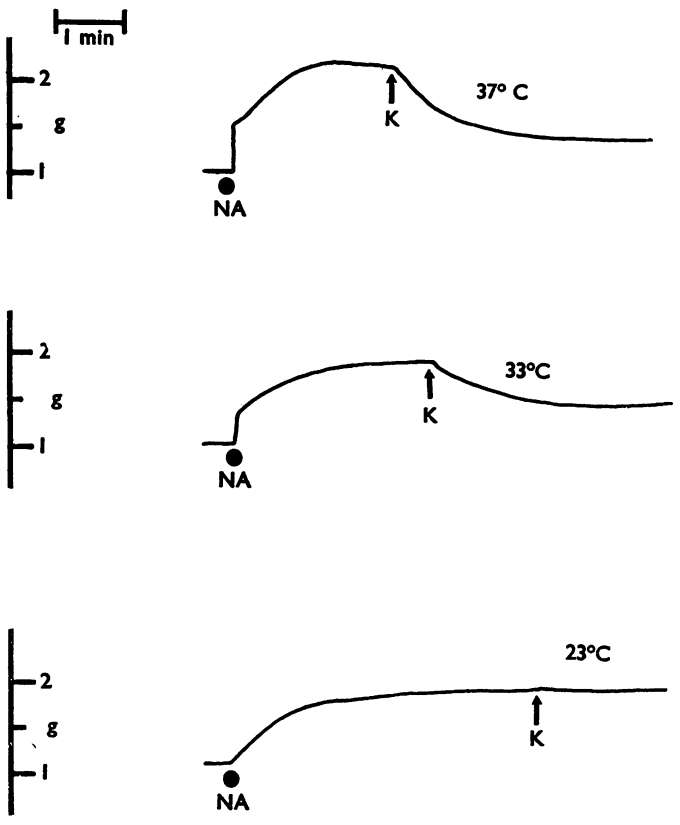

FIG. 10. Effect of temperature on relaxation due to KCl. Noradrenaline, 1 μg/ml (NA), added to K-free medium bathing spleen strips at various ambient temperatures. KCl, 42 mM (K), added as indicated.

Effect of low chloride medium

Four experiments were done to see if chloride played a substantial part in the relaxation induced by potassium. The effect of 2 mM KCl in causing relaxation of the noradrenaline-induced contraction was first determined in the usual way in 4 strips, each from a different cat, in K-free medium containing the normal concentration of NaCl. The strips were then stimulated once more with noradrenaline and then exposed to K-free medium in which NaCl was replaced by an isosmolar concentration of sodium isethionate, thus lowering the chloride concentration in the bathing fluid to 7·2 mM. After 30 min in this solution, the strip

was exposed to noradrenaline and the effect of addition of 2 mM KCl again determined. The mean residual active tensions after KCl, expressed as a percentage of the initial response to noradrenaline, were 54% in the isethionate K-free solution and 52% in the K-free solution containing NaCl, which are not significantly different.

Discussion

Several observations made in this study on splenic capsular smooth muscle resembled those made in the rabbit coronary artery by Norton & Detar (1972). In both preparations reduction of the external potassium concentration did not appreciably affect the responses to agonists, but restoration of potassium inhibited the responses. Sensitivity to agonists subsequently returned with continuing exposure to the normal level of potassium. Norton & Detar (1972) proposed that contractility depends on the ratio of intracellular (K_i) to extracellular (K_o) potassium. An increase in this ratio would enhance contractility, and a decrease would reduce it. The persistence of normal sensitivity to agonists with lowered external potassium concentration was explained by assuming an accompanying decrease in intracellular potassium, leaving the ratio K_i/K_o unchanged. When normal potassium concentration is restored the ratio would transiently decrease, leading to inhibition of the muscle. Gradually K_i would increase, the normal K_i/K_o ratio return and sensitivity to agonists would be restored. If this presumed relationship between the K_i/K_o ratio and contractility holds, it may also be predicted that lowering K_o should produce an immediate increase in the responses of the muscle to the agonist, followed later by a return to the normal response. This was observed in the coronary artery, where abrupt reduction of K_o initially potentiated the response to acetylcholine. After 30 min of exposure to the low potassium medium the potentiation was not seen. While this explanation is attractive and could readily account for some of our observations in the spleen it cannot explain others. Thus in spleen strips treated with ouabain or in sodium-free solution containing lithium an increase in potassium concentration from zero should still decrease the ratio of K_i/K_o, resulting in inhibition of the muscle. This did not happen ; either procedure decreased or prevented the inhibition due to potassium. Additional evidence suggests that alteration in K_i/K_o ratio cannot fully explain the inhibition due to potassium in spleen strips. The relaxation caused by potassium in strips contracted by noradrenaline in K-free medium was reduced when the sodium concentration of the solution was decreased. This change in concentration should not alter the K_i/K_o ratio so as to block the inhibition. In view of these discrepancies we sought an alternative explanation for inhibition induced by potassium. Because ouabain (Skou, 1965), lithium (Keynes & Swan, 1959) or low external sodium (Garrahan & Glynn, 1967) inhibit the sodium pump, it appeared possible that this pump was somehow involved in mediating the relaxation due to the rise in external potassium from zero. This possibility is favoured by the similarity of the concentrations of potassium (<6 mM) needed to relax the smooth muscle and to activate the sodium pump (Garrahan & Glynn, 1967). The high Q_{10} of the relaxation induced by potassium also suggests the involvement of an active process. The conditions necessary for allowing the relaxation due to restoration of extracellular potassium concentration, namely exposure of tissue to a K-free medium and stimulation with agonist are both likely to cause downhill movements

of sodium and potassium by inhibiting the sodium pump (Garrahan & Glynn, 1967) and increasing the membrane permeability to ions (Setekleiv, 1970) respectively. Exposure to K-free medium alone for 30 min did not meet with the requirements for allowing the relaxation. The evidence thus suggests that an increase in internal sodium or a decrease in internal potassium or both may be necessary to attain the proper conditions for reactivation of the sodium pump to inhibit these contractions. The reduced effect of potassium in the experiments with lowered sodium content in the external environment suggests that high internal sodium is more important than low internal potassium.

If activation of the sodium pump reduces the responses due to noradrenaline, one might expect that inhibition of the sodium pump by removal of potassium from the environment of the normal resting muscle should increase the response to noradrenaline. This did not occur. This discrepancy suggests that the sodium pump in resting normal smooth muscle cells may behave differently from the sodium pump activated by the addition of potassium to a K-free medium. The difference could be accounted for if the sodium pump in the normal resting muscle is electrically neutral. Inhibition of such a pump would not produce any significant decrease in membrane potential over a short period of time. Hence no change in responsiveness of the muscle would be expected. However, the sodium pump operating under conditions where the internal sodium concentration is higher than normal may become electrogenic. An electrogenic sodium pump has been shown in several tissues such as rat uterus (Taylor *et al.*, 1970) and guinea-pig taenia coli (Casteels *et al.*, 1971) under conditions which are expected to increase internal sodium concentrations. It is conceivable that hyperpolarization caused by an electrogenic pump may be responsible for inhibition of mechanical responses. This is further suggested by our observation that the inhibitory effect of restoration of potassium is greatest initially but gradually decreases with time. Similarly electrogenic sodium pumps shown in the taenia coli (Tomita & Yamamoto, 1971) and rat uterus (Taylor *et al.*, 1970) cause maximum hyperpolarization initially followed by a gradual return of membrane potential to normal levels. In the spleen too, after the initial inhibition of noradrenaline by potassium, the pump probably becomes progressively less electrogenic as restoration of normal concentrations of internal sodium (and also eventually potassium) proceeds. This could account for restoration of normal responsiveness of the strips. The continued presence of noradrenaline after activation of the pump, instead of a single brief test with noradrenaline, prevents the time dependent recovery from the decreased sensitivity to noradrenaline, presumably because the increased membrane permeability due to the agonist impairs the ability of the sodium pump to restore normal internal sodium and potassium concentrations.

Inhibition of smooth muscle and hyperpolarization due to potassium could also be caused by an increased inward chloride movement or by an increase in potassium permeability. The first possibility is ruled out by the inability of removal of external chloride to affect relaxation due to potassium. The second alternative is more important. External potassium increases membrane permeability to potassium in heart (Vassale, 1965) and smooth muscle (Casteels, 1970). This mechanism can be distinguished unequivocally from an electrogenic sodium pump only by comparing the maximum hyperpolarization due to restoration of potassium with the calculated potassium equilibrium potential and also by the study of

'reversal potentials' with the help of the voltage-clamp technique. This has not been possible in the spleen which, being multiunit, is not suitable for the sucrose gap technique. However the marked temperature dependence and the inhibition of relaxation due to potassium by low concentrations of ouabain support the pump mechanism more strongly.

Although we did not find an increase in responsiveness of spleen strips to agonists when exposed to a low potassium environment, this has been shown on rabbit aorta by Dodd & Daniel (1960). This could be explained by the normal presence of an electrogenic component of sodium pumping in the resting state of these muscles or by accidental gain of sodium by these preparations due to handling of tissues.

The lack of change of resting tension of the spleen strips when exposed to potassium-free medium at least during the first 2–3 h differs from the behaviour of the rat portal vein (Axelsson, Wahlstrom, Johansson & Jonsson, 1967), guinea-pig taenia coli (Tomita & Yamamoto, 1971), rabbit detrusor muscle (Paton, 1971) and occasionally the rabbit coronary artery (Norton & Detar, 1972). The reason for this difference is not clear. Apart from the coronary artery, all these muscle preparations which respond to potassium lack by a rapid increase in tension, normally exhibit spontaneous rhythmicity in the bath and are classed as single unit smooth muscles. The spleen strip, in contrast, is multi-unit and does not exhibit spontaneous contractions. It is possible that the contracture of these single unit muscles and their ability to contract spontaneously may be related to the contribution of an electrogenic sodium pump to the resting membrane potential or to a greater sensitivity of the membrane's potassium permeability to external levels of potassium.

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