PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 252–256 (1980)

Calcium Efflux in Rat Tail Artery during Potassium Induced Relaxation (40857)[1]

R. CLINTON WEBB, PAUL M. VANHOUTTE, AND DAVID F. BOHR

Department of Physiology, University of Michigan, Ann Arbor, Michigan 48109 and Department of Medicine, Universitaire Instelling Antwerpen, 2610 Wilrijk, Belgium

Abstract. The current study investigates the mechanism by which the concentration of activator Ca^{2+} is decreased during potassium induced relaxation of vascular smooth muscle. Helically cut strips of rat tail artery were mounted between a fixed base and force transducers; isometric contractions were recorded. The arterial strips relaxed in response to potassium after contraction induced by norepinephrine in potassium-free solution. The efflux of $^{45}Ca^{2+}$ from the strips was stimulated by norepinephrine during the potassium-free cycle. This increase in efflux was prevented during potassium induced relaxation. D-600, an inhibitor of transmembrane Ca^{2+} flux, reduced the magnitude of potassium induced relaxation by 35%. These observations suggest that relaxation in response to potassium is the result of a decrease in membrane permeability to Ca^{2+} coupled with an increase in Ca^{2+} sequestration at intracellular sites.

Isolated vascular smooth muscle relaxes when potassium is added back to a potassium deficient bathing medium (1–4). Measurement of cell membrane potential during this relaxation indicates that the mechanical relaxation is associated with hyperpolarization due to the electrogenic pumping of sodium and potassium (1, 3, 4). The current study investigates the mechanism by which this hyperpolarization causes the decrease in activator Ca^{2+} concentration responsible for relaxation. This decrease in activator Ca^{2+} could result from: (a) an interruption of Ca^{2+} supply to the contractile apparatus; and/or (b) an increased Ca^{2+} removal due to active extrusion or rebinding to cellular stores (5). From measurements of $^{45}Ca^{2+}$ during potassium induced relaxation we have made deductions about the mechanism by which the concentration of activator Ca^{2+} is reduced.

Methods. Adult male albino rats (Sprague–Dawley, 350–400 g, 12–14 weeks old) were used. The rats were killed by a blow to the head and tail arteries were rapidly excised. The arteries were transferred to a dissection dish containing physiological salt solution (bicarbonate– or Tris–PSS, see below and Footnote No. 2) and cut helically into strips (0.8 × 10.0 mm) under a dissecting microscope.

$^{45}Ca^{2+}$ efflux during potassium relaxation. The method used for measurement of $^{45}Ca^{2+}$ efflux from rat tail artery strips during potassium induced relaxation was similar to that described by Deth and Casteels (6). For each experiment, one helical strip was tied to a stainless steel holder and placed in a muscle bath (50 ml, 37°) containing Tris–PSS for 30–60 min. Following this equilibration, the strip was placed in

[1] These studies were supported by NHLBI Grants HL-03756 and HL-18575 and by a grant from the Belgian Minister of Science Management.

[2] It should be noted that the $^{45}Ca^{2+}$ efflux measurements were performed in Tris–PSS, whereas all other experiments were performed in bicarbonate–PSS. Some investigators have recently reported that Tris–PSS may have deleterious effects on isolated vascular smooth muscle (15, 16). This was not observed in our studies (see 17 also). Contractile responses to potassium-free solution and exogenous norepinephrine were the same in bicarbonate–PSS as they were in Tris–PSS. There was no difference in the rate or magnitude of potassium relaxation in the two buffer systems. Furthermore, ouabain inhibits the relaxation in response to potassium to the same degree in bicarbonate–PSS as in Tris–PSS (unpublished observation). These similarities argue that the specific cellular mechanisms producing potassium relaxation are the same in the two buffer systems. Thus, the comparison of flux data and mechanical responses in Tris–PSS (Fig. 1) with mechanical responses in bicarbonate–PSS (Figs. 2 and 3) are justified.

0037-9727/80/070252-05$01.00/0

Tris–PSS (10 ml, 37°) radiolabeled with 10 μCi/ml $^{45}Ca^{2+}$. The strip was allowed to equilibrate ($^{45}Ca^{2+}$ loading) in this solution for 90 min and then transferred to an identical solution for another 90 min. At the end of this 3-hr loading period the stainless steel holder with strip was transferred to a ring stand. The upper end of the strip was connected to a force transducer (Grass FT03) and the resting tension was adjusted to 500 mg. The strip was allowed to equilibrate for 1 hr in 100 ml of unlabeled Tris–PSS (changed every 15 min). The bathing medium was maintained at 37° and aerated with a mixture of 95% O_2 and 5% CO_2. During the equilibration period, the passive force placed on the strip was readjusted to 500 mg. This level of passive force has been determined to give maximum active force in response to vasoactive stimuli (unpublished observations).

Before the efflux measurements were started, the strip was rinsed for an additional 10 min in 100 ml Tris–PSS. Thereafter, the strip was transferred at 2-min intervals through a series of plastic test tubes containing 2 ml of either normal Tris–PSS, potassium-free Tris–PSS, or potassium-rich Tris–PSS (10.0 m*M* KCl) with or without 10^{-7} g/ml norepinephrine (NE). These solutions contained $^{40}Ca^{2+}$ to prevent alterations in membrane function which may occur in Ca^{2+}-free solutions. The radioactivity in the efflux tubes was determined by liquid scintillation spectrometry.

The composition (mmole/liter) of the normal Tris–PSS was as follows: NaCl, 140; KCl, 4.5; $MgCl_2$, 1.2; dextrose, 5.5; $CaNa_2EDTA$, 0.03; Tris–HCl, 5.0; and $CaCl_2$ $2H_2O$, 1.6. The pH of the solution was 7.4. The potassium concentration of the solution was varied without compensating for changes in tonicity. Tris–PSS was used in order to minimize Ca^{2+} precipitation during efflux measurements. $^{45}Ca^{2+}$ (1.0 mCi/ml) was obtained from New England Nuclear.

Effect of D-600 and norepinephrine concentration on potassium relaxation. Helically cut strips of rat tail artery were mounted vertically on a glass or plastic holder in a tissue bath containing bicarbonate–PSS (see Footnote No. 2). The upper ends of the strips were connected to force transducers and the resting tension was adjusted to 500 mg. The bathing medium was maintained at 37° and aerated with a mixture of 95% O_2 and 5% CO_2. The pH of the solution was 7.4 and the composition (mmole/liter) was as follows: NaCl, 118.3; KCl, 4.7; NaH_2PO_4, 1.2; $CaCl_2 2H_2O$, 2.5; $NaHCO_3$, 25; $MgSO_4$, 1.2; dextrose, 11.1; $CaNa_2EDTA$, 0.03. The concentration of potassium in the bath was varied without compensating for changes in tonicity. Before the start of experiments the strips were allowed to equilibrate for 90 min in bicarbonate–PSS. During the equilibration period, the passive force placed on the strips was readjusted to 500 mg to achieve maximum active force in response to vasoactive stimuli.

Norepinephrine bitartrate and D-600 (methoxy derivative of verapamil) were obtained from Fluka AG or Winthrop Laboratories and Aldrich Europa, respectively. D-600 was kept in an ethanolic stock solution. The stock solution was diluted and added to the bicarbonate–PSS to give a final concentration of D-600 of 10^{-6} to 10^{-4} *M*. The bath concentration of ethanol did not exceed 0.1%. This concentration of ethanol had no effect on the mechanical responses of the tail artery strips. Results are expressed as mean values with the standard errors of the mean (SEM). Statistical analyses were performed with the use of Student's *t* test. In all cases, a *P* value less than 0.05 was selected to denote statistical significance between groups.

Results. $^{45}Ca^{2+}$ efflux during potassium relaxation. Figure 1 illustrates the format of the procedure used to produce potassium relaxation. The rat tail artery strips were placed in potassium-free solution for 20 min. Four minutes prior to the introduction of potassium, NE was added to the efflux tubes to give a concentration of 10^{-7} g/ml. This caused a near maximum contraction. When potassium (10.0 m*M*) was included in the efflux media in addition to the NE, an abrupt relaxation of the strips occurred. Following several minutes of relaxation, a rapid increase in the mechanical response of the strips was observed.

The efflux of $^{45}Ca^{2+}$ from the strips during

the potassium induced relaxation is shown in the lower panel of Fig. 1. Upon introduction of the strips to efflux tubes containing potassium-free solution, there was a small increase in the $^{45}Ca^{2+}$ efflux. This small increase in $^{45}Ca^{2+}$ was not statistically significant. When the strips were placed in potassium-free medium containing 10^{-7} g/ml NE, there was an abrupt increase in the efflux of $^{45}Ca^{2+}$. The inclusion of 10.0 m*M* potassium in the efflux tubes caused an initial decrease in the efflux of $^{45}Ca^{2+}$ which paralleled the decrease in mechanical activity (see upper panel). Following several minutes at this low level of efflux, there was a secondary increase in the $^{45}Ca^{2+}$ efflux which preceded and then paralleled the return of contractile activity. When the strips were exposed to control solution, the $^{45}Ca^{2+}$ efflux returned to a low level.

Effect of D-600 and norepinephrine concentration on potassium relaxation. D-600 caused a small decrease in the magnitude of potassium relaxation (lower panel, Fig. 2). There was a decrease in the absolute magnitude of the contractile response to NE (10^{-7} g/ml) during the potassium-free incubation (upper panel, Fig. 2).

Increasing the concentration of exogenous NE from 10^{-9} to 10^{-5} g/ml caused a decrease in the magnitude of the relaxation in response to potassium (lower panel, Fig. 3). The magnitude of the contractile response during the potassium-free incubation increased with increasing NE concentration (upper panel, Fig. 3).

Discussion. Potassium induced relaxation of vascular smooth muscle has been attributed to membrane hyperpolarization due to activation of the electrogenic pumping of sodium and potassium (1–4). The goal of the present study has been to evaluate the mechanism by which this

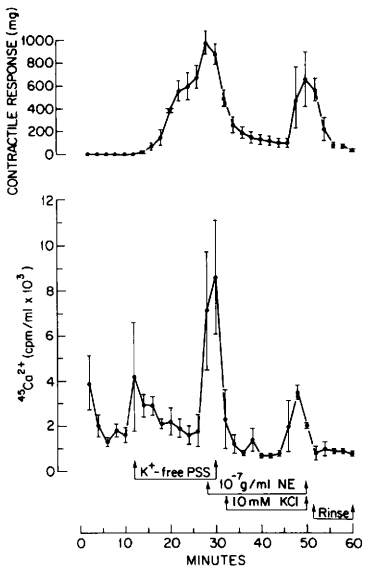

FIG. 1. Efflux of $^{45}Ca^{2+}$ and mechanical activity during potassium relaxation. Helical strips were loaded with $^{45}Ca^{2+}$ as described under Methods. Following equilibration, the strips were transferred at timed intervals through the series of solutions indicated. The upper panel shows the changes in tension of the strips during the potassium induced relaxation and the lower panel shows the efflux of $^{45}Ca^{2+}$ from the strips during this procedure. The values indicated are the means ± standard error of the mean (SEM) for four rats.

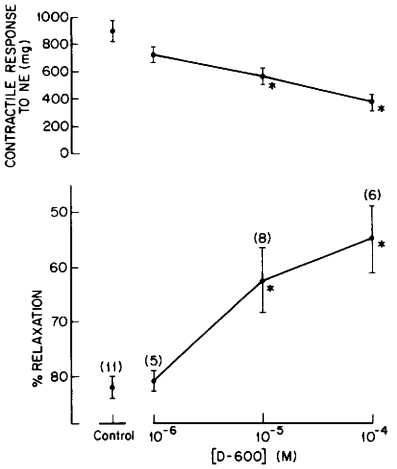

FIG. 2. Effect of D-600 on potassium relaxation. Strips of rat tail artery were exposed to potassium-free solution for 17 min. D-600 (10^{-6} to 10^{-4} *M*) was added to the bath 5 min after the beginning of exposure to potassium-free PSS (12 min before potassium was returned to the bath). Contraction of the strips was induced by 10^{-7} g/ml NE and relaxation was caused by addition of 10.0 m*M* potassium. D-600 produced a concentration-dependent suppression of the magnitude of potassium relaxation (lower panel) and the magnitude of the contractile response to NE (upper panel). Values are the mean ± SEM. The numbers in parentheses are the number of rats used and the asterisks indicate statistical differences from control values ($P < 0.05$).

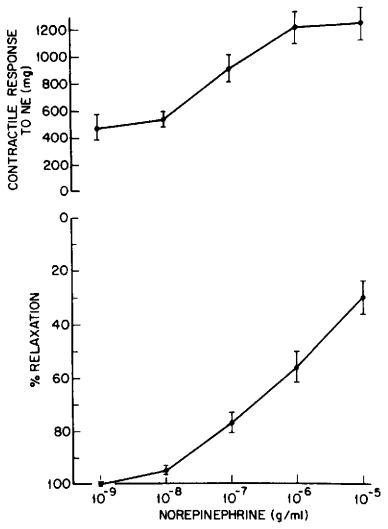

FIG. 3. Norepinephrine concentration and potassium relaxation. Potassium relaxation of rat tail arteries was performed as described in Fig. 2 except that the concentration of NE added to the bath was varied from 10^{-9} g/ml to 10^{-5} g/ml. As the concentration of NE was increased there was a decrease in the magnitude of potassium relaxation (lower panel) and an increase in the contractile response (upper panel). Values are the means ± SEM for six rats.

hyperpolarization causes the decrease in activator Ca^{2+} responsible for relaxation. The results suggest that relaxation induced by potassium causes: (a) closure of passive Ca^{2+} channels; and (b) rebinding of Ca^{2+} to cellular stores.

Potassium-free medium inactivates the electrogenic sodium–potassium pump (1, 2) and leads to sodium accumulation by the smooth muscle cells. Exposure to potassium-free PSS caused: (a) a transient increase in the efflux of $^{45}Ca^{2+}$ which decreased to control levels during the latter portion of the interval; and (b) a gradual contraction which is due to the release of NE from intrinsic nerve endings in the blood vessel wall (7). Addition of exogenous NE to the efflux medium resulted in an increase in contractile tension and a concomitant increase in the efflux of $^{45}Ca^{2+}$ from the strips. Godfraind (8) demonstrated that NE increases, in a similar manner, both the influx and efflux of $^{45}Ca^{2+}$ from rat aortic strips. Similarly, Deth and Casteels (6), Deth and van Breemen (9), and van Breemen and Deth (10) have shown that NE increases $^{45}Ca^{2+}$ efflux from rabbit aortic strips. The source of this $^{45}Ca^{2+}$ is probably intracellular (6, 9, 11) and does not result from mechanical changes in the extracellular space (6, 10, 11). The increase in efflux caused by NE is probably the result of: (a) an opening of passive channels which allows $^{45}Ca^{2+}$ efflux (8); and (b) an increase in the active extrusion of $^{45}Ca^{2+}$ which may accompany the increase in cytoplasmic Ca^{2+} (10).

Upon addition of potassium to the efflux media, an abrupt decrease in contractile tension occurred due to the activation of the electrogenic pump producing membrane hyperpolarization (1, 3, 4). The decrease in the efflux of $^{45}Ca^{2+}$ during potassium induced relaxation suggests that there is a decrease in the number of channels for free Ca^{2+} diffusion into the cell. This hypothesis is strengthened by the observation that D-600 reduced the magnitude of relaxation induced by potassium. Since a high concentration of D-600 did not prevent relaxation completely, hyperpolarization of the membrane must also have an additional effect on activator Ca^{2+} concentration, such as a decrease in the release of Ca^{2+} from cellular storage sites and/or a stimulation of the sequestration of Ca^{2+} into these sites. Following several min of relaxation, there was a return of contractile activity indicating that the electrogenic pump had reduced the intracellular sodium concentration; the pump activity then decreases; the membrane potential returns toward normal; and the presence of NE then causes excitation of the cell and an increase in mechanical response is observed. The efflux of $^{45}Ca^{2+}$ from the strips increased during this return of contractile activity.

Other mechanisms for decreasing the concentration of activator Ca^{2+} do not adequately explain the results of our experiments (see 5 for review). An increase in the activity of an active Ca^{2+} extrusion mechanism would predict that the $^{45}Ca^{2+}$ efflux would increase instead of decrease upon addition of potassium. An increase in the binding of Ca^{2+} by intracellular structures without a change in membrane permeability would suggest that none of the potassium

induced relaxation would be sensitive to inhibition by D-600. A decrease in Ca^{2+} flux across the membrane without involvement of other mechanisms would suggest that the relaxation should be inhibited completely with D-600. A decrease in the delivery of Ca^{2+} from intracellular stores is also unlikely since potassium was readded during the tonic phase of the NE contracture which results from Ca^{2+} of extracellular origin (12). Thus, our data support the hypothesis that potassium relaxation is the result of a decrease in membrane permeability coupled with an increase in Ca^{2+} sequestration at intracellular sites.

Reiner (3) has observed that D-600 inhibited the relaxation induced by potassium in rabbit ear artery. D-600 did not alter the magnitude of membrane hyperpolarization suggesting that hyperpolarization by potassium leads to relaxation by blocking the influx of extracellular Ca^{2+}. More recently, Droogmans and Casteels (13) have observed that the hyperpolarization of the smooth muscle cells in rabbit ear artery by readmission of potassium is not accompanied by a change in the rate of $^{45}Ca^{2+}$ efflux suggesting that hyperpolarization modifies the influx of extracellular Ca^{2+}. The reason for the difference between the present study and those of Reiner (3) and Droogmans and Casteels (13) is not apparent but it may be due to differences in animal species and blood vessels used. The resting membrane potential in smooth muscle cells of rabbit ear artery does not change when the extracellular concentration of potassium is reduced (3, 13). In rat tail artery, reductions in the extracellular concentration of potassium are accompanied by dramatic changes in the resting membrane potential (1, 14). Thus, it may be that the electrogenic sodium–potassium pump is less important in regulating the level of the membrane potential in rabbit ear artery than it is in the rat tail artery.

Since D-600 blocked not only the potassium relaxation but also the contractile response to NE, it is important to determine whether changes in relaxation are the result of changes in the contractile properties of the vascular smooth muscle. The magnitude of potassium relaxation varied inversely with the magnitude of the NE contraction when the contractile state was altered by changing NE concentration. When D-600 was added, the magnitude of potassium relaxation varied directly with the magnitude of the NE contraction suggesting that the decrease in potassium relaxation produced by D-600 is not a simple reflection of an alteration in the magnitude of the NE contractile response.

1. Bonaccorsi, A., Hermsmeyer, K., Aprigliano, O., Smith, C. B., and Bohr, D. F., Blood Vessels **14**, 261 (1977).
2. Webb, R. C., and Bohr, D. F., Blood Vessels **15**, 198 (1978).
3. Reiner, O., Naunyn-Schmiedeberg's Arch. Pharmacol. **303**, 213 (1978).
4. Hendrickx, H., and Casteels, R., Pflug. Arch. **346**, 299 (1974).
5. Bohr, D. F., and Webb, R. C., *in* "Mechanisms of Vasodilation" (P. M. Vanhoutte and I. Leusen, eds.), p. 37. Karger, Basel (1978).
6. Deth, R., and Casteels, R., J. Gen. Physiol. **69**, 401 (1977).
7. Bonaccorsi, A., Hermsmeyer, K., Smith, C. B., and Bohr, D. F., Amer. J. Physiol. **232**, H140 (1977).
8. Godfraind, T., J. Physiol. **260**, 21 (1976).
9. Deth, R., and van Breemen, C., J. Membr. Biol. **30**, 363 (1977).
10. van Breemen, C., and Deth, R., *in* "Ionic Actions on Vascular Smooth Muscle" (E. Betz, ed.), p. 26. Springer-Verlag, Berlin (1976).
11. Potter, J. M., Sparrow, M. P., and Simmonds, W. J., Aust. J. Exp. Biol. Med. Sci. **48**, 429 (1970).
12. Steinsland, O. S., Furchgott, R. F., and Kirpekar, S. M., Circ. Res. **32**, 49 (1973).
13. Droogmans, G., an Casteels, R., J. Gen. Physiol. **74**, 57 (1979).
14. Hermsmeyer, K., Circ. Res. **38**, 362 (1976).
15. Turlapaty, P. D. M. V., Altura, B. T., and Altura, B. M., Amer. J. Physiol. **235**, H208 (1978).
16. Turlapaty, P. D. M. V., Altura, B. T., and Altura, B. M., Amer. J. Physiol. **237**, H410 (1979).
17. Johansson, B., Ljung, B., Hellstrand, P., Uvelius, B., and Sigurdsson, S., Amer. J. Physiol. **237**, H409 (1979).

Received June 14, 1979. P.S.E.B.M. 1980, Vol. 164.