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Monovalent Ion Specificity of the Electrogenic Sodium Pump in Vascular Smooth Muscle[1] (41090)

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Abstract. Experiments were performed to determine the monovalent ion specificity of the electrogenic sodium pump in vascular smooth muscle. Helical strips of rat tail artery relaxed in response to potassium after contraction induced by norepinephrine in potassium-free solution. This relaxation is due to the electrogenic pumping of sodium and potassium which produces membrane hyperpolarization. The magnitude of the relaxation was decreased when the strips were incubated in solutions containing low concentrations of potassium (0.01–5.0 m*M*) instead of zero potassium. Potassium-induced relaxation was inhibited by ouabain. Helical strips also relaxed in response to rubidium, cesium, and ammonium when these were added instead of potassium to a potassium-free solution. The effectiveness of the monovalent ions in producing relaxation was in the following order: rubidium ≥ potassium > ammonium > cesium. Ouabain inhibited the relaxation responses induced by these latter ions. The results suggest: (i) small changes of the external potassium concentration can modify the amplitude of potassium-induced relaxation, presumably by affecting the intensity of the electrogenic pump; and (ii) rubidium, cesium, and ammonium ions can substitute for potassium in producing sodium pump electrogenicity, as evidenced by relaxation after addition of these ions and by inhibition of the relaxation in response to these ions by ouabain.

It now seems reasonably certain that sodium–potassium adenosine triphosphate (Na-K ATPase) provides the mechanism for the electrogenic pumping of sodium and potassium (1, 2). As a result of the activity of this enzyme, the electrogenic pump in vascular smooth muscle maintains high-intracellular potassium and low-intracellular sodium concentrations. Activation of the electrogenic pump hyperpolarizes the membrane since more sodium is moved out of the cell than potassium is moved into the cell. Membrane hyperpolarization under these conditions produces relaxation of vascular smooth muscle (3).

Recent observations (4–6) suggest that potassium-induced relaxation may be used as a functional indicator of Na-K ATPase activity in vascular smooth muscle. In the current study, a comparison was made of the ability of monovalent ions (rubidium, cesium, and ammonium) to substitute for potassium in producing relaxation.

Methods. Adult male and female Sprague–Dawley rats (250–350 g) were used. All animals were killed by a blow to the head and tail arteries (0.7 to 0.8 mm, o.d.) were excised. The arteries were stored in physiological salt solution (PSS) and cut helically into strips (0.8 × 12 mm) under a dissecting microscope. The helical strips were mounted vertically on either a glass or a plastic holder in a tissue bath containing PSS. The upper ends of the strips were connected to force transducers (Grass FT.03) 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, 130; KCl, 4.7; NaH_2PO_4, 1.18; $MgSO_4 \cdot 7H_2O$, 1.17; $CaCl_2 \cdot 2H_2O$, 1.6; $NaHCO_3$, 14.9; dextrose, 5.5; and $CaNa_2EDTA$, 0.03. Potassium concentrations in the bath varied without compensating for changes in tonicity. Before the start of experiments, the strips were allowed to equilibrate for 60–90 min in

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PSS. During the equilibration period, the passive force placed on the strips was readjusted to 500 mg.

The results of these experiments were statistically analyzed by a variety of procedures. Individual means were compared by the use of unpaired t tests. ED_{50} values were determined by linear regression (values are expressed as geometric means). The relationship between crystal ionic radius and relaxation magnitude was determined by linear regression. In all cases, a P value less than 0.05 was considered to be statistically significant.

Drugs used were norepinephrine bitartrate (Winthrop Laboratories) and ouabain (Nutritional Biochemical Corp.).

Results. *Relaxation induced by potassium.* The tracing in Fig. 1 illustrates the format of the procedure used to evaluate the electrogenic sodium pump by the magnitude of potassium-induced relaxation. The rat tail artery, placed in a potassium-free solution, undergoes a gradual contraction in response to intrinsic norepinephrine released from nerve endings in the vessel wall (7). Two minutes prior to the test for potassium-induced relaxation, norepinephrine (10^{-7} g/ml) was added to the muscle bath. This caused a near maximum contraction. When potassium (5.0 mM) was returned to the bath, an abrupt relaxation was observed. The magnitude of this relaxation was quantified as a percentage of the total contraction (resulting from intrinsic and extrinsic norepinephrine) that existed just before the potassium was added. Following a few minutes of relaxation there was a spontaneous return of contractile tension. The magnitude of relaxation in response to 5.0 mM, potassium decreased as the concentration of potassium was increased during the incubation interval (Fig. 2).

Relaxation in response to potassium, rubidium, cesium, and ammonium. Figure 3 illustrates that the monovalent cations, rubidium, cesium, and ammonium, can substitute for potassium in producing relaxation of rat tail artery strips. The amplitude of the relaxation was dependent upon the concentration of the monovalent ion. The effectiveness of the monovalent ions in producing relaxation was in the following order: rubidium $\geq$ potassium $>$ ammonium $>$ cesium. Calculation of ED_{50} values indicated that the relaxation response was more sensitive to potassium and rubidium as compared to ammonium and cesium (Table I). The amplitude of the maximum relaxation in response to the different monovalent ions was significantly correlated with the ionic radius of the respective ion (Fig. 4).

Ouabain, an inhibitor of the electrogenic sodium pump, was found to cause a decrease in the amplitude of relaxation in re-

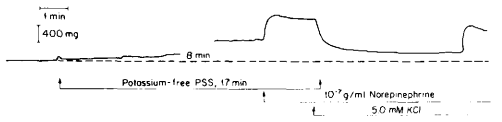

FIG. 1. Potassium relaxation of rat tail artery. A helical strip of rat tail artery was made to contract by exposure to potassium-free PSS. Following 15-min exposure to potassium-free solution, 10^{-7} g/ml norepinephrine was added to the muscle bath. Two minutes later, 5.0 mM potassium was added, and a decrease in tension was observed. After a few minutes an abrupt redevelopment of tension occurred.

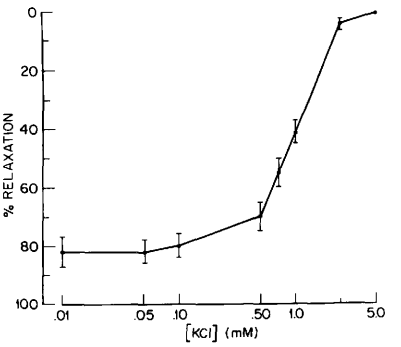

FIG. 2. Effect of potassium concentration on relaxation induced by potassium. Helical strips of rat tail artery were placed in solutions containing different concentrations of potassium (0.01–5.0 mM) for 15 min. The strips were then made to contract in response to 10^{-7} g/ml norepinephrine. Two minutes later, the bath concentration of potassium was increased by 5.0 mM. The magnitude of relaxation following addition of 5.0 mM potassium decreased as the concentration of potassium used during the incubation was increased. Values are the means ± SEM for six rats (two strips per rat).

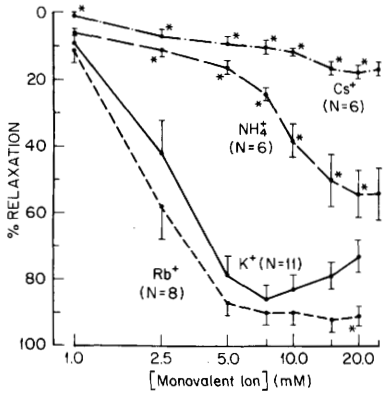

FIG. 3. Relaxation in response to potassium, rubidium, cesium, and ammonium. Helical strips of rat tail artery were placed in potassium-free solution for 17 min. Following 15-min exposure to potassium-free environment, 10^{-7} g/ml norepinephrine was added to the bath. Two minutes later, potassium, rubidium, cesium, or ammonium was added to the bath at the concentrations indicated. Relaxation in response to rubidium and to potassium were similar in magnitude, whereas cesium and ammonium produced relaxations of smaller magnitude. Values are the means ± SEM for 6–11 rats. The asterisks indicate a statistically significant difference from values obtained with potassium ion ($P < 0.05$).

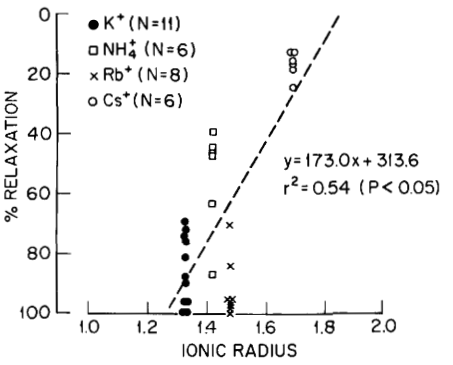

FIG. 4. Ionic radius and relaxation to potassium, rubidium, cesium, and ammonium. The magnitude of the maximal relaxation in response to potassium, rubidium, cesium, and ammonium (data from Fig. 3) was significantly correlated with the ionic radius of the respective monavalent ions. The numbers in parentheses are the number of rats.

sponse to potassium, rubidium, cesium, and ammonium (Fig. 5).

Discussion. The purpose of the present study was to investigate the monovalent ion specificity of the electrogenic sodium pump (and hence, Na-K ATPase) in vascular smooth muscle. The interpretations of the results are based on the observation that potassium-induced relaxation of isolated vascular strips is a functional indicator of pump activity (4–6). Additionally, it is assumed that membrane-bound Na-K ATPase provides the enzymatic mechanism for the active transport of sodium and potassium across the cell membrane (1, 2). These assumptions seem justified since the amplitude of potassium-induced relaxation has been observed to parallel the following

TABLE I. ED_{50} VALUES FOR RELAXATION IN RESPONSE TO MONOVALENT IONS

Monovalent ion	ED_{50}	*N*
Potassium	3.0 ± 3.0	11
Rubidium	2.4 ± 0.2	8
Ammonium	5.5 ± 1.0*	6
Cesium	6.8 ± 0.4*	6

Note. The concentrations of each monovalent ion required to produce half-maximal relaxation were calculated from normalized values presented in Fig. 3. Values are expressed as geometric means ± SEM. Asterisks indicate statistical differences between potassium and the other monovalent ions ($P < 0.05$).

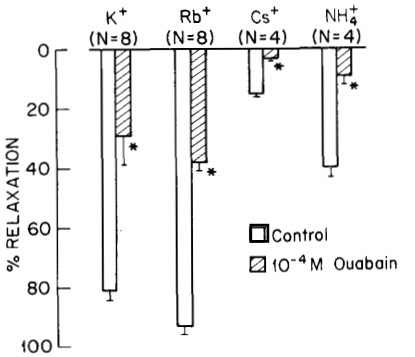

FIG. 5. Effect of ouabain on relaxation induced by potassium, rubidium, cesium, and ammonium. Relaxation in response to potassium, rubidium, cesium, and ammonium (15 m*M*) was performed as described in Fig. 3. Addition of ouabain (10^{-4} *M*) at 5 min into the potassium-free interval (12 min before the addition of the ions) reduced the magnitude of relaxation induced by potassium, rubidium, cesium, and ammonium. Values are the mean ± SEM. The asterisks indicate a statistically significant difference from control values ($P < 0.05$).

variables which are known to influence the activity of Na-K ATPase (5): (i) intracellular sodium concentration; (ii) ouabain administration; (iii) magnesium concentration; (iv) temperature; and (v) potassium concentration.

Relaxation of isolated rat tail artery strips in response to potassium, rubidium, cesium, and ammonium was quantitated following contraction induced by norepinephrine in potassium-free solution. The observations of this investigation may be summarized as follows: (i) small changes of the external potassium concentration can modify the amplitude of potassium-induced relaxation, presumably by affecting the intensity of the electrogenic pump; and (ii) rubidium, cesium, and ammonium can substitute for potassium in producing sodium pump electrogenicity, as evidenced by relaxation after addition of these ions and by inhibition of the relaxation in response to these ions by ouabain.

External potassium concentration and potassium-induced relaxation. Earlier studies (4–6, 8) have shown that the amplitude of potassium-induced relaxation in rat tail artery increases as the concentration of added potassium increases over a range of 1 to 15 m*M*. This relationship probably reflects activation of the pump at a constant intracellular concentration of sodium since the strips were first incubated in potassium-free solution for 17 min. In the present experiments it was observed that the magnitude of relaxation in response to 5.0 m*M* potassium decreased as the potassium concentration of the solution in which the strips were first incubated increased. This latter relationship probably reflects the dependency of pump activity on the concentration of intracellular sodium. It is known that intracellular sodium accumulation is importantly related to the concentration of extracellular potassium and the level of activity of the pump is dependent on the intracellular sodium concentration (1, 2). It is interesting to note that the relationship between the magnitude of relaxation (when added potassium is held constant) and the concentration of potassium used in the incubation interval is sigmoidal (Fig. 2). Na-K ATPase preparations from a variety of sources respond in a similar sigmoidal fashion to increasing concentrations of sodium (9–14).

Relaxation in response to rubidium, cesium, and ammonium. Rubidium, cesium, and ammonium were observed to substitute for potassium in producing ouabain-sensitive relaxation of isolated tail artery strips. The ED_{50} values indicated that the relaxation response was more sensitive to potassium and rubidium as compared to ammonium and cesium. Based on the magnitude of relaxation, the order of effectiveness was: rubidium $\geq$ potassium $>$ ammonium $>$ cesium. The results are consistent with investigations on human red cells (15), skeletal muscle (16, 17), nerve (18), myometrial smooth muscle (19), and gastrointestinal smooth muscle (20), where sodium extrusion has been observed to show a similar monovalent ion specificity.

We also observed that the magnitude of the maximal relaxation was significantly correlated with the ionic radius of the respective ion (Fig. 4). These results suggest that the activation sites for potassium on the external side of the membrane are specific for ion size.

There is little information available concerning the physical nature of potassium and sodium activation sites on the pump. The ionic radii of dehydrated sodium and potassium are about 0.95 and 1.33 Å, respectively. Thus, it is not unreasonable to assume an independence between ion interactions which would allow the sodium pump to bind potassium out of an extracellular matrix containing high sodium and low potassium. Lindenmayer *et al.* (21) have presented evidence for Na-K ATPase preparations isolated from bovine brain that the affinity of the potassium activation sites is related to their size and also that the dehydrated cation as opposed to the hydrated ion binds to the site. This hypothesis agrees well with our observations since the magnitude of relaxation was not correlated with the radii of the hydrated cations (data not shown). Schwartz *et al.* (1) suggest that the hydration sphere is removed from the cations as they interact with the system. Al-

ternatively, Pressman (22) and Winkler and Eigen (23) have suggested that an ionophoretic-like site exists within the pump and substitutes for the hydration sphere of the cation. This would mean that the activity of the pump would be determined in part by the size of the activation site into which the dehydrated cation enters. A similar relationship for potassium activation sites on the electrogenic pump in vascular smooth muscle would explain the effectiveness of these ions in producing ouabain-sensitive relaxation.

1. Schwartz, A., Lindenmayer, G. E., and Allen, J. C., Pharmacol. Rev. **27**, 3 (1975).
2. Wallick, E. T., Lane, L. K., and Schwartz, A., Annu. Rev. Physiol. **41**, 397 (1979).
3. Anderson, D. K., Fed. Proc. **35**, 1294 (1976).
4. Bonaccorsi, A., Hermsmeyer, K., Aprigliano, O., Smith, C. B., and Bohr, D. F., Blood Vessels **14**, 261 (1977).
5. Webb, R. C., and Bohr, D. F., Blood Vessels **15**, 198 (1978).
6. Webb, R. C., and Bohr, D. F., Amer. J. Physiol. **235**, C227 (1978).
7. Bonaccorsi, A., Hermsmeyer, K., Smith, C. B., and Bohr, D. F. Amer. J. Physiol. **232**, H140 (1977).
8. Webb, R. C., and Bohr, D. F., J. Comp. Physiol. **135**, 357 (1980).
9. Garay, R. P., and Garrahan, P. J., J. Physiol. (London) **231**, 297 (1973).
10. Green, A. L., and Taylor, C. B., Biochem. Biophys. Res. Commun. **14**, 118 (1964).
11. Post, R. L., Merrit, C. R., Kinsolving, C. R., and Albright, C. D., J. Biol. Chem. **235**, 1796 (1960).
12. Robinson, J. D., Biochemistry **6**, 3250 (1967).
13. Robinson, J. D., Arch. Biochem. Biophys. **139**, 17 (1970).
14. Skou, J. C., Acta Biochem. Biophys. **23**, 394 (1957).
15. Hoffman, J. F., Amer. J. Med. **41**, 666 (1966).
16. Adrian, R. H., and Slayman, C. L., J. Physiol. (London) **184**, G70 (1966).
17. Beauge, L. A., and Sjodin, R. A., J. Physiol. (London) **194**, 105 (1968).
18. Rang, H. P., and Ritchie, J. M., J. Physiol. (London) **196**, 183 (1968).
19. Taylor, G. S., Paton, D. M., and Daniel, E. E., Comp. Biochem. Physiol. **38A**, 251 (1971).
20. Widdicombe, J. H., J. Physiol. (London) **266**, 235 (1977).
21. Lindenmayer, G. E., Schwartz, A., and Thompson, H. K., J. Physiol. (London) **236**, 1 (1974).
22. Pressman, B. C., Fed. Proc. **27**, 1283 (1968).
23. Winkler, R. and Eigen, M., *in* "Molecules Bioenergetics and Macromolecular Biochemistry" (H. H. Weber, ed.), p. 130. Springer-Verlag, Berlin, (1972).

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