HYPEROSMOTIC APPLICATIONS OF KCl INDUCE VASCULAR SMOOTH MUSCLE CONTRACTION WHICH IS INDEPENDENT OF EXTERNAL Ca

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K-induced contraction is widely used in studies on excitation-contraction coupling in smooth muscle and is also utilized as a standard contraction in the evaluation of smooth muscle relaxants. It has become accepted that K-induced contraction in smooth muscle is the result of Ca influx from the extracellular medium through a voltage-sensitive Ca channel. A solution containing a high concentration of K is prepared either by substituting NaCl with isosmolar KCl or by adding KCl hyperosmotically to the physiological solution. In this communication we report that the hyperosmotic application of KCl induces a contraction in vascular smooth muscle which is independent of external Ca.

Media-intimal layer of rabbit thoracic aorta was prepared as described by Karaki and Urakawa (1). Sections of taenia coli were dissected from the caecum of male guinea pigs. Isometric contractile tension of the smooth muscle preparations was recorded under a resting tension of 1 g for aorta and 0.2 g for taenia coli. The normal physiological solution used was (in mM): NaCl, 136.9; KCl, 5.4; CaCl₂, 1.5; MgCl₂, 1.0; NaHCO₃, 24.0; and glucose, 5.5, pH 7.4, at 37°C, and bubbled with a 95% O₂ and 5% CO₂ mixture (2).

In the aorta, cumulative, hyperosmotic applications of KCl induced a graded contraction which reached a maximum when the concentration of KCl was 40 mM. Further

increase in the concentration of KCl did not change the developed tension level. In a Ca-free solution containing 0.5 mM ethyleneglycol bis (β -aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), cumulative application of KCl unexpectedly induced a graded contraction. As shown in Fig. 1, hyperosmotic applications of 60 mM and 80 mM KCl to the Ca-free solution induced contractions of approximately 15% and 50%, respectively, of that induced in the presence of Ca. Additional increase in the concentration of added KCl further increased the

Fig. 1. Effects of hyperosmotic applications of KCl, NaCl or sucrose on muscle tension in the media-intimal layer of rabbit aorta. Ordinate: isometric muscle tension relative to the maximum contraction induced by KCl in the presence of 1.5 mM Ca. Abscissa: concentration (mM) of added KCl, NaCl or sucrose. Ca-free: Ca depleted solution containing 0.5 mM EGTA. Points are means $\pm$ S.E. of 4 to 6 experiments.

muscle tension. A similar concentration-response relationship in terms of added KCl was obtained in a solution containing both 1.5 mM Ca and 1×10^{-6} M verapamil. The same concentration (1×10^{-6} M) of verapamil completely inhibited the muscle contraction induced by isosmotic 80 mM or 160 mM KCl solution made by substituting KCl for equimolar NaCl in the normal solution. Thus, at least a part of the muscle contraction induced by a hyperosmotic KCl solution was independent of external Ca. Hyperosmotic applications of NaCl or sucrose also induced muscle contractions (Fig. 1). The contractions induced by the hyperosmotic application of NaCl or sucrose were not affected by inhibitors of K-induced contraction (removal of Ca from the medium or the application of 1×10^{-5} M verapamil), an inhibitor of nor-epinephrine-induced contraction (1×10^{-5} M sodium nitroprusside), or nonspecific smooth muscle relaxants (1×10^{-4} M papaverine, 2×10^{-5} M chlorpromazine or 1×10^{-4} M N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7)). However, the contractions induced by the hyperosmotic solutions rapidly relaxed when the muscle strips were washed with normal physiological solution, and the ability of the muscle to respond to nor-epinephrine was not affected by the previous treatment of the muscle with the hyperosmotic solution.

In guinea pig taenia coli, cumulative applications of KCl induced a muscle contraction which reached a maximum when 20 mM KCl was added. As the KCl concentration was increased further, there was little change in developed tension until at 120 mM KCl and above, the tension was sharply decreased as shown in Fig. 2. This may possibly be an effect of hyperosmolarity in inhibiting excitation-contraction coupling (3). In the absence of external Ca (Fig. 2) or in the presence of 1×10^{-6} M verapamil, the hyperosmotic applications of KCl at 80 mM

Fig. 2. Effects of hyperosmotic applications of KCl on muscle tension in the guinea pig taenia coli in the presence or absence of 1.5 mM Ca. Means $\pm$ S.E. of 4 to 6 experiments are shown.

and above induced small contractions.

From these results, it is concluded that hyperosmolarity induces a contraction in smooth muscle, especially in rabbit aorta. The contraction induced in aorta by either NaCl or sucrose in normal Ca was slightly greater than that induced by KCl at the same osmolarity in the absence of added Ca. This may be because KCl is more permeable than NaCl or sucrose in smooth muscle (4). Smooth muscle cells usually behave as perfect osmometers with respect to the addition of non-permeable solutes to the normal medium (5), and hyperosmotic addition of KCl also decreases cell water content (6). The muscle contraction induced by hyperosmolarity (addition of KCl without Ca, addition of NaCl or sucrose) was independent of external Ca and was not affected by various smooth muscle relaxants, suggesting that this contraction may not be attributed to events related to a physiological excitation-contraction coupling process. One possible explanation for this contraction is an osmotic shrinkage of the muscle tissue. However, we do not have available data to explain the difference between rabbit aorta and guinea pig taenia coli. In previous reports (7, 8), it

was found that isosmotic substitution of KCl for NaCl inhibits smooth muscle contraction by inhibiting the co-transport of glucose with Na and/or by the swelling of the smooth muscle cells induced by high concentration of permeable ions, K and Cl. Therefore, care should be taken with the Na concentration as well as the osmolarity of the medium when a high concentration of K is used in studies of smooth muscle contraction.

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