Potassium–induced Contraction in Smooth Muscle

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Summary

High K-induced contractions in the smooth muscle of rabbit aorta and guinea pig taenia coli may be described as follows: High K depolarizes the smooth muscle cell membrane and opens voltage dependent Ca channels, resulting in an influx of extracellular Ca and an activation of contractile machinery. A part of the cellular Ca is taken up by mitochondria. Oxygen consumption of the muscle increases to compensate for the ATP consumed by contraction. Hyperosmotic high K solution induces osmotic shrinkage of the cell which uncouples membrane excitation from contraction. Isosmotic high K, Na deficient solution induces swelling of the cell and also inhibits the synport of Na and glucose resulting in an ATP deficiency; both of the changes could inhibit muscle contractile tension. Na deficiency may also stimulate Ca influx coupled to Na efflux in some smooth muscle preparations although such mechanism does not seem to play an important role in rabbit aorta and guinea pig taenia coli.

Introduction

It has been established that a physiological saline solution containing a high concentration of potassium (high K solution) depolarizes the plasma membrane of excitable cells and induces contraction in skeletal (Frank, 1960), cardiac (Niedergerke, 1956) and smooth muscles (Edman & Schild, 1962). In smooth muscle preparations, the high K-induced contraction is widely employed to study excitation–contraction coupling and is also utilized as a standard contraction for the evaluation of various smooth muscle relaxants (Weiss, 1975, 1977). High K solution is usually made either by adding KCl hyperosmotically to a physiological solution (hyperosmotic high K solution) or by substituting an appropriate amount of Na in the physiological solution by equimolar K (isosmotic high K, low Na solution). Although, these two solutions differ considerably in their Na concentration and tonicity, and consequently affect smooth muscle function differently, little attention has been accumulated on these differences (e.g., see Karaki et al., 1981a). In this review, the changes induced by hyperosmotic and isosmotic high K solutions in membrane potential, contraction, water content, metabolism and Ca movement are compared in two of the most widely used smooth muscle preparations, rabbit
thoracic aorta and guinea pig taenia coli.

**Membrane potential**

Increasing the external K concentration decreases the membrane potential according to the Nernst or Goldman equation, and this K-induced depolarization is not affected by changes in the Na concentration in the medium in rabbit aorta (Mekata, 1976) and taenia coli (Holman, 1958). Membrane depolarization induced by K salts with less permeable anions (K₂SO₄ or K-C₂H₅SO₃) is larger than that induced by KCl, while a more permeable salt, KNO₃, induces less depolarization than KCl (Burnstock et al., 1963; Kuriyama, 1963). In rabbit aorta, an increase in external K concentration induces only a graded depolarization (Mekata, 1976). In taenia coli, as well as in other spike-generating smooth muscles like uterus, vas deferens and ureter, an increase in the external K concentration induces a burst of spike discharges followed by a spike-free membrane depolarization (Burnstock et al., 1963; Shimo & Holland, 1966; Bolton, 1972; Johnishi & Sunano, 1978). In guinea pig portal vein, a hyperosmotic solution itself depolarizes the membrane (Kuriyama et al., 1971).

Besides the depolarizing effect, high K solutions show an effect to hyperpolarize the smooth muscle membrane. This effect is clearly seen in the smooth muscle preincubated with low K solutions. The K-induced hyperpolarization is transient and is usually followed by muscle relaxation. This hyperpolarization is attributable to stimulation of electrogenic Na pump (Kuriyama et al., 1971; Bonaccorsi et al., 1977) and also to increase in K permeability (Somlyo et al., 1972).

**Muscle tension**

*Isotonic and isometric contractions*

Smooth muscle contraction is recorded either isotonically using a lever, or isometrically using a force transducer. In an isotonic recording, especially with a light load, muscle relaxation is sometimes obscured. In isometric recording, high-K induced contraction in taenia coli relaxes to approximately 10% of the control contraction when a hypoxic condition is introduced, and the remaining contraction is abolished when the muscle is washed with a normal physiological solution. However, such a hypoxia-induced relaxation of K-induced contraction is not seen in isotonic recording with a light load. Moreover, no relaxation is observed when the high K-contracted taenia is washed with a normal physiological solution under hypoxia. This “catch-like” contraction continues until oxygen is re-introduced (Ishida & Urakawa, 1974). In taenia, a high concentration of ouabain relaxes the K-induced contraction in isometric recording, but not in isotonic recording with a light load (unpublished observation). In guinea pig vas deferens, it is also observed that the high K-induced initial contraction is much larger than the following sustained contraction in isometric recording, although both contractions have comparable magnitude in isotonic recording (Sunano & Shimodan, 1981). By increasing the load on the isotonic recording lever, the results obtained with this method became similar to those obtained with the isometric recording method (Ishida & Urakawa, 1974).

*Initial transient and following sustained contractions*
In guinea pig taenia coli, contractions induced by high K solutions (either hyperosmotic or isosmotic), are rapidly inhibited in a Ca depleted solution (Chujyo & Holland, 1963; Karaki et al., 1966). However, there are some data indicating differences between the initial transient and the following sustained contractions in taenia coli. Substitution of Cl by other anions like Br, NO₃, I or SCN potentiates the K-induced initial transient contraction, whereas the same substitution inhibits the following sustained contraction (Urakawa et al., 1967). Only an initial transient contraction is induced by high K (and also by carbachol) in the presence of Ca antagonists like verapamil (Riemer et al., 1974; Karaki et al., 1984), D600 (Brading & Sneddon, 1980), ruthenium red (Kawamura & Yabu, 1978), cyproheptadine (Lowe et al., 1981) and N²-dansyl-L-arginine-4-t-butylpiperidine amide (TI 233) (Karaki et al., 1983c), inhibitors of aerobic metabolism (Pfaffman et al., 1965; Ferrari & Carpenedo, 1968; Tsuda et al., 1977; Nakagawa et al., 1984), inhibitors of the Na pump like ouabain (Pfaffman et al., 1965; Kishimoto & Urakawa, 1982a), Li substitution for Na (Pfaffman et al., 1965; Kishimoto & Urakawa, 1982a), and ionophores with mitochondrial inhibiting action like monensin (Kishimoto et al., 1982) and X-537A (Ishida & Shibata, 1982; Murakami et al., 1983). The initial transient contraction induced by high K in taenia coli coincides with a burst of spike discharges (Shimo & Holland, 1966). This electrical change, as well as initial transient contraction, is relatively insensitive to the inhibitory effects of Mn (Brading et al., 1969) and D600 (Inomata & Kao, 1976). These results indicate that the sustained contraction induced by high K (and also by carbachol) in taenia coli may be dependent on Na pump activity, aerobic metabolism and Ca influx through a pathway which is sensitive to Ca antagonists. In contrast, the initial transient contraction may be due to different mechanisms.

There are two conflicting reports on the source of Ca for the initial transient contraction induced by high K solution. Urakawa and Holland (1964) reported that this contraction is attributable to a release of cellular Ca, while Imai and Takeda (1967) suggested this contraction to be a result of Ca influx. Ca free, EGTA-containing solutions inhibit the contraction induced not only by high K but also by carbachol at 37°C. However, initial transient contraction induced by carbachol is likely to be due to release of cellular Ca (which is lost rapidly at 37°C) because carbachol induces a transient contraction in Ca-free, high K solution at 20°C, which is not inhibited by either verapamil or La (Ohashi et al., 1974, 1975). Since these experiments were done in high K solutions, high K does not seem to release the carbachol-releasable Ca store. Thus, although there are many similarities in the transient contractions induced by either high K or carbachol, Ca movements responsible for these contractions may differ.

In rabbit aorta, K-induced contraction is rapidly inhibited by removing external Ca, whereas norepinephrine-induced contraction is not readily inhibited in a Ca-deficient solution (Waugh, 1962; Hudgins & Weiss, 1968). Addition of EGTA or lanthanum inhibits the sustained contraction, but not the initial transient contraction induced by norepinephrine (Van Breemen, 1969; Karaki et al., 1979). Thus, in rabbit aorta, K-induced contraction is composed of a single, sustained contraction which seems to be the result of Ca influx, whereas norepinephrine-induced contraction is composed of an initial transient and a following sustained...
contraction; the former seems to be the result of Ca release while the latter may be the result of inward dislocation of superficially bound Ca (Karaki et al., 1979; Karaki, 1981; Karaki & Weiss, 1984; Karaki et al., 1984). Sodium nitroprusside and procaine inhibits both of the norepinephrine-induced Ca movements in aorta (unpublished observation).

**Hyperosmotic high K solution**

Effects of hyperosmotic high K solution on muscle tension in rabbit aorta and guinea pig taenia coli are shown in Fig. 1. In both aorta and taenia coli, hyperosmotic application of KCl initiates a contraction when the K concentration is 15 to 20 mM and the contraction reaches a maximum when 40 to 50 mM KCl is added. Further increase in the KCl concentration, up to 160 mM, does not change the contractile tension in aorta. In taenia coli, however, a concentration-dependent decrease in developed tension is observed when the concentration of KCl is above 80 mM (Karaki et al. 1983a). In taenia coli, the hyperosmotic high K solution does not induce contraction in the absence of Ca or in the presence of verapamil. In aorta, however, the hyperosmotic high K solution induces a concentration-dependent contraction even in the absence of Ca or in the presence of verapamil. The threshold concentration of K to induce contraction in aorta in Ca-depleted solution is 40 mM and when 80 mM KCl is added, a muscle contraction of more than 50% of that induced in the presence of 1.5 mM Ca is induced. Hyperosmotic application of NaCl or sucrose also induces a concentration-dependent contraction in aorta which is not inhibited by Ca removal, verapamil, papaverine or sodium nitroprusside. The contraction is rapidly relaxed by washing the muscle with normal physiological solution and norepinephrine applied thereafter induces a contraction similar to that of control muscle (Karaki et al., 1983a). In canine femoral, superior mesenteric and coronary arteries, hyperosmotic application of sucrose or mannitol also induces concentration-dependent contractions, and these contractions are not inhibited by Ca removal or addition of Ca antagonists like verapamil, nicardipine and diltiazem, but are partially inhibited by papaverine, 2,4-dinitrophenol or chlorpromazine (Nakayama & Kato, 1980). In rat portal vein, hyperosmotic sucrose-induced contraction is not inhibited by Ca removal, but is partially inhibited by a glucose- and oxygen-deprivation (Andersson et al., 1974). In rat aorta, hyperosmotic sucrose-induced contraction is not inhibited by verapamil and is partially inhibited by Ca-removal and by papaverine (Kent et al., 1983). Another effect of hyperosmotic solution is to inhibit the excitation-contraction coupling (Brading, 1970; Somlyo & Somlyo, 1970); the effects of various agonists, like norepinephrine and histamine, to induce smooth muscle contractions are inhibited in hyperosmotic solution although the effects on membrane potential are not altered (Carrol, 1969; Somlyo & Somlyo, 1970; Krishnamurty et al., 1977; Kent et al., 1983). In both aorta and taenia coli, a portion of high K-induced contraction which is dependent on external Ca is inhibited when the osmolarity of the medium is increased (Fig. 1).

**Isosmotic high K, low Na solution**

Effects of isosmotic high K, low Na solution on muscle tension in rabbit aorta and guinea pig taenia coli are shown in Fig. 1. In both aorta and taenia coli, isosmotic application of KCl induces a contraction; a maximum is reached when 40 to 50 mM KCl was applied. Further
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Hyperosmotically added K (mM)

Fig. 1. Steady level of muscle tension induced by hyperosmotically added K (upper and middle) and isosmotically substituted K (lower) in rabbit aorta and guinea pig taenia coli in the presence or absence of external Ca. Steady contractile tension was reached between 15 to 120 min after the addition of high K depending on the concentration of Na and K.

A similar decrease in the sustained tension level is observed during contraction induced by isosmotic high K solution in rabbit trachea (Ueda et al., 1982), guinea pig trachea, urinary bladder and gall bladder (Shimizu et al., 1984). In isotonic record-
ing with a light load, however, the decrease in the sustained contractile tension is rarely seen (unpublished observation).

The muscle contraction induced by isosmotic high K solution in rabbit aorta and guinea pig taenia coli is totally inhibited in a solution without Ca or by verapamil (Karaki et al., 1983a). In guinea pig aorta, however, a part of the contraction induced by isosmotic high K, Na deficient (11.9 mM) solution is not inhibited by verapamil (Ozaki & Urakawa, 1981a). In this smooth muscle preparation, a sustained contraction is induced by a decrease in transmembrane Na gradient, produced either by inhibiting the Na pump (using ouabain or a K deficient solution) or by decreasing the concentration of external Na. This contraction is not inhibited by verapamil and is possibly due to an influx of Ca in exchange for Na efflux (Ozaki & Urakawa, 1979, 1980, 1981a, 1981b; Ozaki et al., 1978). Thus, it is possible that a part of the contraction induced by high K, Na deficient solution in guinea pig aorta is a result of Na-Ca exchange. Such a Na-Ca exchange mechanism, however, does not seem to play an important role in rabbit aorta and guinea pig taenia coli (Karaki & Urakawa, 1977; Karaki et al., 1978a; Ozaki & Urakawa, 1981b; Van Breemen et al., 1979, 1980).

Tris buffer is sometimes substituted for bicarbonate buffer. Recently, it has been suggested that Tris and other artificial buffer substances inhibit smooth muscle contractions, especially those induced by isosmotic high K solution (Altura et al., 1980; Turlapaty et al., 1978, 1979a, b). However, the inhibition observed in the artificial buffer solution is not attributable to the adverse effects of the buffer substances, but to an inadequate method of adjusting the medium pH and to a Na deficiency of the medium due to replacement of NaHCO₃ with Tris (Karaki & Weiss, 1981b; Karaki et al., 1981a, b).

Cell water content

In skeletal muscle, membrane permeability to Na is much smaller than to K and Cl and the distribution of K and Cl in and outside the cell is described by a Donnan equation:

$$\frac{K_{\text{out}}}{K_{\text{in}}} = \frac{Cl_{\text{in}}}{Cl_{\text{out}}}$$

where $K_{\text{out}}$ and $K_{\text{in}}$ represent K ion concentration outside and inside, respectively, of the cell membrane, and $Cl_{\text{out}}$ and $Cl_{\text{in}}$ represent Cl ion concentration outside and inside, respectively, of the cell membrane. When external Na is substituted by K, the increase in $K_{\text{out}}$ is followed by an increase in $K_{\text{in}}$ and also $Cl_{\text{in}}$. Such a change in ionic distribution results in a large increase in wet weight of the tissue due to an influx of water and swelling of the cell (Boyle & Conway, 1941; MacKnight & Leaf, 1977). In smooth muscle, however, an increase in wet weight in isosmotic high K, low Na solution is not observed (Casteels & Kuriyama, 1966; Brading & Tomita, 1968). The discrepancy between skeletal and smooth muscle is explained by measuring the changes in the extracellular space. In smooth muscle, isosmotic high K, Na deficient solution increases cell water content and simultaneously decreases extracellular space and thus maintains the wet weight of the tissue constant within 30 min of the application of isosmotic high K solution (Jones et al., 1973). The rate of swelling is slow in smooth muscle cells; it takes more than 60 min to obtain a significant increase in the wet weight in isosmotic high K, Na deficient solution (Jones et al., 1973; Karaki et al., 1978b; Suzuki et al., 1980, 1981).

In rabbit aorta, the time courses for the decrease in the sustained contraction and the
increase in the cell water content induced in isosmotic high K (120 mM or higher), Na deficient solution are almost identical (Suzuki et al., 1981). When the external NaCl is substituted by a K salt with a highly permeable anion, like KI or KNO₃, both the decrease in the sustained contraction and cell swelling take place more rapidly than in KCl solution. However, when NaCl is substituted by a K salt of a practically impermeable anion, like K₂SO₄ or K propionate, there is little change in either the sustained contraction or the cell water content. Hyperosmotic addition of 50 to 100 mM sucrose completely prevents both of these changes in the isosmotic high K, Na deficient solution (Suzuki et al., 1981). Similar phenomena are found in rabbit trachea (Ueda et al., 1983), guinea pig trachea and gall bladder (Shimizu et al., 1984). Thus, it seems likely that swelling of the smooth muscle cell somehow inhibits K-induced contraction in rabbit aorta and other smooth muscles.

In guinea pig taenia coli, the increase in the cell water content in the isosmotic KCl solution is also prevented by the application of hyperosmotic sucrose. However, the decrease in the sustained contractile tension is only partly prevented by sucrose (Suzuki et al., 1980). Similar results are obtained in guinea pig urinary bladder (Shimizu et al., 1984). As described in the following section, the Na deficiency in the isosmotic high K solution may inhibit glucose uptake of the cell and decrease ATP synthesis, thus inhibiting contraction in taenia coli.

The differential effects of hyperosmotic high K and isosmotic high K solutions on smooth muscle contraction are summarized in Fig. 2.

### Metabolism

Oxygen consumption of taenia coli, 0.43–0.62 μmol/g/min (Bübrin, 1953; Bübrin & Golenhofen, 1967; Saito et al., 1968; Karaki et al., 1982b), is higher than that of aorta, 0.075–0.14 μmol/g/min (Paul, 1980; Karaki et al., 1982b). In the presence of 40 to 80 mM K, oxygen consumption of both tissues increases to 150–280% of the resting level (Saito et al., 1968; Paul, 1980; Karaki et al., 1982b). The ATP content of taenia coli and of aorta are 2 μmol/g and 0.7
μmol/g, respectively, and 40 to 80 mM K does not change or slightly decreases this (Paul, 1980; Karaki et al., 1982b). Under hypoxic conditions, the ATP content of resting muscle does not change, whereas on the addition of K the ATP content rapidly decreases (Karaki et al., 1982b). Therefore, it seems likely that ATP production increases by an aerobic pathway to replenish ATP consumed during K-induced contraction.

In taenia coli, prolonged exposure of the muscle to isosmotic high K, Na deficient solution gradually decreases the sustained contractile tension. When the sustained contraction decreases to 20% or 10% of the maximum contraction, oxygen consumption of the muscle also decreases to 18% or 0%, respectively, of the maximum level. ATP content also decreases during this period. Similar changes are seen when glucose is removed from the medium during the contraction induced by a hyperosmotic high K solution. Addition of 50 mM NaCl or 5.5 mM pyruvate to isosmotic high K, Na deficient solution increases the muscle tension, oxygen consumption and ATP content (Karaki et al., 1982b). These results suggest that co-transport of Na and glucose (Schultz & Curran, 1970) is inhibited in the isosmotic high K, Na deficient solution resulting in a decrease in ATP synthesis. Hypoxia also decreases K-induced contractile tension in taenia coli (Pfaffman et al., 1965; Karaki et al., 1967; Ganeshanandan et al., 1969). Increasing the concentration of glucose to 25 mM or higher under hypoxia partially restores the muscle tension in the presence of Na (Namm & Zucker, 1973; Nasu et al., 1982). The decrease in ATP content under hypoxia is also partially prevented by increasing the glucose concentration (Karaki et al., 1982b; Ishida et al. 1984). Thus, a part of the ATP utilized for muscle contraction seems to be synthesized by an anaerobic glycolytic pathway.

In rabbit aorta, neither glucose removal nor hypoxia inhibits the K-induced contractile tension. ATP content does not change during the K-induced contraction under hypoxia (Karaki et al., 1982b). However, both oxygen consumption and ATP content decrease to 32% and 66%, respectively, of the control levels during K-induced contraction in glucose depleted solution (Karaki et al., 1982b). Since aorta is able to utilize endogenous amino acids and fatty acid as metabolic substrates (Hellstrand et al., 1977; Chase & Odessy, 1981), muscle contractions may be maintained by these substrates in the absence of added glucose. In isosmotic high K, Na deficient solution, both oxygen consumption and ATP content decrease to levels similar to those in glucose-depleted, hyperosmotic high-K solution (Karaki et al., 1982b). These decreases are not prevented even in the presence of hyperosmotically applied sucrose, which inhibits both cell swelling and tension decrease in isosmotic high K, Na deficient solution, suggesting that cell swelling does not affect metabolism in aorta. From these results, it seems likely that, in aorta as in taenia coli, glucose uptake is inhibited in aorta in isosmotic high K, Na deficient solution. However, endogenous substrates may partly support ATP synthesis and maintain the K-induced contraction in aorta. On the other hand, norepinephrine–induced contraction in rabbit aorta is inhibited by either glucose depletion or hypoxia. However, this contraction is not inhibited by cell swelling induced by decreasing the Na concentration to 1/2 of the normal solution without any osmotic substitution (unpublished observation). These results suggest a difference in the excitation-contraction coupling processes in K- versus norepinephrine–induced contractions.
Calcium movements

Tissue Ca content

Tissue Ca content (total tissue Ca content measured by flame photometry or atomic absorption spectrometry) of taenia coli increases during a contraction induced by hyperosmotic 42.7 mM K solution (Urakawa & Holland, 1964; Karaki et al., 1969b). Such an increase is not observed during a contraction induced by isosmotic high K, Na deficient solutions (Urakawa et al., 1968; Karaki et al., 1969a), histamine (Nasu et al., 1971), barium (Karaki et al., 1969b) or acetylcholine (Nasu & Urakawa, 1973). In rabbit aorta, tissue Ca content is not affected by high K solutions (either isosmotic or hyperosmotic), or by norepinephrine (unpublished observations).

Slowly exchangeable ⁴⁵Ca fraction

In taenia coli, it is found that smooth muscle stimulants including high K, carbachol, histamine and Ba increase the amount of ⁴⁵Ca bound to relatively slowly exchangeable sites (t 1/2 = 7 min) possibly located on the membrane surface. Changes in the size of this fraction show a good correlation with changes in muscle tension and this fraction has been suggested to be a depot of Ca extruded from the cell during muscle contraction (Karaki & Urakawa, 1972). A similar ⁴⁵Ca fraction is found to be increase by high K solution in rabbit aorta (Briggs, 1962). Ouabain also increases this Ca fraction (Briggs & Shibata, 1966) which is due to the effect of endogenous catecholamines released by ouabain (Karaki & Urakawa, 1977; Karaki et al., 1978a).

Cellular ⁴⁵Ca fraction and the rate of ⁴⁵Ca uptake

A lanthanum-wash method (Van Breemen & McNaughton, 1970; Van Breemen et al., 1972) is widely employed to displace extracellular ⁴⁵Ca and thus enable determination of the amount of cellular ⁴⁵Ca in smooth muscle. It has been shown that the cellular ⁴⁵Ca (total amount of cellular exchangeable Ca) of smooth muscle, measured by equilibrating the muscle with ⁴⁵Ca for more than 30 min, is increased by high K solutions (for references see Karaki et al., 1982a). In rabbit aorta, isosmotic 20 mM or higher K increases the cellular ⁴⁵Ca in a concentration-dependent manner and when all the Na in the medium is replaced by K, cellular ⁴⁵Ca increases 300–800 nmol/g above the resting level (Van Breemen, 1977; Karaki & Weiss, 1979). Hyperosmotic high K solutions also increase the cellular ⁴⁵Ca in a concentration-dependent manner (Ito et al., 1977). This increase in cellular ⁴⁵Ca is attributable to the influx of Ca through voltage dependent Ca channels since this increment is specifically inhibited by verapamil (Ito et al., 1977; Karaki & Weiss, 1979; Karaki et al., 1982a). The increased cellular ⁴⁵Ca seems to be located mainly in mitochondria because mitochondrial inhibitors like antimycin A, oligomycin, KCN or hypoxia inhibit the increase in cellular ⁴⁵Ca (Karaki & Weiss, 1981a; Karaki et al., 1982a). The K-induced contraction, on the other hand, is not inhibited by the mitochondrial inhibitors, suggesting that the Ca accumulation by mitochondria does not play an important role in muscle contraction (Karaki & Weiss, 1981a; Karaki et al., 1982a). Although a high concentration of K does not increase the total ⁴⁵Ca content under hypoxia, it
increases the rate of \(^{45}\text{Ca}\) uptake (Karaki et al., 1983b) measured by a short (less than 10 min) \(^{45}\text{Ca}\)-incubation period (pulse-label method) (Kroeger et al., 1975). This increase in the rate of \(^{45}\text{Ca}\) uptake is inhibited by verapamil (Karaki et al., 1984). Thus, a high concentration of K has dual effects on Ca movements in rabbit aorta, to increase the rate of \(^{45}\text{Ca}\) uptake and to accumulate \(^{45}\text{Ca}\) in mitochondria. The latter \(^{45}\text{Ca}\) is detected as increase in cellular \(^{45}\text{Ca}\) content. Similar results have been obtained in the intestinal smooth muscle of guinea pig taenia coli (Karaki et al., 1982a, 1984).

An increase in the total cellular \(^{45}\text{Ca}\) has been found only when the plasma membrane of rabbit aorta is depolarized. Other stimulants, such as norepinephrine, histamine and angiotensin II, do not change the cellular \(^{45}\text{Ca}\) in rabbit aorta (Deth & Van Breemen, 1974). Furthermore, the norepinephrine-induced contraction in rabbit aorta is relatively insensitive to verapamil and other organic Ca antagonists (Kalsner et al., 1970; Schumann et al., 1975; Golenhofen & Weston, 1976; Ito et al., 1977; Van Breemen et al., 1981). From these results, it is sometimes concluded that norepinephrine-induced contraction is not dependent on external Ca. However, norepinephrine or histamine, added to an aorta depolarized by isosmotic high K, Na deficient solution, induces a further increase in cellular \(^{45}\text{Ca}\) (Karaki & Weiss, 1979, 1980) although, when the concentration of K is lower than 120 mM, norepinephrine does not show an additive increase in cellular \(^{45}\text{Ca}\) (unpublished observation). Such an additive increase in cellular \(^{45}\text{Ca}\) may be attributable to the accumulation of \(^{45}\text{Ca}\) by mitochondria since the increment is inhibited by mitochondrial inhibitors (unpublished observation). Norepinephrine also increases the rate of \(^{45}\text{Ca}\) uptake (Godfraind, 1976; Karaki et al., 1983b). This increase in the rate of \(^{45}\text{Ca}\) uptake is detected by a modified lanthanum-wash method using a high concentration (50–80.8 mM) of La and/or low temperature (Godfraind, 1976; Karaki et al., 1983b), but is not detected by the original lanthanum-wash method using 2 to 10 mM La and 37°C (Van Breemen & McNaughton, 1970; Van Breemen et al., 1972). Washing the \(^{45}\text{Ca}\)-treated muscle with EGTA at low temperature seems to remove extracellular bound \(^{45}\text{Ca}\) in a manner similar to the modified lanthanum-wash method (Meisher et al., 1981). Such an increase in the rate of \(^{45}\text{Ca}\) uptake is inhibited by neither mitochondrial inhibitors (Karaki & Weiss, 1985) nor by verapamil (Karaki et al., 1984). These results suggest that the sustained contraction induced by norepinephrine is due to an influx of Ca through a Ca channel which is insensitive to verapamil, and that this Ca is not accumulated in the cell (or mitochondria) during the norepinephrine-induced contraction of polarized rabbit aorta.

Ca movements in rabbit aorta during contraction induced by high K and norepinephrine are summarized in Fig. 3.

**Calcium channels**

Organic Ca antagonists are specific inhibitors of K-induced contraction in smooth muscle. The increases in both the amount of cellular Ca and the rate of Ca exchange induced by high K solution are inhibited by verapamil or D600 in rabbit aorta (Van Breemen et al., 1972; Ito et al., 1977; Karaki & Weiss, 1979; Thorens & Haeusler, 1979; Meisher et al., 1981; Karaki et al., 1982b). On the other hand, verapamil or D600 has little effect on the norepinephrine-induced Ca movements in rabbit aorta (Karaki & Weiss, 1980; Meisher et al., 1981; Karaki
et al., 1984). The latter changes in Ca movements are inhibited by sodium nitroprusside (Karaki & Weiss, 1980; Karaki et al., 1984). These results support the suggestion that there are two types of Ca channels in smooth muscle, i.e., a voltage-dependent Ca channel and a receptor-linked Ca channel (Weiss, 1977, 1981; Bolton, 1979; Van Breemen et al., 1979; Triggle, 1981). Furthermore, it seems quite likely that the organic Ca antagonists and sodium nitroprusside are specific inhibitors of the respective channels in rabbit aorta (see also Golenhofen, 1981). Although there seems to be two types of Ca channels in other types of smooth muscle, such a relationship between Ca channels and specific inhibitors is seen only in rabbit aorta. In most other vascular smooth muscles, K-induced contraction is inhibited by both verapamil and sodium nitroprusside while norepinephrine–induced contraction is also inhibited by both verapamil and sodium nitroprusside (Karaki & Weiss, 1984; Karaki et al., 1984). Therefore, voltage-dependent Ca channels and receptor-linked Ca channels in these smooth muscles seem to have common characteristics, or these two Ca channels are not functionally separated. In guinea pig taenia coli (and other intestinal smooth muscles), organic Ca antagonists inhibit both the sustained contraction and the Ca movements induced by high K solution and by histamine, while sodium nitroprusside has little effect on these changes (Karaki et al., 1984). Thus, taenia coli does not seem to have a Ca channel which is inhibited by sodium nitroprusside (for further details see Karaki & Weiss, 1984; Karaki et al., 1984).

Accumulation of Na in the cell, induced by ouabain or vanadate, is reported to inhibit the
voltage–dependent Ca channel (Kishimoto & Urakawa, 1982a; Kishimoto et al., 1980; Ueda et al., 1982). Intracellular Li may have a similar but more potent effect than intracellular Na (Kishimoto & Urakawa, 1982b). In contrast, bassianolide, a cyclodepsipeptide, inhibits the sustained contractions induced by various receptor–agonists (i.e., acetylcholine, histamine, serotonin and norepinephrine) whereas this inhibitor has no effect on the contraction induced by high K solution in guinea pig taenia coli, ileum and vas deferens (Nakajo et al., 1982, 1983). These findings further support the concept of different pathways of Ca influx in the contraction induced by a high concentration of K and by receptor–agonists.

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