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IONIC MECHANISMS INVOLVED IN THE STRONTIUM-INDUCED SPIKE AND PLATEAU IN THE SMOOTH MUSCLE OF RAT PORTAL VEIN

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SUMMARY

1. The action of Sr on the smooth muscle of rat portal vein was studied electrophysiologically using micro-electrodes.

2. By replacing Ca with Sr (2·5 mM), the spontaneous membrane activity was altered and spikes were followed by a long lasting plateau potential. The mechanisms which generated the spike and the plateau in the Sr-induced activity were elucidated.

3. As the concentration of Sr was increased, the peak potential and the maximum rates of rise and fall of the initial spike in each discharge increased. The peak potential varied by 15·2 mV with a 10-fold change in $[Sr]_0$.

4. As there was a decrease in the membrane resistance during the plateau, an increase in the permeability of the membrane for Sr, Cl or Na could be responsible for generation of the plateau.

5. The amplitude of the plateau decreased with increase in the concentration of Sr, remained unchanged in a low-Cl solution, but was diminished in a low-Na solution.

6. Mn (1–2 mM) inhibited not only the spike but also the plateau.

7. TEA (20 mM) shifted the plateau potential in a positive direction and the plateau became permanent. When inward currents were applied in the presence of TEA, spikes with large overshoots and small rates of fall were induced.

8. These results indicate that Sr and K conductances of the membrane generate the spike and that slow-inactivating voltage-dependent Na conductance produces the plateau.

INTRODUCTION

Calcium may be the main charge carrier for the spike discharge in various smooth muscles. The entrance of Ca would then activate contractile mechanisms or release stored Ca or do both. Calcium which is bound on the cell membrane is likely to play an important role in the control of ionic permeability of the membrane (Brading, Bülbring & Tomita, 1969*a*, *b*; Bülbring & Tomita, 1970). Because Ca has various and complex actions on the physiological processes of smooth muscle, the role of Ca in the function of smooth muscle is still poorly understood.

Some divalent cations can substitute for Ca, albeit not completely. In the guinea-pig taenia coli, Ba which was effective in producing the spike could not replace Ca for

the action of adrenaline (Bülbring & Tomita, 1968), and either Sr and Ba had a weaker stabilizing action than did Ca (Hotta & Tsukui, 1968). In the guinea-pig ureter, Sr could replace Ca completely for production of the spike, but only partially for the control of the membrane Na conductance (Kuriyama & Tomita, 1970). Thus, by replacing Ca with such ions, the electrical and mechanical activities of smooth muscle are probably altered. Although the phenomena which can be observed under such conditions are not physiological, they can still aid in elucidating the physiological functions of smooth muscle.

In the smooth muscle of rat portal vein, Uvelius, Sigurdsson & Johansson (1974) reported that Sr and Ba could maintain the spontaneous electrical and mechanical activities in the absence of Ca. However, they did not discuss changes in the electrical activity induced by the replacement of Ca with Sr. In the present experiments, the Sr-induced membrane activity of the smooth muscle of rat portal vein was examined in an attempt to clarify the ionic mechanisms involved in the generation of the spike and plateau seen in the presence of Sr. Our observations suggest that production of the spike depends upon Sr and K conductances of the membrane and that plateau is mainly due to Na current passing through a slow-inactivating voltage-dependent channel.

METHODS

Male Wistar rats, 12–27 weeks of age, were exsanguinated and the portal-mesenteric vein, about 15 mm in length, was excised and soaked in aerated Krebs solution. Under a binocular microscope, the vein was slit along the longitudinal axis and the adventitial connective tissue was removed. The vein was then placed in a perfusion chamber and its proximal (liver) portion was pinned to a silicone rubber block to suppress movement. The distal (mesenteric) end of the specimen was attached to an isometric force transducer. Measurements were begun only after a 60 min stabilizing period.

The volume of the perfusion chamber was 0·75 ml. and the flow rate of the perfusate was 3 ml./min (perfusion pump). When the perfusate was replaced, 90% of the solution in the chamber was changed within 60 sec and 99% was changed within 100 sec. The temperature of the perfusate was maintained at 35 °C throughout the experiments.

For observation of the membrane activity we used a conventional micro-electrode filled with 3 M-KCl and with a tip resistance of 50–100 MΩ. This electrode was impaled from the outer surface of the proximal portion of the specimen.

A method similar to that described by Abe & Tomita (1968) was used for the current injection. The stimulating electrodes consisted of parallel silver plates, 1 cm apart. Thus, the chamber was divided into two compartments, one for recording and the other for stimulating. The specimen passed through holes in the plates and the perfusate flowed through these holes. Although the surface exposed to the recording compartment was coated with an insulating layer of epoxy resin, the leakage current in this compartment was significantly large. To counteract the influence of current leakage, the potential difference between two micro-electrodes was measured. One electrode was impaled into a cell and the other placed in the solution in close proximity to the cell. The relative values of the stimulating current were obtained by recording the voltage gradient in the solution with two silver electrodes, 2·5 mm apart, placed in the stimulating compartment.

Solutions used were as follows (concentrations in mM).

(a) Standard Ca-Krebs solution: NaCl, 120·7; KCl, 5·9; $MgCl_2$, 1·2; $CaCl_2$, 2·5; NaH_2PO_4, 1·2; $NaHCO_3$, 15·5; glucose, 11·5 (Casteels, Kitamura, Kuriyama & Suzuki, 1977). The solution was gassed with 95% O_2 and 5% CO_2, and had a pH of 7·2–7·3 at 35 °C. When Sr was added to the Ca-Krebs solution, a stock solution of 1 M-$SrCl_2$ was used without any other correction.

(b) Standard Sr-Krebs solution: this solution contained 2·5 mM-$SrCl_2$ instead of 2·5 mM-$CaCl_2$. Other components were the same as those of the standard Ca-Krebs solution. When the

concentration of $SrCl_2$ was modified, the osmolality of the solution was kept constant by changing the concentration of NaCl. When tetraethylammonium chloride (TEA, Wako Pure Chemicals, Osaka) was added, equimolar NaCl was omitted.

(c) Tris-buffered Sr-Krebs solution: NaCl, 120·7; $KHCO_3$, 5·9; $MgCl_2$, 1·2; $SrCl_2$, 2·5; tris (hydroxymethyl)aminomethane (Tris), 16·7; glucose, 11·5. The solution was gassed with 95% O_2 and 5% CO_2, and the pH was adjusted to 7·2–7·3 at 35 °C with HCl. This solution was used in experiments involving Mn, and also as a standard solution when Na was replaced with Tris. When Mn was applied, a stock solution of 1 M-$MnCl_2$ was added to the Tris-buffered Sr-solution, in appropriate quantities. Na-free Sr-solution was prepared by replacement of Na with isotonic Tris. Na-deficient Sr solution was prepared by mixing the standard solution and Na-free solution, in appropriate proportions.

(d) Cl-deficient Sr solution: sodium benzenesulphonate, 126·6; $KHCO_3$, 5·9; $MgCl_2$, 1·2; $SrCl_2$, 2·5; NaH_2PO_4, 1·2; $NaHCO_3$, 9·6; glucose, 11·5. The solution was gassed with 95% O_2 and 5% CO_2, and had a pH of 7·2–7·3 at 35 °C.

Phentolamine mesylate (10^{-5} M), atropine sulphate (10^{-6} M) and propranolol hydrochloride (10^{-5} M) were added to all solutions to eliminate neural effects.

RESULTS

Effect of Sr on the spontaneous activity

The smooth muscle of the rat portal-mesenteric vein exhibited spontaneous electrical and mechanical activity in standard Ca-Krebs solution (Fig. 1*A*, *a*). The electrical activity consisted of a small slow-depolarization of the membrane with a burst discharge of spikes on the top. When 2·5 mM-$SrCl_2$ was applied, the duration of the burst increased and the contraction was prolonged (Fig. 1*A*, *b*). After all the Ca had been removed (2·5 mM-Sr remained), the electrical activity changed and spikes were followed by a long lasting plateau potential (Fig. 1*A*, *c*). Mechanisms which generated the spike and the plateau of the Sr-induced activity in Ca-free solution were thus given attention.

Fig. 1*B* shows the detailed configuration of the Sr-induced membrane activity. As a train of spikes appeared, the spikes were superimposed on a large depolarization wave. The spike discharge was soon inhibited and the membrane potential reached an elevated stable level, i.e. plateau. The mean of this plateau potential was $-23{\cdot}1 \pm 3{\cdot}9$ mV (S.D. of an observation, $n = 63$). The duration of the plateau varied with the preparation, but was dominant for 10–30 sec. The plateau potential decreased gradually and small spikes appeared. The membrane then abruptly repolarized and the potential reached its most negative level. The mean of this value was $-60{\cdot}6 \pm 3{\cdot}7$ mV (S.D. of an observation, $n = 63$), and was more negative than that in standard Ca-Krebs solution ($-48{\cdot}6 \pm 1{\cdot}5$ mV; S.D. of an observation, $n = 103$).

Sr-induced activity at various concentrations of Sr

When the external Sr concentration was reduced from 2·5 to 1·0 mM (Fig. 2*A*), the peak potential of the initial spike in each intermittent discharge decreased. Both the maximum rates of rise and fall of the initial spike also decreased. Although the duration of the plateau decreased, the amplitude was not affected. Tension development also decreased with a low concentration of Sr.

Fig. 2*B* shows the activity in the presence of Sr at 2·5, 10 and 20 mM. With increase in concentration, the peak potential and the maximum rates of rise and fall of the initial spike increased, as did the duration of the plateau. The amplitude of the

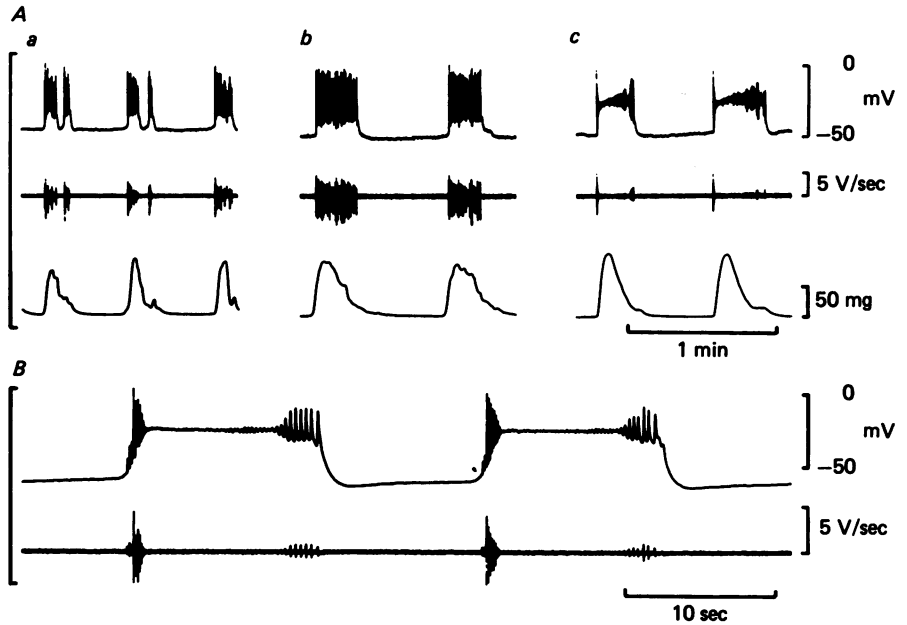

Fig. 1. *A*, the effect of Ca and/or Sr on the spontaneous activity. The upper trace shows the electrical activity, the middle trace the dV/dt relation of the electrical activity, and the lower trace the mechanical activity. The activities: *a*, in Ca, 2·5 mM; *b*, in Ca, 2·5 mM+Sr, 2·5 mM; *c*, in Sr, 2·5 mM. *B*, the electrical activity (upper trace) and its dV/dt relation (lower trace) in Ca-free 2·5 mM-Sr solution with expanded time-scale. *A* and *B* were recordings from different preparations.

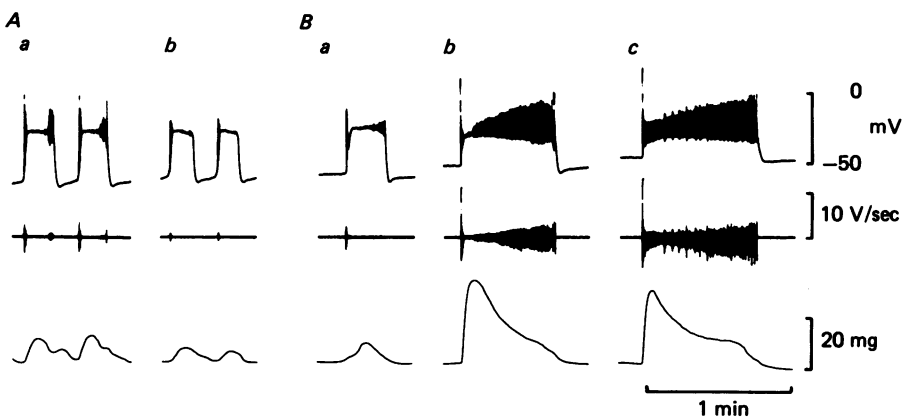

Fig. 2. The Sr-induced activity at various concentrations of Sr. *A* and *B* were recordings from different preparations. The upper trace shows the electrical activity, the middle trace the dV/dt relation of the electrical activity, and the lower trace the mechanical activity. The activities were *A*:*a*, in Sr, 2·5 mM; *b*, in Sr, 1·0 mM; *B*:*a*, in Sr, 2·5 mM; *b*, in Sr, 10 mM; and *c*, in Sr, 20 mM.

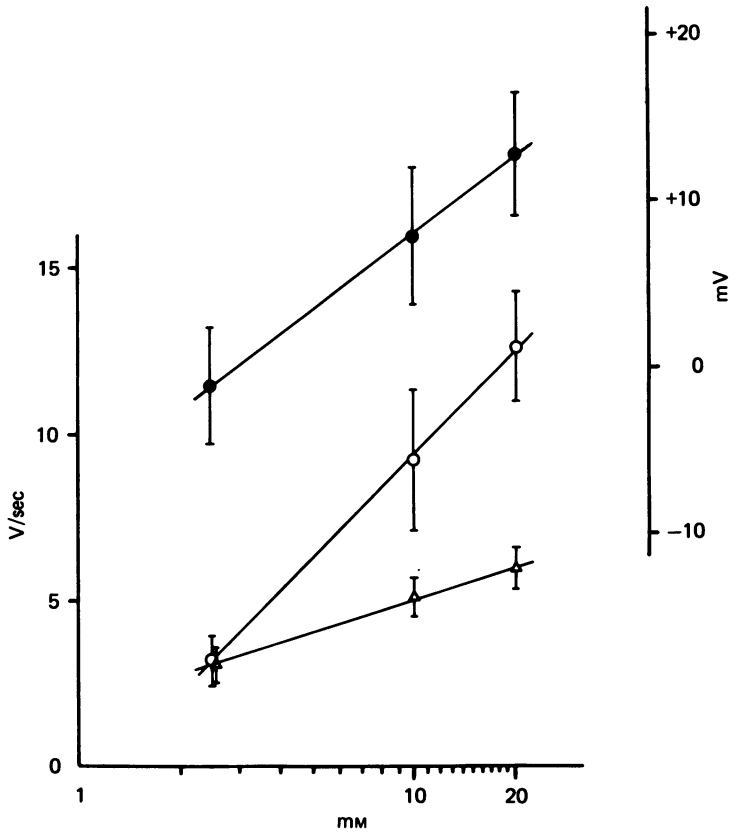

Fig. 3. Dependence of the peak potential (●), and the maximum rates of rise (○) and fall (△) of the initial spikes upon $[Sr]_0$. $[Sr]_0$ is represented on a logarithmic scale on the abscissa, the peak potential on a linear scale on the right ordinate, and the rates of rise and fall on a linear scale on the left ordinate. This Figure was prepared using data obtained from the same preparation as Fig. 2*B*. Each point shows the mean ± S.D. Linear regression lines were fitted by the least-squares method. Correlation coefficients for the peak potential and the maximum rates of rise and fall were 0·84, 0·95 and 0·91, respectively ($P = 0·0005$).

plateau decreased and frequent spike discharges appeared in the presence of high concentrations of Sr. The developed tension increased in Sr-rich solutions.

It is known that Sr can enter the cell through the so-called Ca channel and will activate the contractile mechanism (Uvelius *et al.* 1974). Thus, the spike discharge that was observed in a Sr-containing Ca-free solution was assumed to be produced by the inward Sr current. The peak potentials and the maximum rates of rise and fall of the initial spikes obtained from a single cell were then plotted against the logarithm of $[Sr]_0$ (Fig. 3). There were linear relationships between these three characteristics of the initial spikes and log $[Sr]_0$. The peak potential and the maximum rates of rise and fall increased with increases in the external Sr concentration, with slopes of 15·2 mV, 10·3 V/sec and 3·3 V/sec for a 10-fold change in the concentration, respectively.

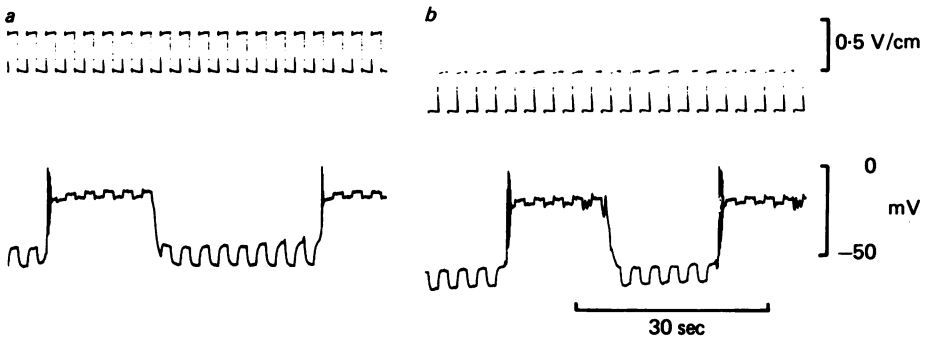

Fig. 4. Change of the membrane resistance during the Sr-induced activity. Successive electrotonic potentials induced by outward (*a*) and inward (*b*) current injection (upper trace) are shown in the electrical recording (lower trace).

Changes in the membrane resistance during the Sr-induced activity

The amplitudes of the electrotonic potentials induced by successive application of outward and inward current decreased during the plateau (Fig. 4*a* and *b*). This finding suggested that the membrane resistance decreased during the plateau, i.e. the ionic permeability of the membrane was enhanced. When the membrane permeability for an ion increases, the equilibrium potential of which is positive or at least less negative than the resting membrane potential, the membrane depolarizes. Na and Sr have positive equilibrium potentials, and Cl has a less negative equilibrium potential than the resting membrane potential (Wahlström, 1973). It seems unlikely that the enhanced membrane permeability for Sr was the main factor in production of the plateau, as the amplitude of the plateau did not decrease when external Sr was reduced but did decrease when the external Sr was increased. The dependence of the Sr-induced activity on Cl and Na was then examined.

Effect of Cl deficiency on the Sr-induced activity

Fig. 5 shows the effect of Cl-deficient solution on the Sr-induced activity. When the external concentration of Cl was decreased from 134 to 7·4 mM, the duration of the plateau was transiently prolonged, and membrane activity with normal plateau duration reappeared after about 10 min. Although the resting membrane potential shifted in the negative direction, the level of the plateau potential was more or less the same as that of the control. Chloride did not seem to be a main factor in plateau generation. The decrease of the developed tension seen in Cl-deficient solution may be the result of hyperpolarization of the membrane which would in turn decrease the number of cells contracting synchronously.

Effect of Na depletion on the Sr-induced activity

When the external Na concentration was reduced from 120·7 to 20 mM by replacement with Tris, the membrane was depolarized slightly during the resting phase and the amplitude of the plateau was markedly decreased (Fig. 6*b*). The membrane activity in a 20 mM-Na Sr-Krebs solution was similar to that in standard Ca-Krebs solution. With complete removal of Na the spontaneous activity transiently

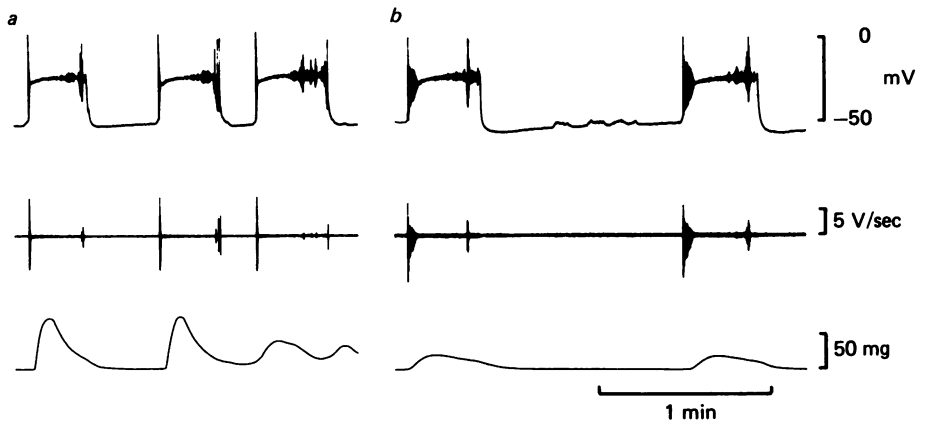

Fig. 5. Effect of Cl deficiency on the Sr-induced activity. The upper trace shows the electrical activity, the middle trace the dV/dt relation of the electrical activity, and the lower trace the mechanical activity. Cl was replaced with benzenesulphonate. The activities; *a*, in Cl, 134 mM and *b*, in Cl, 7·4 mM.

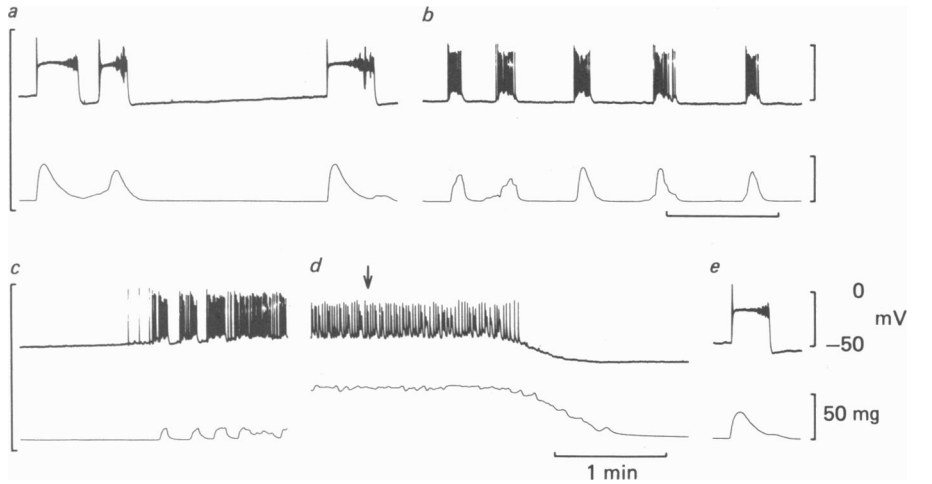

Fig. 6. Effect of Na depletion on the Sr-induced activity. The upper trace shows the electrical activity and the lower trace the mechanical activity. Na was replaced with Tris. The Sr-induced activities: *a*, in Na, 120·7 mM and *b*, in Na, 20 mM. At the beginning of *c*, all Na was replaced. The interval between *c* and *d* was about 5 min. At the arrow in *d*, 120·7 mM-Na solution was re-introduced. The interval between *d* and *e* was about 6 min, and *e* shows recovery of the Sr-induced activity.

ceased. The membrane then depolarized gradually, and successive spike discharges and a sustained contracture appeared (Fig. 6, *c* and *d*). These changes were fully reversible when the external Na was replaced (Fig. 6*e*). Na seemed to play important roles both in maintenance of the intermittent activity and in production of the plateau.

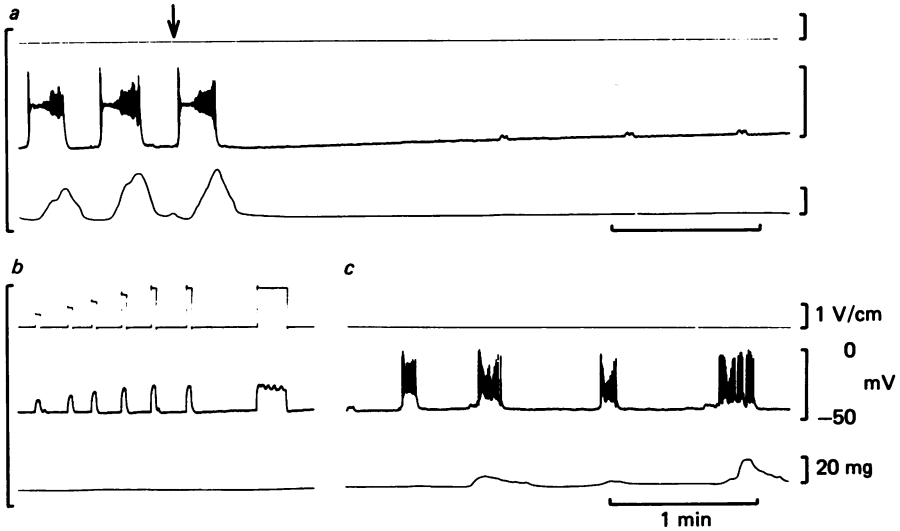

Fig. 7. Effect of Mn on the Sr-induced activity. The upper trace shows the injected current, the middle trace the electrical activity, and the lower trace the mechanical activity. At the arrow in *a*, Mn (1 mM) was added to 2·5 mM-Sr solution. *b*, depolarizing current failed to evoke membrane activity in the presence of 1 mM-Mn. *c*, the activity in 20 mM-Sr solution with 1 mM-Mn.

Effect of Mn on the Sr-induced activity

Mn blocks the Ca channel in various smooth muscles (Brading *et al.* 1969*b*; Osa, 1973). To examine the involvement of the Ca channel in the Sr-induced activity, Mn was added to the solution (Fig. 7). In the presence of 1 mM-Mn the spontaneous activity ceased and the membrane gradually depolarized. Soon small intermittent depolarization waves appeared, but neither the spike nor the plateau appeared (*a*). These small depolarization waves persisted in a 2 mM-Mn solution (not illustrated). To examine the possibility that Mn inhibited only the mechanism related to the spontaneity, depolarizing currents were applied to evoke the action potential. However, an active response could not be obtained (Fig. 7*b*). The concentration of Sr was then increased to 20 mM in the presence of 1 mM-Mn (*c*). Although spontaneous burst discharges with contractions appeared under these conditions, the amplitude of the plateau was diminished. In Sr solution, the spikes seem to be generated by the Sr current which enters the cell through the Mn-sensitive Ca channel. Mn also has an effect on generation of the plateau.

Effect of TEA on the Sr-induced activity

TEA reduces both resting K conductance and voltage-sensitive K conductance in various smooth muscles (Harder & Sperelakis, 1979; Imaizumi & Watanabe, 1981). To clarify the contribution of the K conductances in the Sr-induced activity, the effect of TEA was examined. With application of 20 mM-TEA the plateau became permanent and the depolarized membrane approached zero potential (Fig. 8*A*, *a*). Under this condition, small abortive repolarizations of the membrane were intermit-

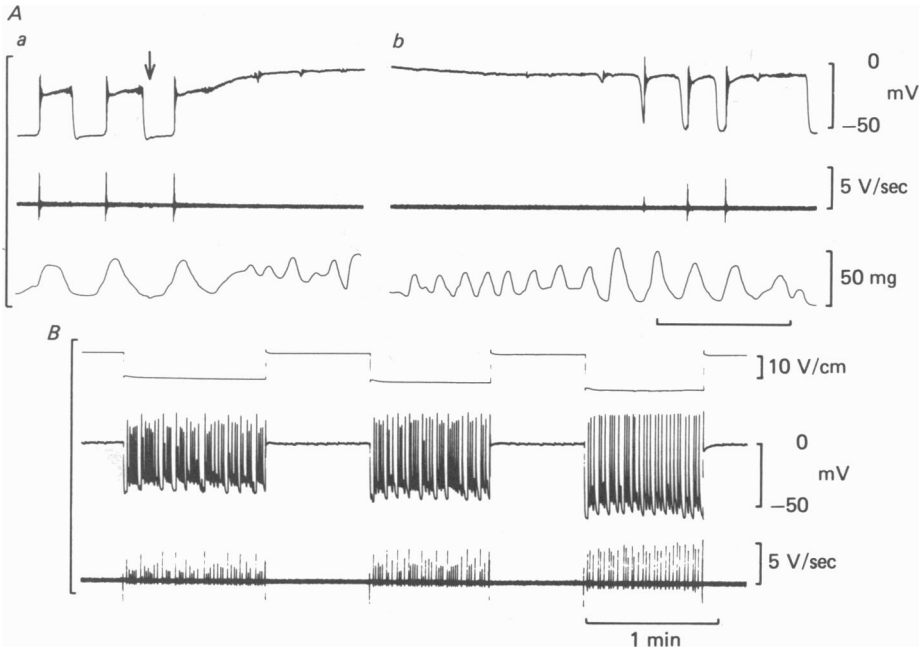

Fig. 8. Effect of TEA on the Sr-induced activity. *A* and *B* were the recordings from different preparations. *A*, the upper trace shows the electrical activity, the middle trace the dV/dt relation of the electrical activity and the lower trace the mechanical activity. At the arrow in *a*, TEA was introduced (20 mM), and at the beginning of *b*, TEA was washed out. *B*, the upper trace shows the injected current, the middle trace the electrical activity, and the lower trace the dV/dt relation of the electrical activity. The recording was obtained with 20 mM-TEA in the solution.

tently observed. This change was completely reversed after washout of TEA (Fig. 8*A*, *b*).

When the membrane was depolarized by 20 mM-TEA, inward currents were applied to repolarize the membrane (Fig. 8*B*). Frequent discharges of spikes were induced by the repolarization and on these spikes, the overshoots (up to 25 mV) were larger than those of the spontaneous spikes in the absence of TEA. Moreover, their maximum rates of fall were small compared to their rates of rise. An increase in the K conductance which can be inhibited by TEA is thus probably involved in determining the peak and the falling phase of the spike in Sr solution.

DISCUSSION

The replacement of Ca with Sr altered the spontaneous electrical activity of the smooth muscle of rat portal vein. Under such conditions, a burst discharge of spikes was followed by a long lasting plateau potential. With this change in electrical activity, the contraction was enhanced and prolonged. We shall discuss mainly the ionic movements that occur during the production of the spike and the plateau.

In the barnacle muscle fibre which produces Ca spikes, Sr could carry more or less the same amount of current as did Ca, through the Ca channel (Hagiwara, Fukuda

& Eaton, 1974). If the membrane channel involved in spike generation is highly selective for a divalent cation, the peak potential should approach the equilibrium potential for the ion, and it should increase by 30·5 mV for every 10-fold increase in the concentration of the ion at 35 °C (Reuter, 1973). Because the internal concentration of Sr is unknown, its equilibrium potential cannot be calculated validly. However, it can be assumed that it is more positive than +100 mV in the solution containing 2·5 mM-Sr. If this assumption is valid, the peak potentials of the initial spikes observed in the present study would be far negative than the equilibrium potential of Sr. Moreover, the observed 15·2 mV variation of the peak potential for a 10-fold change in $[Sr]_0$ is approximately half of the calculated value for the Sr-sensitive spike. These results can be explained in at least two ways. The channel responsible for the inward current may have a significant permeability to monovalent cation, namely Na, or there may be an overlapping outward K current which reduces the net inward current during the upstroke of the spike. Although the involvement of the inward Na current in spike generation cannot be excluded, the finding that 20 mM-TEA increased the overshoot of the spike and decreased its maximum rate of fall favours the existence of the TEA-sensitive K conductance which contributes to the spike.

In the smooth muscle of the guinea-pig ureter, the action potential in normal Ca solution was characterized by an initial spike component and a subsequent plateau component (Shuba, 1977). Using the voltage-clamp method, Bury & Shuba (1976) found that a slow inward Na current contributed to the plateau in the ureter smooth muscle and that this current was not blocked by 2 mM-Mn. Replacement of Ca with Sr prolonged this plateau up to 20 sec, and there was a large increase in the membrane conductance during the plateau (Kuriyama & Tomita, 1970). The Sr-induced plateau potential in the present study had characteristics resembling those of the plateau seen in the case of the ureter. The membrane conductance increased during the plateau and the amplitude of the plateau was sensitive to the external Na concentration. Thus, an inward Na current may also be the main factor in the generation of this plateau in the rat portal vein.

Na-dependent plateau potentials were also observed in the guinea-pig taenia coli soaked in Ca-free solutions containing 0·5–1·0 mM Mg (Bülbring & Tomita, 1970), and in small intestines of the cat and the guinea-pig, and stomachs of the skate, the toad and the frog which were soaked in a Ca-free solution with a Ca-chelating agent (Prosser, Kreulen, Weigel & Yau, 1977). While the former was a potential evoked by a depolarizing current pulse, the latter was a spontaneous activity and was blocked by Ca-channel blockers such as Mn, Co, La, verapamil or D-600. These authors concluded that when bound Ca was removed by a chelator, Na might enter rhythmically through the Ca channel. Because the Sr-induced plateau seen in the present study was markedly suppressed by 1 mM-Mn, one may speculate that the plateau is produced by a Na current which enters the cell through the Ca channel responsible for the spike, as suggested in the case of the chelator-induced potential. This explanation is, however, highly improbable for the following reasons. The spike inward current which passes through the Ca channel differs from the inward current responsible for the plateau, since (i) its size was increased as the extracellular Sr was increased, whereas the plateau was reduced, and (ii) increasing the concentration of Sr in the presence of Mn resulted in reappearance of the spike but not the plateau. If the plateau inward current also passes through a Ca channel, there would have

to be two types of Ca channel. In the guinea-pig taenia coli, Brading & Sneddon (1981) found two types of voltage-sensitive Ca channel, one rapidly inactivating and the other with little inactivation and responsible for Ca entry during prolonged depolarization. This new type of Ca channel is, however, unlikely to be responsible for the plateau inward current, because 1 mM-Mn had little effect on this type of Ca channel yet did block the spike generation completely (Brading & Sneddon, 1981).

In the guinea-pig taenia coli, membrane Na conductance is controlled by Ca bound in the membrane (Brading *et al.* 1969*a*, *b*; Bülbring & Tomita, 1970). Sr increases the duration of the plateau in the guinea-pig ureter by affecting the process which controls Na conductance (Kuriyama & Tomita, 1970). If the membrane has a voltage-dependent Na channel which is usually masked by Ca bound in the membrane (stabilizing action of Ca), and Sr is less potent than Ca in suppressing Na conductance, the plateau might be produced by activation of this Na conductance. In addition to the stabilizing action of Ca, we could also account for the prevention of the plateau generation by Ca if this muscle has the $[Ca]_i$-sensitive K conductance system found in the guinea-pig taenia coli (Inomata & Kao, 1979) and in the rat myometrium (Mironneau & Savineau, 1980). Ca influx caused by spike discharge activates the K conductance, and the membrane is repolarized, thus preventing plateau generation. Because Sr seems to be less potent than Ca at activating the K conductance in molluscan neurones (Gorman & Hermann, 1979), the replacement of Ca with Sr may lead to a depolarization of the membrane. In either case, when Ca is replaced by Sr, the depolarization caused by spike discharge activates the Na channel and the membrane is depolarized to a certain level, i.e. plateau potential. The Ca-binding site which controls the Na conductance is located at the outer layer of the membrane (Brading *et al.* 1969*a*). The effect of Mn on plateau generation might be explained if Mn, like Ca, binds to this site and suppresses the Na conductance.

How then is the plateau terminated? Presumably by either a slow inactivation of the plateau Na current, or a slow activation of an outward K current. The finding that TEA prevented the termination does not necessarily mean that an outward K current plays an important role in the repolarization. TEA may reduce the resting K conductance and depolarize the membrane to the extent that the Na channel is activated. Time-dependent inactivation of the Na channel is likely to be involved in the repolarization, since small abortive repolarizations were observed in the presence of TEA.

Since Ca-sensitive non-selective channels have been detected in cultured cardiac cells (Colquhoun, Neher, Reuter & Stevens, 1981), such channels may be involved in generation of the plateau.

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