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MECHANISM OF SLOW DISCHARGES OF SHEEP CAROTID ARTERY

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SUMMARY

1. Single and double sucrose-gap methods were used to follow changes in membrane potential and conductance of smooth muscle of sheep carotid arteries.

2. K depolarization induced discharges lasting several seconds in various solutions containing Mn, or Mg, or Ca and procaine, sometimes with no other added cations and with only sulphate or ethanesulphonate as anions.

3. Membrane conductance usually rose substantially above resting level in the early part of these discharges, but fell towards resting level during the later part.

4. Large depolarizing currents caused proportionately less voltage displacement than small currents, and reduced voltage displacements induced by superimposed current pulses, even in Cl and HCO_3 free solutions, indicating activation of K conductance by depolarization.

5. When procaine was added, or Ca replaced by Mn or Mg, conductance was lower both at rest and on depolarization, and the increase on depolarization often underwent slow inactivation which it never did in simple Ca containing solutions.

6. The results indicate that a slowly inactivated inward current dependent on Ca, Mn or Mg was largely responsible for the slow discharges, and that procaine, Mn or Mg assisted the discharges by reducing the normal rapid outward-rectifying K conductance and allowing it to inactivate on prolonged depolarization.

INTRODUCTION

Slow waves of depolarization in intestinal smooth muscles, first described by Alvarez & Mahoney (1922), are Na dependent to some degree (Daniel, 1965, dog intestine; Job, 1969, cat intestine; Ohba, Sakamoto & Tomita, 1977, guinea-pig stomach). Assessment of membrane conductance by injection of an occasional pulse of current during such waves has given varied results; Bolton (1975) reported an increase in conductance during slow waves induced by acetylcholine in guinea-pig intestine, and Connor, Prosser & Weems (1974) no change in conductance during spontaneous slow waves in cat duodenum. The present paper describes slow waves in arterial smooth muscle which did not require Na but were dependent on Ca, Mn or Mg, and were generally associated with a time-dependent change in conductance consisting of an initial increase with a later fall.

The commonest type of electrical discharge in sheep carotid arteries is a spike lasting about a second or less, requiring either Na or Ca, and associated with Na influx in Na containing solutions (Keatinge, 1968*a*, *b*). In physiological solutions

the arteries sometimes also produce slow waves and contractions lasting many seconds, particularly during anoxia when they may function to displace arterial obstructions (Keatinge, 1964). It was recently noticed that such slow waves could be elicited from the arteries in Na and Ca free Tris solution provided Mn or Mg were present (Keatinge, 1976). In the present experiments a single sucrose-gap apparatus was first used to determine the ability of Mn, Mg, Ca and Na to sustain slow waves. A double sucrose-gap apparatus was then used to inject repeated alternating hyperpolarizing and depolarizing current pulses in order to follow changes in conductance throughout these and so to assess the role of increased inward current or decreased outward current in producing them.

These arteries (Mekata & Keatinge, 1975) like rabbit carotid and aorta (Mekata, 1971, 1976) normally show rapid outward-going rectification which tends to suppress all forms of discharge. It is attributable to K conductance which increases on depolarization and can be reduced by procaine (Cooper, Goodford, Hardy, Herring, Hind & Keatinge, 1974; Jacobs & Keatinge, 1974). The double sucrose-gap apparatus has now been used to follow conductance during prolonged injection of depolarizing current, in order to see whether any important changes in the usual behaviour of these K channels takes place in solutions which support slow discharges.

METHODS

Arteries and recording methods

Common carotid arteries were removed from sheep within 10 min of the animal being killed by exsanguination at a slaughterhouse. Adventitia was removed and a helical strip approximately 30 mm long and 1·5 mm wide was cut from the media.

Electrical and mechanical records were made from some of these strips with the single sucrose-gap apparatus described previously (Keatinge, 1964); the strip in the test section of this apparatus was 10 mm long, and was under tension of 2·5 g. The electrical output was picked up by Ag/AgCl electrodes, fed into a d.c. amplifier of 500 MΩ input resistance, and displayed with the output of the isotonic mechanical transducer by a S.E. Laboratories ultra-violet multichannel recording galvanometer (frequency response flat to 200 Hz).

Other studies were made with a double sucrose-gap apparatus (Fig. 1). The length of the centre chamber of this, containing the test portion of artery strip, was 3 mm. This is approximately the space constant of these strips at rest (Graham & Keatinge, 1975; Mekata & Keatinge, 1975) and therefore close to the optimum for the 'node' in a double sucrose-gap (McGuigan & Tsien, 1974). The segment of artery in the centre chamber, assuming a thickness of 302 μm (Keatinge, 1966) will, therefore, have contained 822 mm^2 cell membrane (Keatinge, 1968*b*); this figure was used to relate currents and conductances to area of cell membrane throughout the present paper. Each sucrose section was 8 mm long. Junctions between the fluid in the centre section, and sucrose solution in the adjacent sections, were sealed by silicone rubber sheets 0·1 mm thick, the artery strip being pulled through a pinhole in each sheet. Junctions between the sucrose sections and the pools of KCl in which the ends of the artery strip rested were sealed by plugs of Plasticine after the strip was in place. The e.m.f. between one of these pools of KCl and the fluid in the centre section of the apparatus was picked up by Ag/AgCl electrodes and recorded by the same amplifier and recorder used with the single sucrose-gap apparatus. Current was provided by three Neurolog NL 800 constant current units; the outputs, arranged in parallel, were injected through a second pair of Ag/AgCl electrodes, one in the second pool of KCl and one in the test solution in the centre part of the apparatus. One of these units provided current pulses, usually of 500 msec duration with a pause of 500 msec between them. The direction of alternate pulses from this unit was reversed by a reversing switch, so that successive pulses were opposite in direction. The other two units were used to inject prolonged depolarizing or hyperpolarizing current and were operated manually. With arteries in standard saline, the change in membrane potential was complete, or nearly so, within 500 msec of the onset of injection of small constant

currents (see Fig. 6) but in solutions with Mn, Mg or procaine, when membrane conductance was lower, it was often not complete in this time. The size of voltage displacement during 500 msec pulses would then over-estimate the absolute value of membrane conductance, but the direction of any change in the size of the displacement will indicate the direction of any change in conductance.

In both single and double sucrose-gap experiments the test portion of artery was kept at $36{\cdot}5 \pm 0{\cdot}5$ °C, and after being put into the apparatus was left in standard saline at this temperature for 90 min before any measurements were made. After a change in the composition of a solution the tissue was left in the new solution for at least 25 min before tests were made.

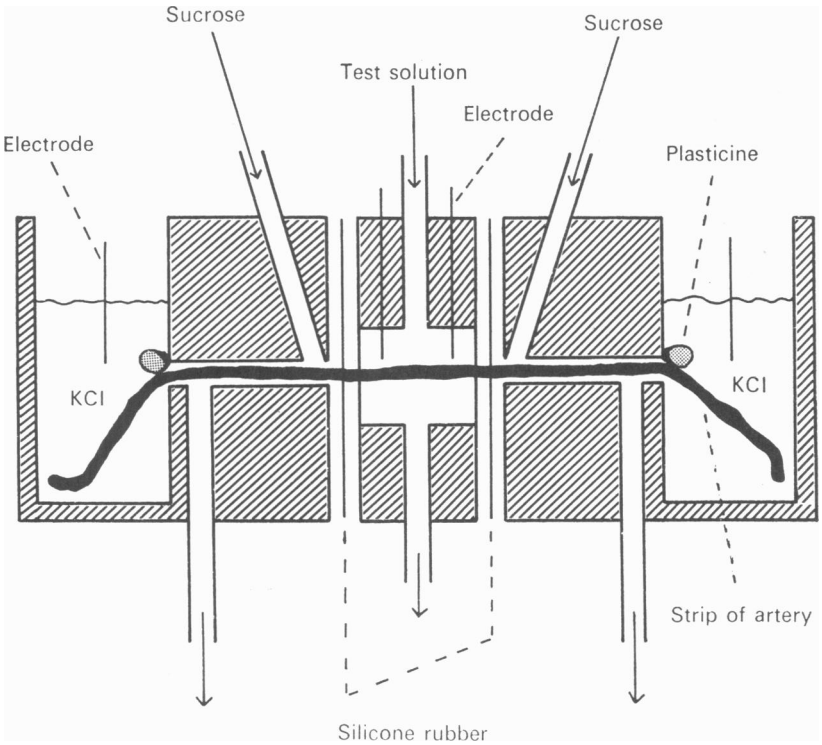

Fig. 1. Double sucrose-gap apparatus. See text for details; not to scale.

Solutions and drugs

'Standard saline' used in the initial stages of all experiments contained (mM): NaCl 133; $NaHCO_3$ 15, KCl 4·7, $CaCl_2$ 1·25, glucose 7·5. Ca-free saline had the same composition except that $CaCl_2$ was omitted and the solution was shaken with a little chelating resin (Chelex 100, Biorad Laboratories) to remove traces of Ca; the resin was then allowed to settle and the solution decanted from it. Tris Cl solution was free of Na and contained (mM): Tris (Trizma base, Sigma) 148; $KHCO_3$ 4·7, glucose 7·5; HCl was added to bring the pH to 7·35 during gassing with 95% O_2 + 5% CO_2. KCl solution contained (mM): KCl 137·7, $KHCO_3$ 15, glucose 7·5. These solutions were gassed continuously with 95% O_2 + 5% CO_2 during use.

Tris ethanesulphonate solution was free of Na and contained (mM): Tris 152·7, glucose 7·5; ethanesulphonic acid (Aldrich Chemical Co.) was added to bring the pH to 7·35. K ethanesulphonate solution was free of Na and contained (mM): KOH 152·7, glucose 7·5; ethanesulphonic acid was added to bring the pH to 7·35. These HCO_3 free solutions were gassed with 100% O_2.

Increases of K concentration in solutions based on Ca free saline, Tris Cl, or Tris ethanesulphonate were produced by mixing with KCl or K ethanesulphonate solution as appropriate. Concentrated $CaCl_2$, $MnCl_2$ or $MgCl_2$, Ca ethanesulphonate, Mn ethanesulphonate and Mg

ethanesulphonate solutions were prepared and added to the main solutions as required. The last three were prepared by neutralizing CaO, $Mn(OH)_2$ or $Mg(OH)_2$ respectively with ethanesulphonic acid. Solid $Mn(OH)_2$ and $Mg(OH)_2$ were prepared in turn by adding KOH to $MnSO_4$ or $MgSO_4$ solution, centrifuging and washing the resulting precipitate twice with deionized water; deoxygenated water and solutions were used in the case of Mn, in order to minimize oxidation of Mn^{2+} to Mn^{3+} at alkaline pH. For saline, K, or Tris based solutions with Procaine (B.D.H.), the main cation (Na, K or Tris), was reduced by the same molar concentration as that of the procaine added. This was necessary to avoid serious changes in osmotic pressure, as the concentration of procaine used was sometimes as high as 80 mM. The pH of the final Cl or ethanesulphonate solutions were adjusted to 7·35 by HCl or ethanesulphonic acid respectively during gassing by the appropriate gas mixture (95 % O_2 + 5 % CO_2, or 100 % O_2).

Unbuffered solutions containing a single cation and anion were made up as follows. Procaine sulphate solution contained (mM) procaine 105, sucrose 155, glucose 7·5; H_2SO_4 was added to bring pH to 7·35. $MnSO_4$ solution contained (mM): $MnSO_4$ 40, sucrose 225, glucose 7·5; solid $Mn(OH)_2$ was added to bring pH to 7·35. $MgSO_4$ solution contained (mM): $MgSO_4$ 40, sucrose 225, glucose 7·5; $Mg(OH)_2$ was added to bring pH to 7·35. These solutions had ionic strengths and osmotic pressures close to those of the Na, K and Tris based solutions. K depolarization of arteries in these solutions was produced by mixing them with K ethanesulphonate solution (containing 5 mM-Mn or Mg or Ca ethanesulphonate as appropriate). These solutions were gassed by 100 % O_2.

The sucrose solution used to perfuse the sucrose-gap contained (mM): sucrose 305, glucose 7·8.

Chemicals used were Analar except when otherwise stated. The water used was glass distilled, further purified by passage through 'Elgastat' deionizing resin.

Statistical comparisons

Results obtained under one set of conditions were paired with results from strips of the same artery under different conditions. The paired comparisons were made by the *t* test, except in the case of ethanesulphonate solutions in which the distribution of conductance was markedly skewed and the sign test was accordingly used.

RESULTS

Slow discharges in solution containing divalent ions

Artery strips in the single sucrose-gap apparatus were exposed to Tris Cl solution (Na free) for 20–30 min. K concentration was then increased from the initial 4·7 mM successively to 93·5 and 152·7 mM by partial and then complete replacement of Tris. This caused depolarization but never induced discharges in the absence of divalent ions (ten experiments). Nor did it induce discharges when the solutions contained Ca 5 mM (twenty experiments), or when they contained procaine 10 mM without Ca (eight experiments). However, when the solutions contained both Ca 5 mM and procaine discharges were obtained. Electrical discharges were recorded in nineteen of twenty-six such experiments with Ca 5 mM, with concentrations of procaine varying from 10 to 80 mM. The duration of those discharges varied. Spike discharges lasting about 1 sec or less were commoner with the lower concentrations of procaine, and slow discharges lasting several seconds were commoner with the higher concentrations of procaine, but both types were often seen in a single experiment. Spikes were then usually obtained at moderate levels of depolarization and slow discharges on further depolarization. Fig. 2*A*, *B* gives examples. It also shows that discharges in Ca containing solutions were often followed by contractions. Similar K depolarization in Tris Cl solution containing Mn or Mg produced slow discharges, even in the absence of procaine. Discharges lasting several seconds were

obtained in one of two such experiments with Mn 5 mM, and in seven of eight such experiments with Mg mM. As the examples in Fig. 2*C*, *D* show, these slow discharges usually had an initial abrupt upstroke. This occasionally took the form of a small initial spike, but was always followed by a plateau or wave of depolarization lasting several seconds. Individual discharges in Ca free Tris solution with Mn or Mg were never followed by significant contractions. Very similar results were obtained in Na as in Tris based solutions containing Mn or Mg. K depolarization induced discharges lasting several seconds in both of two experiments with Mn 5 mM in Ca free saline, and in five of eleven experiments with Mg 5 mM in Ca free saline.

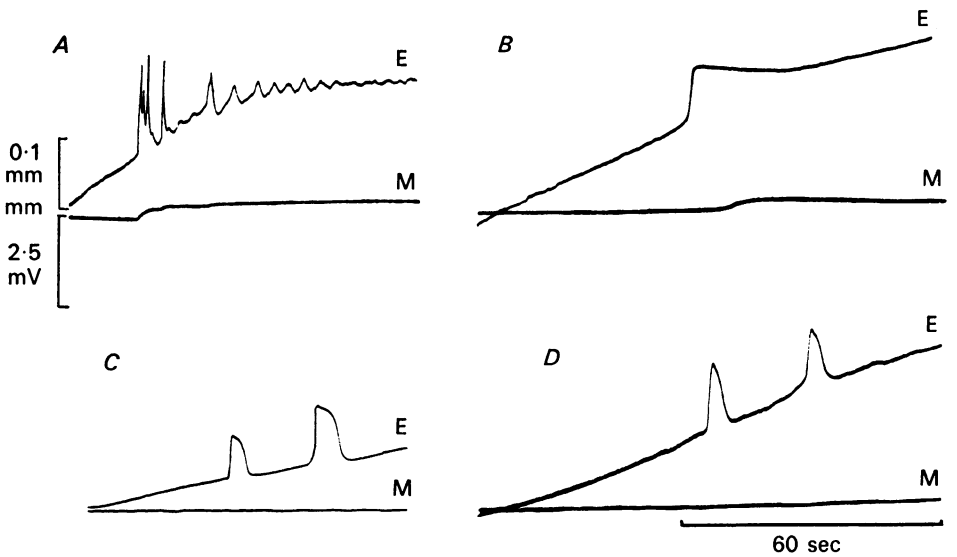

Fig. 2. Discharges induced by K depolarization in solutions containing divalent cations. Single sucrose-gap.
Tris Cl solution (Na free); K increasing from 4·7 to 93·5 mM.
A, with Ca 5 mM + procaine 10 mM.
B, with Ca 5 mM + procaine 40 mM.
C, with Mn 5 mM.
D, with Mg 5 mM.
E = electrical, M = mechanical trace.

In Na based solution as in Tris, no slow waves were obtained in the absence of divalent ions, but unlike Tris solution the Na allowed spike discharges lasting one second or less. These spikes appeared spontaneously within 20–30 min in Ca free saline in nine out of sixteen experiments, and were induced by adding EDTA 5 mM in two out of three of the rest and by K depolarization in two out of four of the rest. The only indication of a slow component to discharges in this solution was occasional grouping of spikes into pairs or larger series. Fig. 3 shows an example. Procaine did not affect the pattern. Similar addition of EDTA, followed by depolarization with K 93·5 mM in Ca free saline with procaine 10–20 mM, produced spike discharges similar to those obtained without procaine (three out of six experiments). Small contractions sometimes followed sustained electrical activity in Ca free saline, perhaps because the depolarization released small amounts of bound or sequestered

Ca, but individual discharges did not produce a detectable contraction, in this as in other Ca free solutions.

Membrane conductance during slow discharges

Slow discharges were then induced by K depolarization of arteries in the double sucrose-gap apparatus, while alternating 500 msec pulses of constant current were injected across one gap to follow membrane conductance. Voltage displacements produced by such pulses decreased in size during K depolarization, indicating an increase in conductance. They also decreased in size during the early part of the larger slow discharges, but returned towards starting level and occasionally to, or just above it, during the later part of these discharges. Conductance, therefore, generally increased during the early part, indicating that inward current rather than decreased

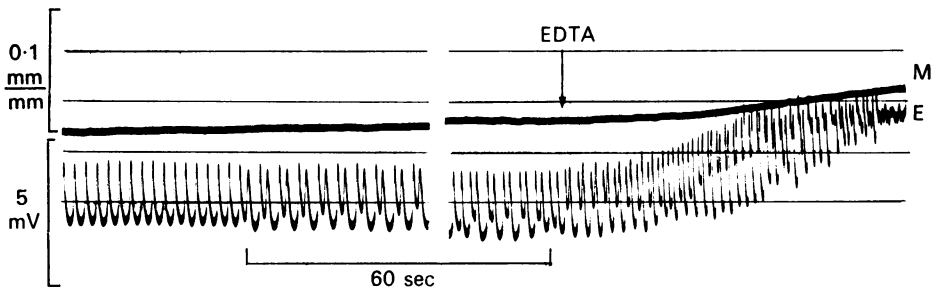

Fig. 3. Single and grouped spike discharges in Ca free saline.
Single sucrose-gap.
EDTA 5 mM added at arrow.
E = electrical, M = mechanical trace.

outward current caused depolarization at this stage, and fell to near resting level in the later part of the slow discharges. The extent of the increase was variable, and some slow discharges with a slow rise and small amplitude were accompanied by no clear change in conductance at any stage.

Fig. 4 shows examples of slow discharges with an initial increase in conductance which were recorded during K depolarization with Mn or Mg 5–10 mM present. Such discharges were seen in four out of ten experiments with Ca free saline + Mn 5 mM; in each of two with Tris Cl (Na free) + Mn 5 mM + Ca 5 mM; in one out of two with Tris Cl (Na free) + Mn 10 mM; one with Tris Cl (Na free) + Mg 10 mM; in five out of eleven with unbuffered Tris ethanesulphonate (Na free) + Mn 5–10 mM (Cl and HCO_3 free); and in one out of two with Tris ethanesulphonate solution (Na free) + Mg 10 mM. In order to see whether the early inward current required Na or Tris as well as the divalent ions, similar experiments were made in unbuffered $MnSO_4$ or $MgSO_4$ (40 mM) solution with only sucrose and glucose added. Depolarization of arteries in these solutions was brought about by replacing part and then all of the original solution by K ethanesulphonate solution containing $MnSO_4$ or $MgSO_4$ (5 mM) as appropriate; it sometimes caused slow discharges similar to those seen with Na or Tris present. Fig. 5*A*, *B* shows examples; similar discharges with a clear early increase of conductance in the larger discharges were obtained in one out of five such experiments with $MnSO_4$ and in three out of five with $MgSO_4$. Similar K depolarization

of arteries in procaine sulphate (105 mM) solution with $CaSO_4$ 5 mM produced similar discharges (two out of ten experiments; Fig. 5*C*).

Rectifying properties of K channels

Voltage deflexions produced by injection of prolonged constant currents were measured in order to assess conductance changes due to channels permeable to ions near to equilibrium distribution across the membrane, such as K ions. This was done

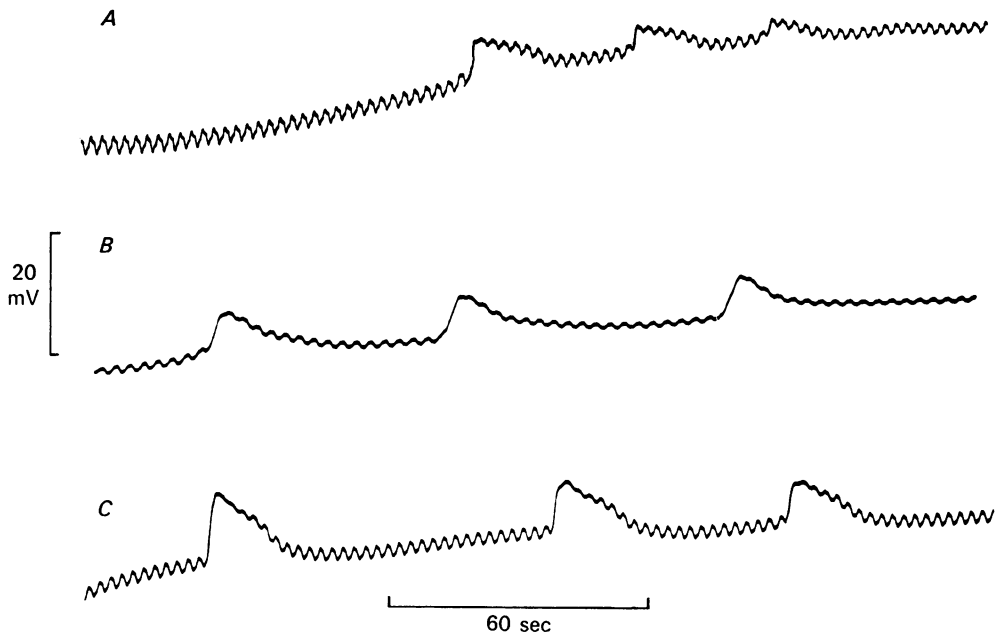

Fig. 4. Conductance during slow discharges in saline and Tris solutions containing Mn or Mg.
Double sucrose-gap.
Current pulses of 3 nA/mm² cell membrane and 500 msec duration injected every second in alternate directions.
A, Ca free saline + Mn 5 mM initially. K increasing from 4·7 to 93·5 mM.
B, Tris ethanesulphonate (Na, Cl and HCO_3 free) + Mn 5 mM initially. K increasing from 0 to 93·5 mM.
C, Tris ethanesulphonate (Na, Cl and HCO_3 free) + Mg 5 mM initially. K increasing from 0 to 93·5 mM.

first in solutions with physiological K concentration, 4·7 mM. The currents used, up to 60 nA/mm², rarely activated discharges. Preparations in which discharges were induced were discarded. Fig. 6*A* (left) shows that in standard saline (Ca 1·25 mM) small hyperpolarizing and depolarizing currents produced voltage deflexions that were of similar size and were complete in approximately 500 msec. The size of the deflexions in five such experiments gave resting conductance as $2{\cdot}30 \pm 0{\cdot}61$ μmho/mm² (mean ± S.E.). Larger depolarizing currents caused proportionately less voltage deflexion; in the five experiments, membrane conductance calculated from the size of the injected current and the resulting depolarization ($5{\cdot}3 \pm 1{\cdot}0$ mV) had increased to $13{\cdot}8 \pm 3{\cdot}9$ μmho/mm² 1 sec after the onset of 60 nA/mm² depolarizing current.

Conductance then either changed little or increased when the depolarizing current was continued, and was $29{\cdot}3 \pm 14{\cdot}9$ μmho/mm^2 after 30 sec (means $\pm$ S.E., five experiments). Five otherwise similar experiments in which alternating current pulses were superimposed throughout showed smaller resulting voltage pulsations throughout the injection of depolarizing current of 60 nA/mm^2 than without current injection, giving further evidence of increased conductance on depolarization. Fig. 6*A* (right) shows an example.

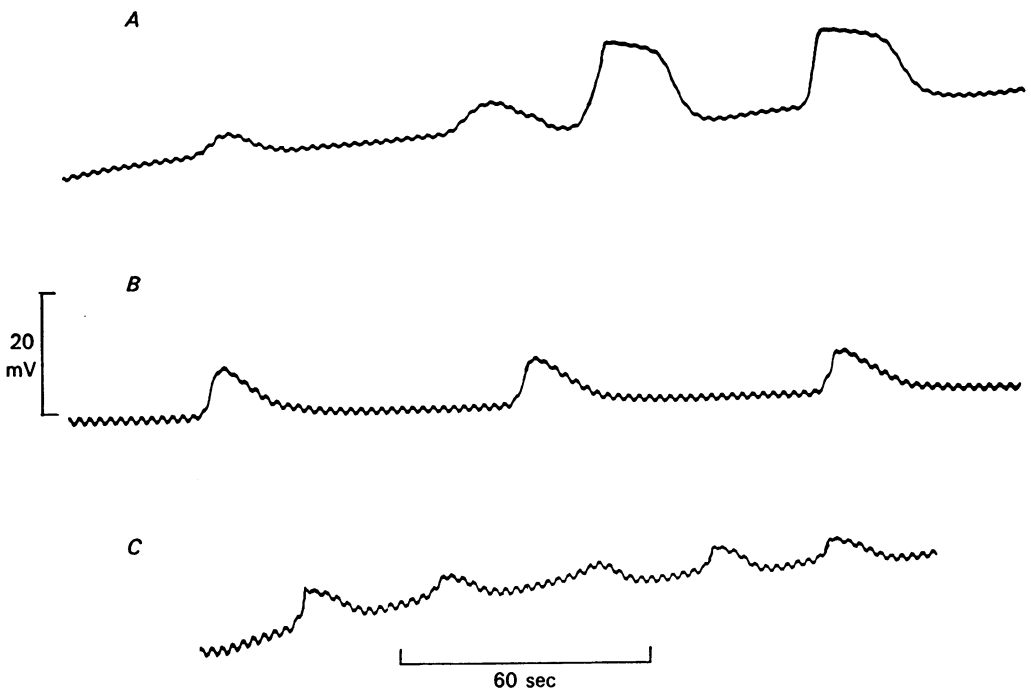

Fig. 5. Slow discharges sometimes induced by K depolarization in solutions with no other cations except Mn, or Mg, or Ca + procaine.
Double sucrose-gap. Current pulses 3 nA/mm^2.
A, $MnSO_4$ 40 mM initially. Mn 19, K 91·6, SO_4 16, ethanesulphonate 97·6 mM at end.
B, $MgSO_4$ 40 mM initially. Mg 19, K 91·6, SO_4 16, ethanesulphonate 97·6 mM at end.
C, Ca 5 mM, procaine 105 mM, SO_4 57·5 mM initially; Ca 5, procaine 66·0, K 67·6, SO_4 23, ethanesulphonate 97·6 mM at end.

Fig. 6*B* shows that injection of small currents produced larger voltage deflexions in Ca-free saline containing Mn 5 mM than similar current produced in the Ca containing saline. Resting conductance in five experiments in Mn saline was $0{\cdot}56 \pm 0{\cdot}24$ μmho/mm^2 (mean $\pm$ S.E.), significantly lower than in standard saline ($P < 0{\cdot}05$ by the paired t test). Although conductance again increased, to $4{\cdot}5 \pm 1{\cdot}2$ μmho/mm^2, 1 sec after starting to inject 60 nA/mm^2 depolarizing current in Mn saline, it was still significantly lower ($P < 0{\cdot}05$) than at the same stage of the corresponding experiment in standard saline. The most striking difference, though, was that in Mn saline the size of the voltage deflexion subsequently increased in four out of five experiments as the depolarizing current continued, suggesting that the increased

K conductance on depolarization underwent slow inactivation when depolarization was maintained. The conductance one second after onset of the 60 nA/mm² depolarizing current, calculated from injected current and voltage displacement, declined by $0{\cdot}53 \pm 0{\cdot}15$ μmho/mm² ($P < 0{\cdot}05$) in the five experiments when the current was sustained for 30 sec. The increasing voltage deflexion is very unlikely to have been due to activation of an inward current as it was sustained for 30 sec and more while the slow inward currents underwent considerable inactivation in a few seconds. A further indication of slowly decreasing conductance during sustained depolarization in Mn saline was provided by four similar experiments in which current pulses were superimposed on the sustained current injection and the voltage oscillations produced by these were found to increase during the sustained depolarization. Fig. 6*B* (right) shows an example.

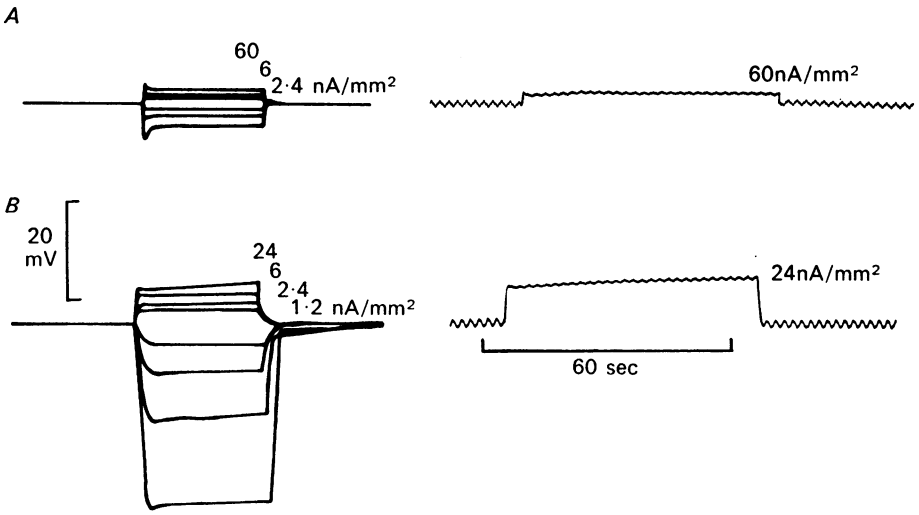

Fig. 6. Conductance changes induced in Ca or Mn containing saline by prolonged current injection.
Double sucrose-gap.
A, standard saline (Ca 1·25 mM). Superimposed 500 msec current pulses of 6 nA/mm² were injected in alternate directions throughout experiment on right.
B, Ca free saline + Mn 5 mM. Current pulses of 1·2 nA/mm² injected throughout experiment on right.

Table 1 shows results from similar experiments in Tris ethanesulphonate solutions (Na, Cl and HCO_3 free, and containing K 4·7 mM). With Ca 5 mM present conductance in this solution, as in standard saline with Ca, usually increased further when the depolarizing current was maintained for 30 sec. Replacement of Ca 5 mM by Mn 5 mM or Mg 5 mM, or the addition of procaine 40 mM to Ca 5 mM, all decreased mean conductance both at rest, and throughout depolarization by current of 60 nA/mm². The Mn, Mg or procaine did not prevent mean conductance from initially increasing on depolarization, but all of them caused that increase to be followed by a slow decrease. Since the solutions contained no Cl or HCO_3, changes in K rather than Cl or HCO_3 channels seem to have been responsible for these effects of Mn, Mg and procaine.

Discharges induced by injected current

When similar depolarizing currents were applied in $MnSO_4$, $MgSO_4$ or procaine sulphate + Ca 5 mM solution after partial K depolarization (K 61–94 mM) they again induced depolarization with increases in conductance, but slow discharges with further increases in conductivity were sometimes superimposed on these. Fig. 7*A* shows an example; the voltage displacements produced by current pulses show that conductance was increased above resting level throughout application of depolarizing current even in the absence of slow waves. As with current injections in solutions of lower K content, in the absence of a discharge the voltage displacement produced by steady injection of 60 nA/mm² was proportionately less than that produced by 6 nA/mm², indicating outward-going rectification. Such rectification could most easily

TABLE 1. Effect of Mn, Mg and procaine on membrane conductance of arteries in Tris ethanesulphonate solution (Cl and HCO_3 free, containing K 4·7 mM)

		Membrane conductance (μmho/mm²)		
		At rest	During depolarizing current of 60 nA/mm²	
Solution	Number of experiments		Initially	Increase during next 30 sec
Tris ethanesulphonate + Ca 5 mM	9	2·37 ± 1·25	7·82 ± 3·37	2·10 ± 1·34
Tris ethanesulphonate + Mn 5 mM	6	*0·34 ± 0·15	2·23 ± 0·62	* − 0·68 ± 0·28
Tris ethanesulphonate + Mg 5 mM	6	*0·81 ± 0·26	4·65 ± 1·53	* − 0·69 ± 0·46
Tris ethanesulphonate + procaine 40 mM + Ca 5 mM	6	0·39 ± 0·10	*1·63 ± 0·24	* − 0·10 ± 0·08

* Differs $P < 0·05$ from value in Tris ethanesulphonate + Ca (top line) by paired sign test; six pairs in each comparison.

be explained by increased conductance to an ion with an equilibrium potential at or negative to the membrane potential present during injection of the current. This ion was presumably K in this Cl-free solution, indicating that K equilibrium potential was at or negative to membrane potential at the time the discharges appeared. The inward-rectifying conductance responsible for the slow discharges would, therefore, involve some other ion, presumably Mg in this solution. Similar results were obtained in one experiment with $MnSO_4$, three with $MgSO_4$ and one with procaine sulphate + Ca.

Slow discharges were sometimes also produced in these solutions by terminating hyperpolarizing current (one out of three experiments with $MnSO_4$, one out of five with $MgSO_4$, three out of five with procaine + Ca). Fig. 7*B* shows an example, and

Fig. 7*C* shows a spontaneous discharge from the same artery. These last two discharges were interesting in being unusually prolonged; a progressive fall in conductance during the plateau is well seen, particularly in 7*B*.

No substantial slow discharges were ever induced by depolarizing or hyperpolarizing currents in similar experiments in Ca free saline, Tris ethanesulphonate, or procaine sulphate solutions, without divalent cations (five experiments in each case, with K 61–94 mM) although small negative overshoots in potential (1–2 mV) followed termination of hyperpolarizing current in two of the five experiments with procaine sulphate solution.

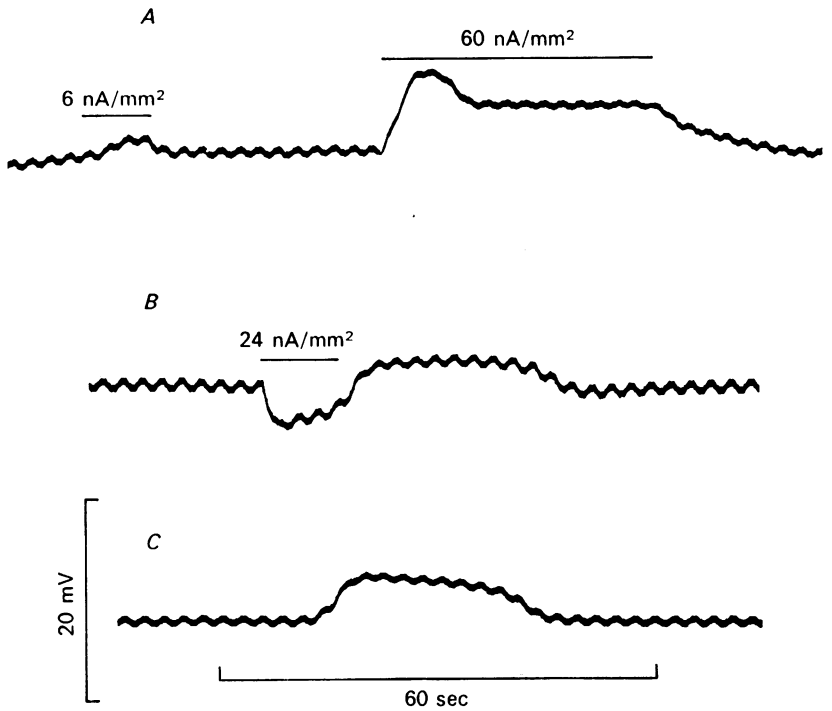

Fig. 7. Slow discharges induced by prolonged current injection.
Double sucrose-gap. Current pulses 6 nA/mm² superimposed throughout.
A, discharge induced by depolarizing current.
B, discharge on terminating hyperpolarizing current.
C, spontaneous discharge (same artery as *B*).
Solution contained Mg 19, K 91·6, SO_4 16, ethanesulphonate 97·6 mM in all experiments.

As examples in Figs. 4, 5 and 7 illustrate, when divalent ions were present there were no obvious systematic differences between slow discharges in otherwise comparable Cl and HCO_3 containing as opposed to Cl and HCO_3 free solutions, Na containing as opposed to Na free solutions, and Tris containing as opposed to (Tris + Na) free solutions. The size and shape of discharges, and the change in conductance during them, often varied considerably between different discharges in any given solution.

DISCUSSION

The fact that the slow discharges required Mn, Mg or Ca and that conductance increased in the early part of the larger ones, immediately suggested that their initial depolarizing phase was produced by a slowly inactivated inward current which could be carried by any of these divalent ions. Since the discharges were obtained after as long as 40 min in solutions that contained no cations except Mn or Mg, and the K used to reduce membrane potential, the only major alternatives to Mn or Mg as carriers of the inward current would be extracellular K or intracellular anions such as Cl or HCO_3, and some evidence was obtained against both of these alternatives. Although the inactivating K conductance observed in some arteries would theoretically be capable of producing discharges based on inward K current in a cell which was hyperpolarized with respect to K equilibrium potential, slow discharges were recorded when there was evidence that the cells were not hyperpolarized (Fig. 7*A*). The fact that the discharges were not significantly affected by removal of extracellular Cl and HCO_3 makes it unlikely that inward Cl or HCO_3 currents were responsible for them. Evidence that Ca as well as Mn or Mg could sustain slow discharges was less clear because another cation, procaine, was needed to reduce K conductance before slow discharges could be obtained in Ca containing solutions free of Mn or Mg. However, supporting evidence that Ca did carry slow inward current was provided by contractions that accompanied slow discharges in Ca containing solutions but not in Ca free solutions with Mn or Mg. The slow inward channel, therefore, seems to have a rather non-specific selectivity for divalent ions, and it is interesting that there were indications that it might utilize Na as well to some extent.

Although inward current was the primary cause of at least the larger slow discharges, alterations in background K permeability contributed to them. The experiments with prolonged current injection showed that Mn, Mg or procaine generally reduced K conductance both at rest and on depolarization. It was of particular interest that in the presence of these agents the increase of K conductance produced by depolarization often inactivated slowly, and that this could prolong slow discharges by many seconds while the slow inward current inactivated (e.g. Fig. 7*B*, *C*). The simplest explanation for these effects of Mn and Mg is that they reduced intracellular Ca and that this in turn allowed Ca dependent K channels to close, leaving other K channels which were not Ca dependent but were opened and then slowly inactivated by prolonged depolarization. Procaine probably blocked the Ca dependent channels directly. Intracellular Ca is known to open K gates in other tissues (e.g. squid axon, Tasaki, Watanabe & Lerman, 1967; skate electric organ, Clusin & Bennett, 1977), and procaine reduces K efflux from depolarized arteries in Ca containing solution (Cooper *et al.*, 1974). Inactivation of K conductance must have approximately matched activation of this conductance during the depolarizing phase of some small discharges with very slow onset, in which total conductance did not change significantly at any point (e.g. Fig. 5*A*). This inactivation of K conductance must have been as important as inward current in producing these discharges. Inward current during them was small. Assuming that the slow inward current had an equilibrium potential 100 mV positive to resting potential, conductance associated with inward current would be only 3% of background conductance during a 3 mV discharge

such as the smallest in Fig. 5*A*. This would not have been detectable against changes of background K conductance. None of the present findings exclude an electrogenic pump as a factor in the slow discharges, such as has been suggested in slow waves of intestine, but the fact that conductance was approximately constant during some slow discharges does not make it necessary to postulate an electrogenic component.

The finding that Mg could sustain slow inward current in these arteries appears to be unique; Mg ions are not believed to pass through voltage-dependent channels in other tissues. In other respects, the electrical behaviour of the arteries shows some resemblances to that of cardiac muscle. Cardiac muscle like the arteries has a slowly inactivating inward channel able to use Ca, and K channels which can be opened by moderate and closed by larger depolarization (see Weidmann, 1951; Hutter & Noble, 1960; Orkand & Niedergerke, 1964; Niedergerke, Page & Talbot, 1969; Rougier, Vassort, Garnier, Gargouil ; Coraboeuf, 1969; Kohlhardt, Bayer, Krause & Fleckenstein, 1972; Weidmann, 1974; Trautwein, McDonald & Tripathi, 1975; McAllister, Noble & Tsien, 1975; Beeler & Reuter, 1977). Cardiac muscle, like these arteries (Keatinge, 1968*a*, *b*), has a largely Na based fast inward current which can produce spike discharges. Even so, the present results show major differences between such processes in the arteries and the heart. Not only does Mg fail to sustain slow inward current in the heart (Ochi, 1976) but Mn, which can do so to some degree, usually shortens the cardiac action potential in Ca containing solution by blocking Ca current (Carmeliet & Vereecke, 1969; Yanaga & Holland, 1969), rather than lengthening discharges as it did in the arteries. Also the fall of K conductance in cardiac muscle on depolarization is so rapid that its time course cannot be measured and may be instantaneous, while the fall was extremely slow in the arteries. The fall in K conductance of striated muscle on depolarization, first described by Katz (1949) does take place slowly after an initial increase in conductance, as in the arteries (Nakajima, Iwasaki & Obata, 1962) but in the absence of a slow inward channel in striated muscle does not do so in time to prolong discharges significantly in that tissue.

In some respects the arteries differed even more from intestinal smooth muscle than from cardiac muscle. The largely Na dependent spikes and largely Ca and Mg dependent slow waves of the arteries contrast with largely Ca dependent spikes (Holman, 1957; Bulbring & Kuriyama, 1963; Golenhofen & Petranyi, 1969; Bulbring & Tomita, 1970) and Na dependent slow waves (Daniel, 1965; Connor *et al.* 1974) of intestine. The significance of Mg as a carrier of slow inward current in the arteries is unclear, but the additional use of Ca to carry this current will help to provide the mechanical response to sustained depolarization which occurs not only during slow discharges but during the action of vasoconstrictor agents and injury (Keatinge, 1964; Graham & Keatinge, 1975). Na, as the most abundant cation, is clearly a convenient carrier for fast inward current in the arteries. Slow waves of intestine provide conducted background 'pacesetter' activity of relatively constant frequency and rate of transmission; they do not induce significant contraction unless they trigger spikes, which enable propulsive mechanical activity to be coupled to the slow waves when required (Bass & Wiley, 1965; Grivel & Ruckebusch, 1972). The Na based slow waves and Ca based spikes of intestine provide a convenient basis for slow waves that do not cause contraction and spikes that do. Less information is available on other smooth muscles. The basis of uterine slow waves in particular is

unknown although their fast inward current is Na based like that of the arteries (Anderson, 1969; Anderson, Ramon & Snyder, 1971; Kao & McCulloch, 1975); Na may carry slow inward current in guinea-pig ureter, since removal of Na shortens its plateau discharges (Shuba, 1977*a*, *b*).

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