INWARD RECTIFICATION IN SUBMUCOSAL ARTERIOLES OF GUINEA-PIG ILEUM

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SUMMARY

1. The current–voltage relationships of short segments of submucosal arteriole of guinea-pig have been determined using constant current and voltage clamp techniques.

2. The current–voltage relationships were non-linear over the membrane potential range -60 to -90 mV, the conductance increasing with hyperpolarization.

3. The membrane potential ranges over which the membrane conductance increased were changed by changing the external concentration of potassium ions.

4. Changing the external concentrations of sodium and chloride ions had no effect on the arteriolar current–voltage relationships.

5. The hyperpolarization-activated conductance increase was prevented by low concentrations of barium ions.

6. It is suggested that these arterioles display potassium-selective inward rectification and that the rectifier supplies the dominant resting potassium conductance. The properties of this rectifier are compared with those of other tissues.

INTRODUCTION

The membranes of some excitable cells display inward or anomalous rectification. This was first described in skeletal muscle fibres by Katz (1949); a specific increase in membrane conductance to potassium ions occurs at potentials negative of the equilibrium potential for potassium ions (E_K). Potassium-selective inward rectification was subsequently demonstrated in starfish eggs (Hagiwara & Takahashi, 1974) and olfactory cortex neurones (Constanti & Galvan, 1983). This type of rectification is prevented by barium ions (Hagiwara, Miyazaki, Moody & Patlak, 1978) and its activation voltage shifted to more negative potentials as the external potassium concentration is decreased (Hagiwara, Miyazaki & Rosenthal, 1976; Hille, 1984).

A different type of hyperpolarization-activated inward rectifier has been demonstrated in cardiac muscle (DiFrancesco, 1981, 1985), mouse sensory neurones (Mayer & Westbrook, 1983) and in isolated smooth muscle cells of rabbit jejunum (Benham, Bolton, Denbigh & Lang, 1987). Again the rectifier .is activated by

hyperpolarization but the resultant current has a reversal potential near -20 mV: the current results from an increase in both sodium and potassium conductance (DiFrancesco, 1985). Changing the external potassium concentration changes the amount of current flowing at a given potential but does not change the activation potential of the rectifier. The cardiac and jejunal smooth muscle rectifiers are further distinguished from potassium-selective inward rectifiers in that they require high concentrations of barium ions to block them (Yanagihara & Irisawa, 1980; DiFrancesco, 1985; Benham *et al.* 1987).

A previous report on the membrane properties of arterioles has indicated that the current–voltage relationships of these arterioles are only linear for very small disturbances from resting potential (Hirst & Neild, 1978). This paper describes the current–voltage relationships of arterioles examined over a wider range of membrane potentials. Rectification was found to occur at membrane potentials near rest. Like that of skeletal muscle and starfish egg, rectification was selective to potassium ions and inactivated by barium ions. However, the activation voltage of the arteriolar inward rectifier was positive of the resting membrane potential and the rectifier provides the dominant potassium conductance at rest.

METHODS

All experiments were carried out using short, isolated segments of submucosal arteriole of the small intestine of guinea-pigs (weight 100–200 g). These arterioles lie in the same connective tissue sheet as the submucous plexus. After dissecting a sheet of tissue free (for details see Hirst, 1977), it was pinned in a shallow chamber (volume approximately 1 ml). The preparations were viewed using an inverted compound microscope and maintained by flowing warmed (bath temperature 35 °C) physiological saline (composition in mM: NaCl, 120; KCl, 5·0; CaCl$_2$, 2·5; MgCl$_2$, 2·0; NaHCO$_3$, 25; NaH$_2$PO$_4$, 0·1; glucose, 11), bubbled with 95 % O$_2$–5 % CO$_2$, at a rate of 5 ml/min. In some experiments the external potassium concentrations was varied (range 1·25–10 mM); this was done by changing the potassium chloride concentration, without osmotic compensation. In other experiments the sodium chloride was replaced by an equiosmotic amount of sucrose; in yet other experiments barium chloride (0·25–1 mM), manganese chloride (2 mM) or tetraethyl-ammonium chloride (10 mM, Sigma Chemicals) were added to the physiological saline. The segments (length 100–200 μm) were cut using a fragment of razor and allowed to equilibrate for 1 h. During this time the cut smooth muscle cells electrically sealed and the segments relaxed (Hirst & Neild, 1978, 1980).

Intracellular recordings were made using microelectrodes, filled with 0·5 M-KCl, with resistances in the range 80–120 MΩ. In each experiment the tips of electrodes were dipped in silicone fluid before use and the depth of physiological fluid over the tissue lowered to 50 μm. These procedures reduced microelectrode tip capacitance (Finkel, Hirst & van Helden, 1984). Electrophysiological data were collected using a single-electrode current clamp/single-electrode voltage clamp (Axon Instruments): details of use and precautions taken during data collection have been described (Finkel *et al.* 1984). After the initial experiments, in which it became apparent that we were not studying a phenomenon with rapid time dependence, a switching rate of 0·5 kHz was routinely used. Data were subsequently filtered with a low-pass filter (cut-off frequency 300 Hz), digitized and stored for later analysis using an IBM/AT computer. Data acquisition and analysis routines were programmed using the DAOS (Laboratory Software Associates) software package (Version 7·0).

RESULTS

General observations

After impaling a segment of arteriole, the resting potential was initially between -40 and -60 mV. Sufficient current, 0·1–0·4 nA, was passed through the recording
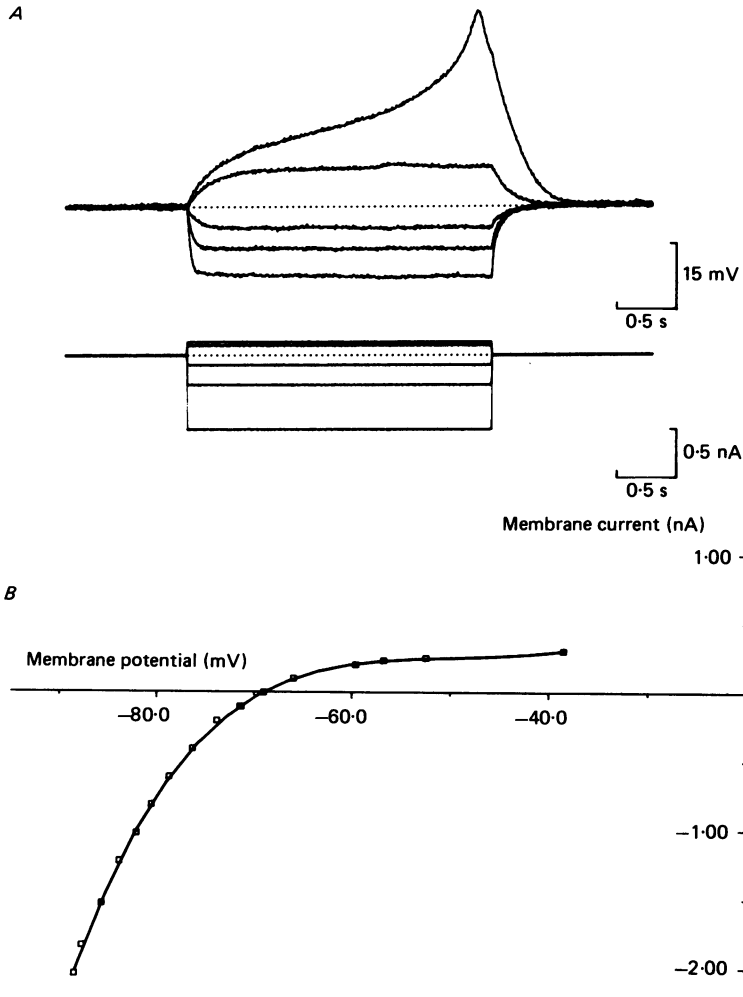
Fig. 1. Changes in membrane potential resulting from current injection into a short segment of submucosal arteriole (A) and relationship between steady-state membrane potential change and current (B). It can be seen that progressively larger hyperpolarizing currents were required to produce similar increments of membrane potential change. Conversely only small-amplitude depolarizing currents were required to produce appreciable depolarizations. The lower part of the figure shows the current–voltage relationship determined by measuring the membrane potential change just at the termination of the current pulse and plotting it against current intensity.

electrode to hold the membrane potential at -70 mV, the resting value recorded from these arterioles with higher-resistance electrodes (Hirst & van Helden, 1982). As the electrode 'sealed', the holding current was reduced to zero. Recordings were accepted if the resting membrane potential, with no current flowing, settled to a value more negative than -65 mV. Those with resting membrane potentials less negative than this were discarded. The mean membrane potential of the preparations used was -70.5 mV (S.E.M. ± 0.9 mV, $n = 28$). This value is similar to that reported previously (Kuriyama & Suzuki, 1981; Hirst & van Helden, 1982).
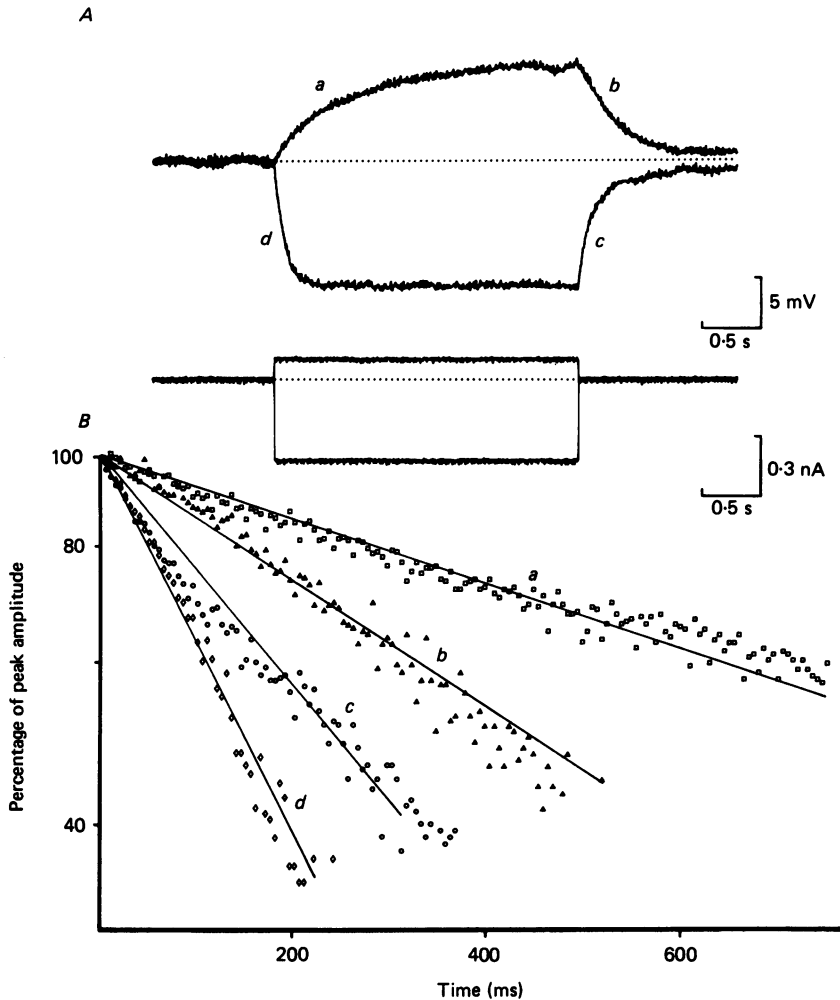
Fig. 2. Electrotonic potentials (*A*) and logarithmic plots of their rising and falling phases (*B*). The rate of membrane potential change during depolarization (*A a*) can be seen to be slower than during return to resting potential (*A b*). This results in the logarithmic plots of the potentials having different slopes (*B a* and *b*). Similarly the aspects of the hyperpolarizing potentials had faster time courses both on their make (*A c*; *B c*) and break (*A d*; *B d*). It will be appreciated that the description of the time courses of these electrotonic potentials by simple exponentials is an approximation. Charging, or discharging, of the membrane capacitance will be via a variable membrane resistance whose value is determined by the absolute membrane potential at any instant in time. A more accurate description of the time courses of the electrotonic potentials is given in the Appendix (see for example Fig. 9).

When small-amplitude hyperpolarizing current pulses (< 0·1 nA, duration 1–2 s) were passed through the recording electrode, preparations were shown to have input resistances of some 60–120 MΩ. Using larger-amplitude current pulses, it became apparent that the current–voltage relations were non-linear in the membrane potential range −50 to −100 mV. Examples of electrotonic potentials and

membrane currents are shown in Fig. 1*A*; the current–voltage relationship for this preparation is shown in Fig. 1*B*. It can be seen that the membrane resistance increased with depolarization and decreased with hyperpolarization. At potentials more positive than −50 mV, the electrotonic potentials became contaminated with regenerative potential changes. Following the addition of tetraethylammonium ions (TEA, 10 mM) to the superfusion fluid, a two-component action potential, similar to that recorded from rat cerebral arterioles (Hirst, Silverberg & van Helden, 1986), was detected. The ionic basis of these action potentials, and their variation in properties with location, will be dealt with in a later paper.

In addition to the non-linearity of the current–voltage relationships, the time courses of the electrotonic potentials showed a number of asymmetries. During the flow of depolarizing current the electrotonic potential had a slow time course, often reaching a steady-state value after several seconds. In contrast, after the break of depolarizing current flow the membrane potential change had a more rapid time course, the membrane potential returning to its resting value in about 1 s (Fig. 2*A*). With hyperpolarizing currents, the time courses of the electrotonic potentials were more rapid than those resulting from depolarizing currents. Again differences in time courses of the electrotonic potentials at the make and break of current flow were apparent. The membrane potential change at the onset of current flow had a faster time course than that following the break of current flow (Fig. 2*A*). The time courses of these aspects could each be approximately described by a single exponential; each exponential approximation had a different time constant (Fig. 2*B*). In subsequent experiments the basis of these phenomena was examined using a single-electrode voltage clamp technique.

Current–voltage relationships of submucosal arterioles

Short segments of submucosal arteriole were voltage clamped at their resting potential. The membrane potential was stepped over a range of potentials and the resulting membrane current recorded. Voltage command steps of +30 to −60 mV from rest were applied, thus the absolute of membrane potentials examined was −40 to −130 mV. On occasions only part of the range of hyperpolarizing potentials could be examined because the microelectrode tip potential failed to settle during the passage of the intense currents required to produce membrane hyperpolarization (see Finkel *et al.* 1984). In the depolarizing range, membrane currents became contaminated with a mixture of delayed rectifier and inward calcium currents (see Hirst *et al.* 1986). The delayed rectifier current could be suppressed by TEA (10 mM), and this was added in a number of experiments.

Examples of membrane potential steps and the associated currents are shown in Fig. 3*A*. It can be seen that, for command steps of a given amplitude, larger hyperpolarizing currents were required than were depolarizing currents. It can also be seen from these traces that the membrane currents did not change as a function of time unless very large hyperpolarizing steps were applied. The current–voltage relationship is shown in Fig. 3*B*. In this example, over the membrane potential range −60 to −105 mV (voltage command steps +10 to −35 mV from rest), the relationship between membrane potential and current could be described by a single exponential (see Appendix), with a voltage constant of 11·3 mV. The voltage
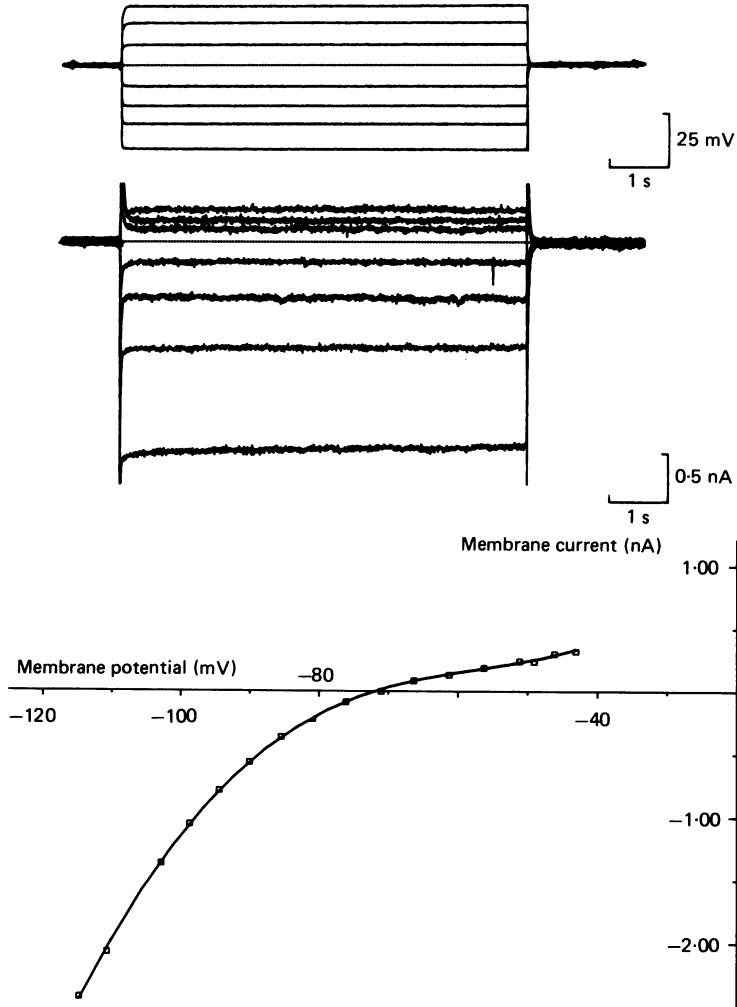
Fig. 3. Membrane currents recorded under voltage clamp from a short segment of submucosal arteriole. The upper family of traces in *A* illustrate the membrane potential changes and the lower, the associated membrane currents; the resting potential was −71 mV. Note that larger currents flowed during hyperpolarization than during depolarization and only with the largest hyperpolarizing step (40 mV from rest) was slight time-dependent inactivation noted. The lower part of the figure (*B*) shows the plot of membrane potential change against membrane current determined at the end of the voltage step. The current–voltage relationship is non-linear with the slope conductance at hyperpolarized potentials being ten times greater than at depolarized potentials. For further details see text.

constant specifies the depolarization required to reduce the current flowing through the voltage-sensitive conductance to 1/e of its previous value. At membrane potentials more negative than −105 mV, the relationship became linear with a slope resistance of 12·2 MΩ. The mean voltage constant from this series of experiments (*n* = 8) was 10·7 ± 1·1 mV (mean ± S.E.M); the exponentials were fitted over the

membrane potential range $-62 \cdot 6 \pm 2 \cdot 5$ to $-95 \cdot 6 \pm 2 \cdot 6$ mV the slope resistance in the hyperpolarized quadrant was $11 \pm 1 \cdot 4$ MΩ. After the addition of TEA (10 mM) to the perfusion fluid, it was frequently possible to detect a linear portion in the current–voltage relationship in the potential range -50 to -60 mV. On occasions this range of potentials was dominated by inward currents. Where detected, the linear range in the depolarized quadrant had a mean slope resistance of $131 \cdot 5 \pm 21 \cdot 2$ MΩ ($n = 6$). This concentration of TEA partially inactivated the rectifier. The voltage constant of the current–voltage relationship was $13 \cdot 0 \pm 0 \cdot 5$ mV ($n = 9$); the linear slope resistance determined at hyperpolarized potentials was $15 \cdot 1 \pm 2 \cdot 1$ MΩ ($n = 9$).
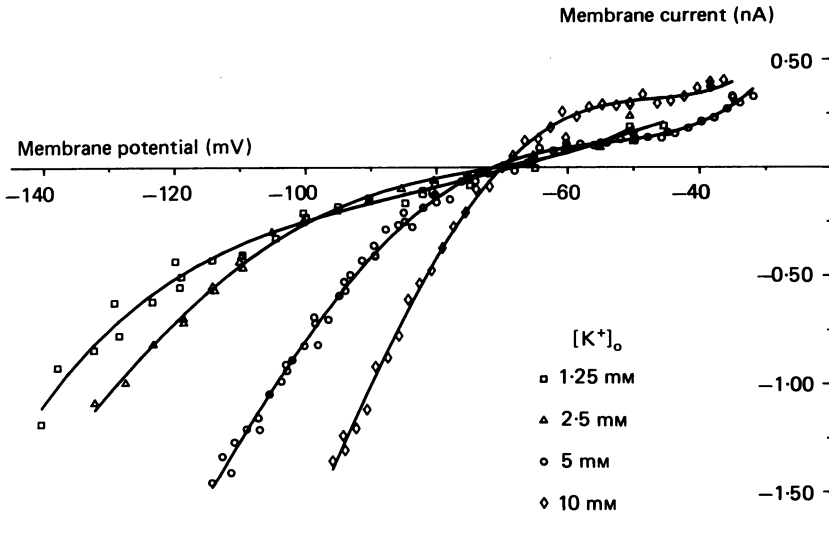

Fig. 4. Effect of changing the external concentration of potassium on the current–voltage relationship of a submucosal arteriole. Each of the curves was determined by holding the membrane potential at -70 mV and applying the appropriate command steps (changes in membrane potential). It can be seen that the activation voltages for inward rectification are changed when $[K^+]_o$ was changed; as $[K^+]_o$ was reduced, so activation moved to more negative potentials.

An incidental observation made during the experiments was that, in some preparations which had been set up for several hours, resting membrane potentials of -90 mV were detected (see also Finkel *et al.* 1984). These preparations had very low input resistances (< 20 MΩ). When their current–voltage relationships were examined, no rectification could be detected.

To test whether any slow time-dependent changes in membrane current occurred, voltage clamp steps of 25 s duration were applied. With steps covering the membrane potential range -60 to -90 mV, time-dependent changes in the membrane currents were not detected. With larger hyperpolarizing command steps (-40 to -80 mV from rest) a slow partial inactivation of membrane current was detected. At membrane potentials of -130 and -150 mV the membrane currents fell to about 60% of their initial values over the 25 s period. This behaviour has not been investigated further; the membrane potentials required to produce time-dependent inactivation were clearly well outside normal physiological ranges.

Membrane resistance was estimated at a number of potentials either by use of voltage steps or constant current pulses. It was found that less current was required to produce a voltage deviation at a depolarized potential than at resting membrane potential. At a hyperpolarized potential more current was required. Similarly, in current clamp mode, constant current pulses produced larger membrane potential changes at depolarized potentials than at rest. Conversely, at hyperpolarized potentials, they produced smaller potential changes. These experiments suggest that the non-linearity of the current–voltage relationships reflects the suppression of an outward current at depolarized potentials.

Effect of changes in external ion concentrations on arteriolar rectification

The current–voltage relationships of arterioles were changed by changing the external potassium concentration ($[K^+]_o$). In this series of experiments the external potassium concentration was doubled ($[K^+]_o = 10$ mM), halved ($[K^+]_o = 2\cdot5$ mM) or reduced to one-quarter ($[K^+]_o = 1\cdot25$ mM) of the control value ($[K^+]_o = 5$ mM). In each experiment the membrane potential was held at the resting value measured in control solution. The different potassium concentrations used in these experiments produced only small changes in membrane potential (see Hirst & van Helden, 1982), which were reflected as small changes in holding current. A decrease in $[K^+]_o$ decreased the slope conductance measured at resting potential; conversely an increase in $[K^+]_o$ increased the slope conductance measured at resting potential. The current–voltage relationships determined in four different potassium solutions from a single preparation are shown in Fig. 4. It can be seen that the activation curve for the rectifier was shifted to more negative potentials as $[K^+]_o$ was reduced and to more positive potentials as $[K^+]_o$ was increased.

When the external concentrations of sodium and chloride ions were simultaneously reduced, by substituting the sodium chloride in the physiological solution with sucrose, the current–voltage relationships were unchanged. This indicates that arteriolar rectification does not involve either of these ions and suggests that the conductance change is potassium selective. Rectification also persisted in the presence of manganese ions (2 mM), suggesting that it did not depend upon calcium entry.

In other tissues, potassium-selective inward rectification is prevented by low concentrations of barium ions (Hagiwara *et al.* 1978). This was also the case for arterioles. Barium ions (0·25–1 mM) rapidly and reversibly prevented rectifier activation over the membrane potential ranges −60 to 120 mV (Fig. 5). In the presence of barium, hyperpolarizing holding currents (0·15–0·3 nA) were required to maintain membrane potentials at −70 mV. In voltage-recording mode, barium (0·25–1 mM) was found to cause a depolarization of 20–30 mV. As barium both prevented inward rectification and caused depolarization, the potassium current flowing through the recitifer must be a major determinant of normal resting potential.

Interaction between inward rectifier, arteriolar action potentials, and excitatory junction potentials

The previous experiments have indicated that a potassium-selective rectifier is partly activated at normal resting potential and that the activation/membrane

potential relationship can be reset by making small changes to the external potassium concentration. Two different experiments were carried out to see if changes in rectifier activation affected arteriolar excitability. These arterioles generate two-component action potentials in the presence of TEA; a rapid component is followed by a plateau component (see also Hirst, 1977). In the first set of experiments the interaction between the rectifier and action potentials was examined. Action potentials were initiated at different membrane potentials, and hence on different parts of the rectifier activation curve. An experiment is illustrated by the upper left three traces of Fig. 6A a–c. It can be seen that at a depolarized
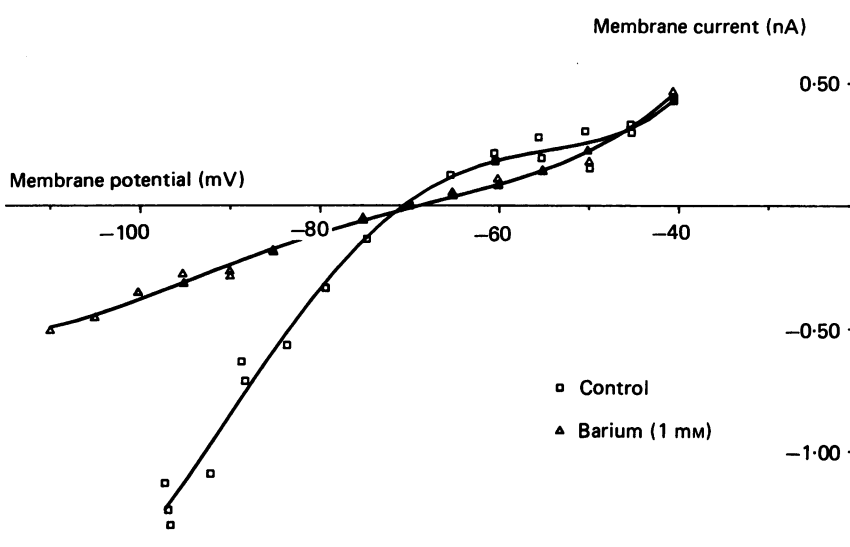

Fig. 5. Abolition of inward rectification by barium ions. The control curve (□) was obtained with the membrane potential held at −70 mV. After the addition of barium (1 mM) to the perfusion fluid a holding current (0·17 nA) was required to hold the membrane potential at −70 mV. The current–voltage relationship was now linear (△) up to depolarized potentials where the preparations started to display delayed rectification.

potential (rectifier inactive) the duration of the action potential was much longer than that of an action potential initiated at resting membrane potential. Conversely at hyperpolarized potentials the action potential had a briefer time course. In the second set of experiments (Fig. 6B) action potentials were initiated in solutions containing different external concentrations of potassium. The membrane potential was held at −70 mV by passing the appropriate steady current, if required. Action potentials were then initiated in half-normal potassium (rectifier inactive, activation being shifted to more negative potentials, see Fig. 4), normal potassium (rectifier partly activated) and twice normal potassium (rectifier fully activated). It can be seen from the traces shown in the lower left of Fig. 6B a–c, that the action potential duration is progressively shortened as the rectifier is progressively activated. These observations confirm that the rectifier is partly activated at normal resting potential and show that the degree of its activation affects arteriolar excitability.

Another indication of the effect of the inward rectifier on arteriolar excitability came from experiments where the time courses of decay of EJPs were examined.
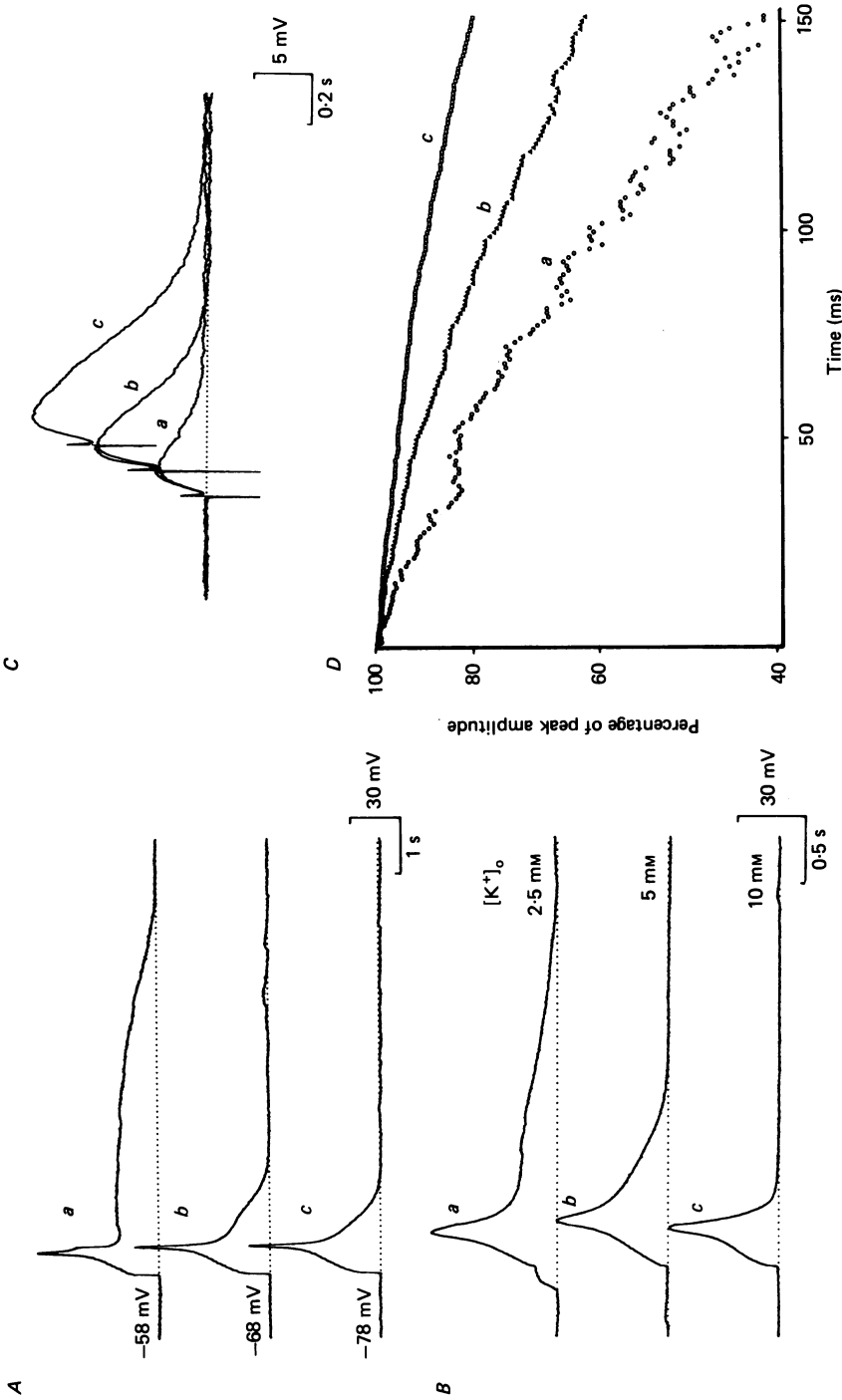
Fig. 6. Action potentials and excitatory junction potentials (EJPs) recorded from submucosal arteriole with varying degrees of rectifier activation. In each of the six traces shown in the left-hand column of the figure (A a–c; B a–c) a depolarizing current pulse of sufficient amplitude to just initiate an action potential was passed through the recording electrode. A a–c, action potentials were initiated at different membrane potentials. It can be seen that as the membrane was progressively depolarized, that is the potassium conductance progressively reduced, the plateau component of the action potential was prolonged. B a–c, action potentials were initiated in solutions containing different external concentrations of potassium; the resting membrane potential was held constant at −70 mV by passing the appropriate steady current. It can be seen that as the inward rectifier was progressively inactivated at −70 mV by reducing $[K^+]_o$ the plateau component was enhanced. TEA (10 mM) was present throughout. C and D, the effect of summing EJPs on the time course of their decay. C, three superimposed records show EJPs produced by one (a), two (b) and three (c) supramaximal perivascular stimuli. D, a logarithmic plot of their initial decays. It can be seen that the larger the EJP the slower the initial rate of decay.

Perivascular nerves were stimulated with single or short trains of impulses. Thus successive EJPs summed to give larger potential changes, hence moving the membrane potential to different regions of the rectifier activation curve. A set of records is shown in Fig. 6*C*, along with logarithmic plots of their initial decays (Fig. 6*D*). It can be seen that as EJPs summed to give larger potential changes, the initial rates of decay of potential were slowed. The time courses of the excitatory junctional currents which underlie EJPs are unaffected by changes of membrane potential in this range (Finkel *et al.* 1984). Since the time course of decay of EJPs reflects the passive membrane properties of an arteriole (Hirst & Neild, 1978), these observations suggest that changes in membrane potential in physiological ranges change the membrane time constant. Evidently the membrane time constant of a submucosal arteriole is not a true constant but rather it varies as a function of membrane potential.

DISCUSSION

The membranes of submucosal arterioles showed voltage-dependent rectification. When depolarized from rest, their resistance increased; when hyperpolarized their resistance decreased. Rectification was reflected by a non-linear membrane potential–membrane current relationship which, over the potential range -60 to -90 mV, could be described by a single-exponential function. Outside the range -60 to -90 mV the membrane potential–membrane current relationships became linear, the slope resistance at hyperpolarized potentials being about a tenth of that at depolarized potentials.

The rectifying properties of arterioles can be distinguished from those described for single isolated cells of rabbit jejunum (Benham *et al.* 1987). The hyperpolarization-activated rectifier of jejunal cells like that of cardiac muscle (DiFrancesco, 1985), results in a relatively non-selective cation conductance increase. Although the amplitudes of the rectifying currents of jejunal and cardiac muscle are changed by changing the external concentration of potassium ions, their activation voltages are unchanged. This contrasts with the arteriolar rectifier for which changing the external potassium concentration over a small range (1·25–10 mM) caused substantial changes in the membrane potentials at which the rectifier was activated. The two rectifiers can be further distinguished by the kinetics of their activation. Within the limits of our experimental resolution, the 10–20 ms required for settling of capacitive transients (Finkel *et al.* 1984), the activation of the arteriolar rectifier was instantaneous. The only time dependence detected was a slow inactivation at very negative potentials. The jejunal rectifier has a slow onset of activation, the time constant of which is voltage dependent. Furthermore the arteriolar rectifier is readily blocked by low concentrations of barium ions and unaffected by changing the external concentration of sodium (and chloride) ions.

The arteriolar rectifier resembles the inward rectifier described in skeletal muscle and in the starfish egg (Katz, 1949; Hagiwara & Takashi, 1974; Standen & Stanfield, 1978; Hille, 1984). With each, the activation curves are shifted to more negative or positive potentials as the external potassium concentration is decreased or increased. Each rectifier is not influenced by changes in the external concentrations of either

sodium or chloride ions. Each results from the activation of a selective potassium conductance and is blocked by barium ions. The general form of the voltage dependence of potassium current flowing through the arteriolar inward rectifier is given by examining the difference between current–voltage curves obtained in control solution and after barium blockade (Fig. 5). The true potassium current would be obtained after correction for current flow through other resting channels. For membrane voltages negative to E_K (about -90 mV) the rectifier current will be inward. For membrane voltages between E_K and the activation voltage of the rectifier (about -50 mV) the current will be outward. At more positive potentials, the rectifier will pass no current. This form, encountered in our data, agrees well with Hille's analysis for a three-site long pore model with an internal blocking ion which cannot cross the outermost potential barrier (Hille & Schwarz, 1978; Hille, 1984). Alternatively, a charged carrier mechanism for potassium ion transfer could also give rise to a rectifier current characteristic of this form (Adrian, 1969). The passing observation that in 'aged' tissues the resting potential is more negative and depolarization does not increase the membrane conductance might suggest that a metabolic product is required for closure of the rectifier. A further hint that an intracellular metabolic product might be involved is the observation that potassium channels which are activated by muscarinic agonists rectify over similar membrane potential ranges (Sakmann, Noma & Trautwein, 1983): these channels have specific metabolic requirements before they can be activated (Pfaffinger, Martin, Hunter, Nathanson & Hille, 1985).

There are differences between the starfish and arteriolar rectifiers. The inward rectifier of starfish egg has two components, one instantaneous and the other with a slow onset. Only an instantaneous component was detected in arterioles. Most potassium-selective inward rectifiers are only appreciably activated at potentials more negative than the Nernst potential for potassium ions unless the internal potassium concentration is reduced (Hagiwara & Yoshi, 1979; Leech & Stanfield, 1981). In contrast the arteriolar rectifier is partly activated at the normal resting potential, some 20 mV positive of the equilibrium potential for potassium ions. It should be pointed out that the Nernst potential for potassium ions has not been directly determined in vascular muscle. However, measurements of internal ion concentrations suggest that the internal concentration of potassium ions is around 140 mM (Casteels, 1981). Ion substitution experiments imply that much of this internal potassium is free and suggest that with a normal external potassium concentration (5 mM) the Nernst equilibrium potential for this ion is about -90 mV (Hirst & van Helden, 1982). At this potential the arteriolar rectifier is fully activated.

The presence of an inward rectifier provides an explanation for many of the unusual observations made when the ionic dependence of the resting membrane potential of submucosal arterioles was examined. Reducing the external potassium concentration increased arteriolar membrane resistance and caused membrane depolarization (Hirst & van Helden, 1982). This can be explained by a shift to more negative potentials for the activation of the arteriolar rectifier and suggests that at potentials positive of E_K the rectifying potassium conductance contributes to the normal resting potential. The observed effects of barium ions support this view:

barium produces membrane depolarization at the same time as it inactivates the rectifier. It is not clear why the rectifier takes on its characteristic degree of activation in control solution.

The asymmetrical time courses of electrotonic potentials recorded from arterioles can be explained by changes in rectifier activation (Fig. 2). As the membrane potential is depolarized during the passage of depolarizing current, the membrane resistance is increased which in turn increases the membrane time constant. During depolarization the membrane time constant will cascade through a range of increasing values. Conversely on cessation of depolarizing current as the membrane starts to repolarize towards its resting value its resistance will fall and allow a more rapid reaccumulation of charge. During the flow of hyperpolarizing current the membrane time constant will progressively fall as the membrane potential is hyperpolarized and the rate of membrane potential change will be rapid. These conditions have been modelled using a simple resistance and capacitance each in parallel with a resistance whose value varies as an exponential function of the voltage across the network. The calculations and derivation of the time courses of electrotonic potentials are given in the attached Appendix.

In a more general framework the properties of the arteriolar potassium rectifier may provide an explanation for increased arterial excitability that occurs during hypokalaemia. A decrease in serum potassium concentration will move the activation curve for the rectifier to a more negative level. Thus the resting outward current will be reduced and action potentials will be prolonged (Fig. 6). The inward rectifier will also cause the integrative properties of an arteriole to vary as a function of the rate of arrival of synaptic information (see Neild, 1983). As the arteriolar membrane is depolarized, for example during an EJP (Fig. 6), the membrane resistance increases. Both the time constant and the length constant of the depolarized region of the arteriole will increase. This will lead to a compounding amplification of excitatory synaptic information: an unusual occurrence in information processing.

<div align="center">APPENDIX</div>

Calculation of the time course of the voltage response to a step of applied current

The membrane can be considered to have a set of voltage-independent ohmic channels, not blocked by barium and not affected by changes in extracellular potassium concentration. Their total conductance, average value 7·6 nS, is referred to as the resting conductance (G_r). It should be noted that this value may overestimate the true resting conductance: measurements taken in this region were frequently contaminated by delayed potassium rectifier current. A second conductance, which is blocked by barium and whose voltage activation profile is modified by changes in extracellular potassium concentration, is activated at membrane potentials more negative than about -50 mV ($[K^+]_o = 5$ mM). This rectifying conductance ($g_{K,V}$) increases with further negative voltage to a maximum at about -90 mV, with an average value of 66 nS. Thus the entire current–voltage relationship can be considered to be made up of three regions (Fig. 7): (1) Voltage-sensitive inward-rectifying conductance approaches zero (membrane potentials more positive than -50 mV). (2) Voltage-sensitive inward rectifying conductance is

partially activated (membrane potentials in the range −50 to −90 mV). (3) Voltage-sensitive inward-rectifying conductance is fully activated (membrane potentials more negative than −90 mV). The regions 1 and 3 of the current–voltage relationship are fitted by straight lines. Region 2 can be fitted by a single exponential.
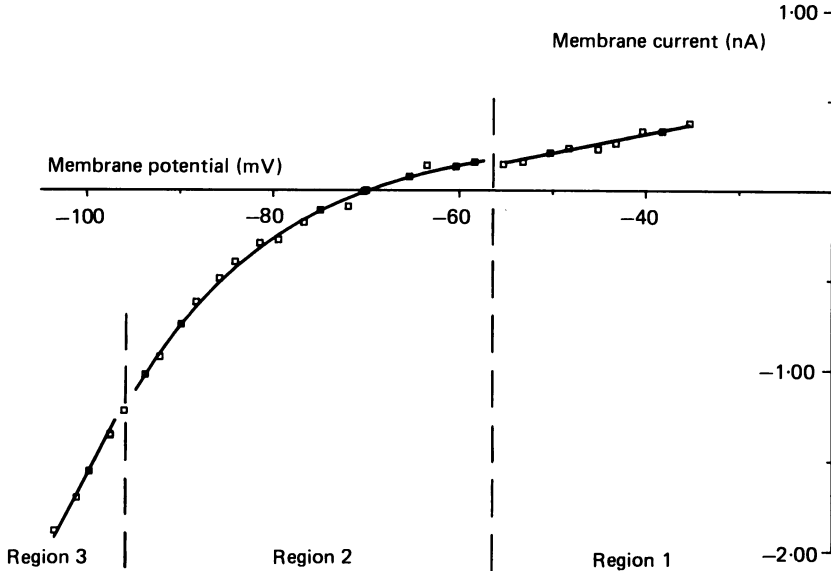

Fig. 7. Analysis of a membrane current–membrane voltage relationship obtained from a submucosal arteriole. The relationship is divided into three regions. Region 1 is a linear region detected at potentials where the voltage-sensitive conductance is zero. Region 2 covers the progressive activation of the inward rectifier. In this region there is an approximately exponential dependence of current upon voltage. Region 3 is a second linear region detected when the voltage-sensitive conductance is fully activated.

The simplified equivalent circuit for the arterial cell membrane is shown in Fig. 8. The current leaving node X must be zero at any time t.

$$i_1 + i_2 + i_3 - I_t = 0, \tag{1}$$

where i_1 is the voltage-independent membrane current which flows through the resting conductance (G_r), i_2 is the membrane capacitance current, i_3 is the voltage-dependent membrane current which flows through the rectifying conductance ($g_{K,V}$), and I_t is the current applied through the electrode.

Inserting values in eqn (1) and rearranging gives the following differential equation, the solution of which is the required membrane potential response:

$$C\, \mathrm{d}V_t/\mathrm{d}_t + (V_t - E_r)\, G_r + (V_t - E_K)\, g_{K,V} = I_t, \tag{2}$$

where C is total membrane capacitance, V_t is the membrane potential, E_r is the resting membrane potential in the absence of voltage-dependent conductances, G_r is the resting conductance, E_K is the reversal potential for potassium ions, $g_{K,V}$ is the rectifying conductance, and $(V_t - E_K)\, g_{K,V}$, which is the voltage-sensitive current, i_3, is equal to zero for $V_t > -50$ mV, or $P \exp(V_t/Q)$ for -90 mV $< V_t < -50$ mV

(where the amplitude P and voltage constant Q can be determined from experimental data), or $(V_t - E_K)g_{K,V_{max}}$ for $V_t < -90$ mV (where $g_{K,V_{max}}$ is value of the voltage-sensitive conductance when fully activated).

There are no standard procedures for obtaining analytic solutions to equations of the form of eqn (2) because the term $g_{K,V}$ introduces an intractable non-linearity. However, solutions for specific data sets can be obtained using numerical procedures. This was done using the Runge–Kutta method, specifically Kutta's 2-parameter formulae of 4th order accuracy (Hildebrand, 1956).
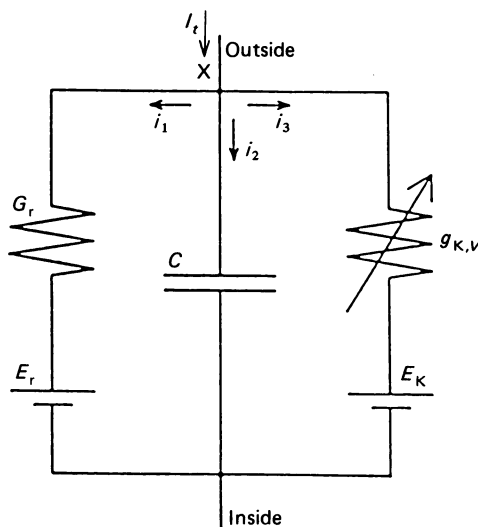

Fig. 8. Simplified equivalent circuit for the membrane. Part of the applied current I_t flows through the resting conductance G_r, part alters the charge on the membrane capacitance C, and the remainder flows through the rectifying conductance, $g_{K,V}$. The battery, E_K, represents the reversal potential for potassium ions and E_r represents the resting membrane potential when the voltage-sensitive conductance is zero.

G_r, P and Q can be determined from the experimental current–voltage relationship. E_r can be estimated from current–voltage curves obtained when the rectifier has been inactivated by barium ions. To fit a series of electrotonic potentials a value of total membrane capacitance, C, was chosen for goodness of fit for a single voltage response. The appropriateness of this choice was tested by fitting other electrotonic potentials of the series using the same value for C.

The different rates of change of membrane potential which occur when current steps of either polarity are applied and released are illustrated by the data points shown in Fig. 9A (see also Fig. 2A). In the example Fig. 9A, the numerical solutions (continuous lines) shown were generated using eqn (2) with a value of 3·8 nF for total membrane capacitance. This value gave good fits to the data for a range of injected currents. Figure 9B shows the four decays in the two traces replotted on a logarithmic voltage axis and inverted where necessary for comparison. The numerical solutions are not truly described by single exponentials. Thus the semilogarithmic
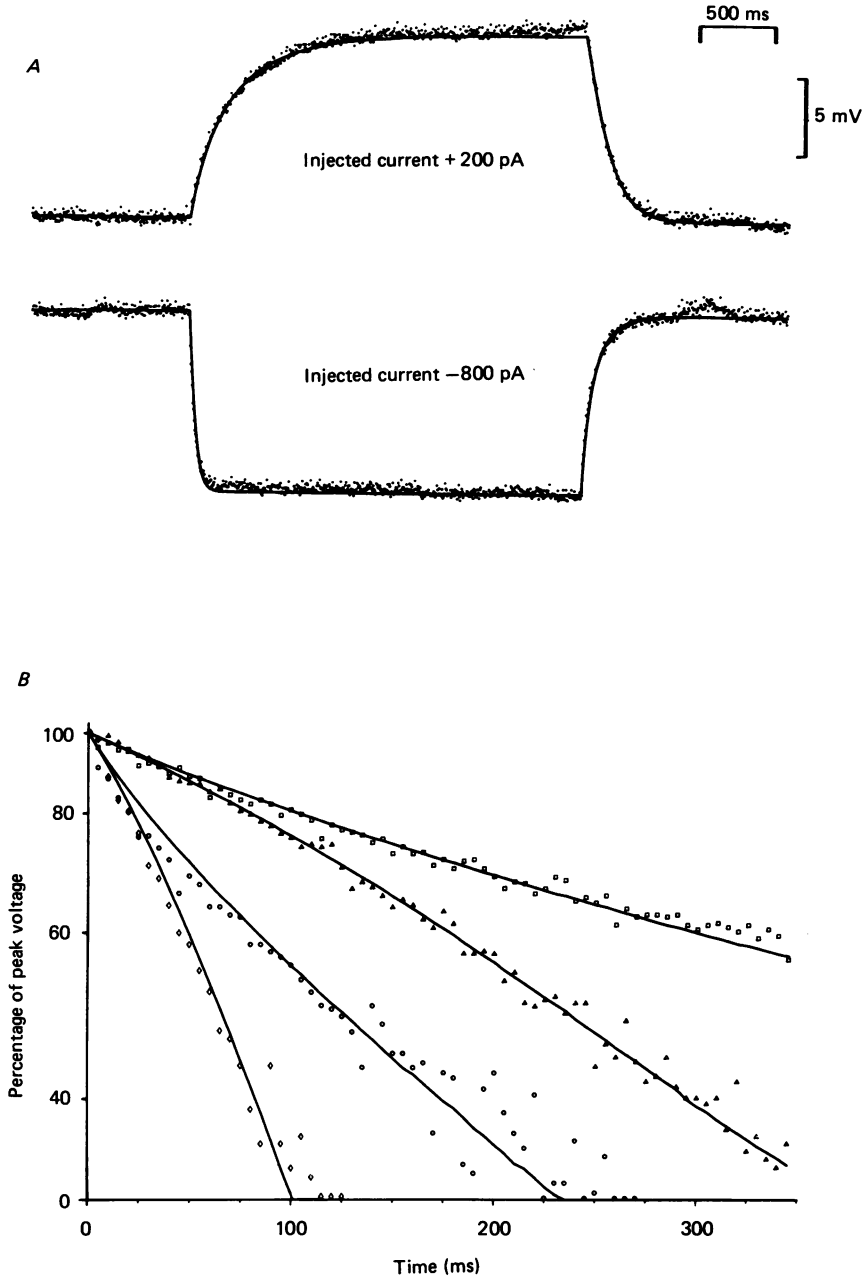
Fig. 9. Recorded and computed voltage responses to depolarizing and hyperpolarizing constant-current pulses (A). The dotted traces represent electrotonic potentials recorded from a short segment of arteriole produced by +200 and −800 pA. The continuous lines are computed using the methods specified in text. The transient sections of both traces, normalized and inverted for comparison where necessary, are replotted on a logarithmic amplitude axis (B). The symbolized plots are data, and the continuous lines are computed results. Irregularities in the computed results reflect noise on the measured membrane current traces (not shown) which were used in the computations.

plots of the electrotonic potentials and the numerical solutions are not straight lines but show slight convexities when positive charge is removed from the membrane capacitance and concavities when positive charge accumulates.

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