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THE ACTION POTENTIAL AND UNDERLYING IONIC CURRENTS IN PROXIMAL RAT MIDDLE CEREBRAL ARTERIOLES

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SUMMARY

1. The active and passive electrical properties of isolated segments (length 120–220 μm, diameter 60–150 μm) of proximal rat middle cerebral arterioles (< 1 mm from parent artery) were analysed using a single-electrode current or voltage clamp.

2. The voltage response to a current step exhibited an exponential time course. The mean resistance and time constant was 102 MΩ and 265 ms corresponding to approximate specific resistance and capacitance of 60 kΩ/cm and 4 μF/cm^2.

3. Membrane resistance was constant in the range -55 to -80 mV. At potentials more negative than -80 mV there was a decrease in membrane resistance resulting in activation of an inward rectifier. At membrane potentials less negative than -50 mV the membrane resistance decreased; larger depolarizations (> -40 mV) initiated small regenerative responses.

4. External application of tetraethylammonium chloride caused membrane depolarization (10–15 mV), spontaneous discharge of action potentials and rhythmic arteriolar constriction. Action potentials studied with the membrane held at -60 mV had a large rapid depolarizing component, an after-depolarization and a small slower after-hyperpolarization.

5. Tetrodotoxin (TTX) had no effect on the action potential. However, both the fast and slow components of the action potential were suppressed by extracellular removal of calcium ions and/or addition of cobalt ions, nifedipine or verapamil.

6. Voltage-clamp studies demonstrated an inward rectifying current at membrane potentials more negative than -80 mV. At depolarized potentials at least four separate currents were activated; two separate calcium currents and two outward currents.

INTRODUCTION

Many smooth muscles generate action potentials or regenerative membrane potential changes in response to membrane depolarization (for review see Bulbring & Shuba, 1976). Such potential changes persist in the presence of tetrodotoxin, suggesting either that inward sodium movement is not responsible for the upstroke of the action potential or that inward sodium channels in smooth muscle are distinct

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from those found in most other excitable tissues (cf. Redfern, Lundh & Thesleff, 1970). Smooth muscle action potentials also persist after the removal of sodium ions from the extracellular fluid (for review see Tomita, 1981). They are however dependent on the presence of calcium ions and are depressed in amplitude by the addition of divalent cations such as cobalt or manganese. Since these procedures depress calcium movements in other excitable tissues it seems likely that inward calcium movement underlies the regenerative responses detected in smooth muscles.

Action potentials or local responses have been recorded from a number of arterial smooth muscle preparations usually initiated by membrane depolarization resulting from sympathetic nerve activity (Bell, 1969; Speden, 1970; Hirst, 1977; Holman & Suprenant, 1979; Hirst, Neild & Silverberg, 1982; Hill, Hirst & van Helden, 1983). Action potentials have also been recorded from sheep carotid arteries (Keatinge, 1968, 1978). These studies indicated that the voltage-dependent conductances activated by depolarization were complex. The action potentials had both rapid and slow components which were calcium dependent. A role for sodium ions was also indicated which may result from a low selectivity of inward channels, but as with other smooth muscles, tetrodotoxin failed to abolish action potentials. The regenerative changes in membrane potential were opposed by both a calcium dependent and a calcium independent rectifier. Action potentials recorded from rabbit ear artery, when initiated as a consequence of exposure to tetraethylammonium ions, were both increased in amplitude and had faster rates of rise in the presence of an increased extracellular concentration of calcium ions (Droogmans, Raeymaekers & Casteels, 1977).

This report presents data obtained from arterioles originating from the middle cerebral artery of the rat. These vessels had small diameters ($<100\ \mu m$) and their wall thicknesses were similar to those of systemic arterioles (Hirst, 1977, Hua & Cragg, 1980). As such, when cut into short segments, they behaved as very short cables (Hirst & Neild, 1978); the membrane currents they generate as a result of changes of membrane potential have been measured using a voltage-clamp technique (see Finkel, Hirst & van Helden, 1984).

The observations made are in accord with those made on sheep carotid arteries (Keatinge, 1968, 1978) and suggest that these arterioles, if they are close to the point of their origination from the middle cerebral artery (see Hill, Hirst, Silverberg & van Helden, 1986), possess two distinct inward calcium currents, an outward rectifying potassium current and a slow calcium-activated potassium current.

METHODS

Experiments were carried out on arterioles which arose from the middle cerebral artery of rats (male or female 170–300 g). Rats were anaesthetized with sodium pentobarbitone (50 mg/1 kg body weight I.P.) and exsanguinated. The brain was rapidly removed and transferred to a dissecting bath containing gassed physiological salt solution (composition, mM: NaCl, 120; KCl, 5; $CaCl_2$, 2·5; $MgCl_2$, 2; NaH_2PO_4, 1; $NaHCO_3$, 25; glucose, 11; gassed with 95% O_2:5% CO_2; pH 7·2–7·4). The pia, to which was attached one of the internal carotid arteries and the middle cerebral artery along with the arterioles originating from these arteries, were dissected free of underlying brain tissue (see Fig. 1, Hill *et al.* 1986). The preparation was pinned pial surface downwards, in a recording chamber (volume 0·1 ml) and viewed using an inverted compound microscope (for details see Hirst, Holman

& Spence, 1974). Physiological saline, warmed to 37 °C, was flowed continuously through the chamber at a rate of 4–6 ml/min. The arterioles were cut into short segments (length 120–220 μm) using a fragment of razor blade that had been glued to a glass rod held in a micromanipulator. For the studies described in this report only the segments within 1 mm of their origination from the middle cerebral artery were used.

Intracellular recordings were made using micro-electrodes pulled from fibre containing glass tubes (Clark Electromedical, GC150F-15) and filled with 0·5 M-KCl. Electrodes with resistances in the range 100–140 MΩ were used. After filling, electrode tips were dipped in silicone fluid to prevent 'creep' of tissue fluid up the outside of the electrodes. Both this procedure and lowering the level of tissue fluid flowing over the preparation to 50 μm reduced the micro-electrode tip capacitance. Electro-physiological data were collected using a single-electrode current clamp/single-electrode voltage clamp (Axoclamp 1, Axon Industries). For details of use and precautions, see Finkel *et al.* 1984. Data were digitized and stored on disk for subsequent analysis. The membrane currents recorded from voltage-clamp experiments were in all cases corrected for linear membrane leakage and charging transients by subtracting an appropriate scaled current determined from a 10 mV hyperpolarizing step.

In some experiments, the following drugs were used: tetraethylammonium chloride (TEA, Sigma), verapamil hydrochloride (Knoll AG), nifedipine (Bayer) and tetrodotoxin (TTX, Sigma). All solutions containing nifedipine were light protected. In the experiments where calcium chloride was substituted for by either barium chloride, cobalt chloride or manganese chloride, the sodium bicarbonate concentration used in both test and control solutions was reduced to one quarter, osmotic compensation being made by addition of the appropriate amount of sodium chloride. This substitution changed the pH by less than 0·1 pH units.

RESULTS

Passive electrical properties

In each experiment, a segment of arteriole close to its origination from the middle cerebral artery was impaled with an intracellular recording electrode. Immediately after impalement, a resting membrane potential of -40 to -50 mV was recorded, constant current pulses passed indicated that the input resistance was low (< 50 MΩ) and the time constant short (< 100 ms). Over the next few minutes, the membrane potential increased (to be in the range -55 to -70 mV, mean -63 mV, S.E. of mean ± 2 mV, $n = 23$) as did both the input resistance and time constant. To avoid variations between preparations, after stabilization, all membrane potentials were adjusted to have a value of -60 mV by passing the appropriate steady polarizing current through the recording electrode. Similarly in all voltage clamp studies, the holding potential was set to be -60 mV.

Each of the segments used had lengths of less than 220 μm. As the electrical length constant of arteries and arterioles is about 1·5 mm (Casteels, Kitamura, Kuriyama & Suzuki, 1977; Hirst & Neild, 1978; Holman & Suprenant, 1979), the middle cerebral arteriole preparations would be expected to be electrically short. This was the case, when current pulses (0·05–0·1 nA, duration 1–2 s) were passed through the recording electrode. The time courses of the resulting membrane potential changes could be described by a single exponential (Fig. 1*A*, *C*). The input resistance of the segment illustrated was approximately 180 MΩ and its membrane time constant 300 ms. The mean input resistance of all segments measured was 102 ± 7 MΩ (mean ± 1 S.E. of mean, $n = 23$) and their time constant 265 ± 30 ms. Taking a mean segment length of 200 μm, an arteriolar diameter of 50 μm with a wall thickness of 5 μm, if no allowance is made for membrane in-foldings or membrane surface lying between

adjacent smooth muscle cells, the specific membrane resistance of these cells is calculated to be 60 kΩ cm^2 and the specific membrane capacitance to be 4 $\mu F/cm^2$. These values are similar to those reported for arteriolar smooth muscle of the guinea-pig (Hirst & Neild, 1978). Clearly a more accurate determination would require a more precise description of cell membrane area (for discussion, see Hirst & Neild, 1978; Hua & Cragg, 1980; Hirst & van Helden, 1982).

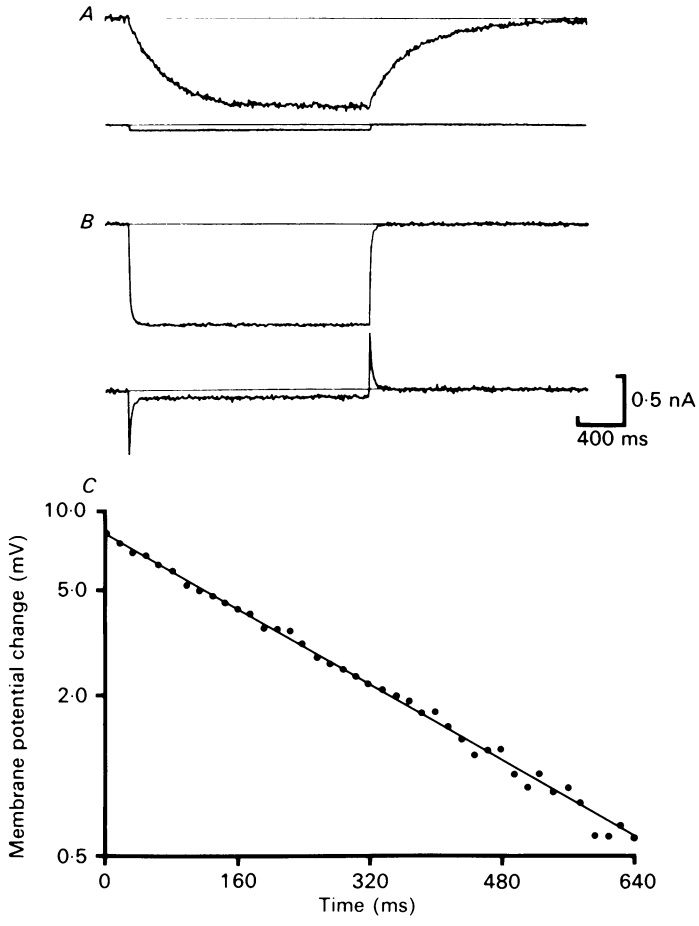

Fig. 1. The passive electrical properties of a segment of middle cerebral arteriole. *A*, the voltage response (upper trace) to a current step (lower trace) injected into an arteriolar segment of length 120 μm, diameter 65 μm and wall thickness of approximately 5 μm. *B*, the current (lower trace) resulting from a voltage step (upper trace) in the same segment but now voltage clamped. *C*, semilogarithmic plot of the transient voltage response to current application (shown in *A*) plotted against time. The line has been fitted by linear regression.

The membrane potential change produced by a 10 mV hyperpolarizing voltage command step along with the associated membrane current is shown in Fig. 1*B*. It can be seen that the charging current lasts for about 20 ms. This reflects the syncytial nature of the preparation. During rapid transient current flow, the segments are not instantaneously isopotential (see Hirst & Neild, 1978; Finkel *et al.* 1984). This

observation places a limit on the accuracy with which the time course of rapid current forms may be determined. However, since the time course of regenerative responses in these arterioles is slow the inaccuracies will be slight.

When the amplitude of the current pulses was varied, it was found that the membrane resistance was constant in the membrane potential range -55 mV to -80 mV. Beyond -80 mV, the membrane resistance decreased by factors of 2–5. This indicates that these arterioles, like submucosal arterioles (Hirst & Neild, 1978) and sheep carotid arteries (Keatinge, 1978), show an anomalous rectification.

Potential changes initiated by membrane depolarization – effects of TEA, TTX and calcium removal

When depolarizing currents were passed through the recording electrode, small regenerative responses were initiated (Fig. 2*A*). The peak amplitude of this regenerative response varied from preparation to preparation. The amplitude of the regenerative responses which exceeded the electronic depolarization produced by maintained current flow, was 16 ± 4 mV with a half width of 60 ± 10 ms (mean ± 1 S.E. of mean, $n = 8$). The threshold for initiation of these regenerative responses occurred at a membrane potential of -36 ± 1 mV ($n = 8$).

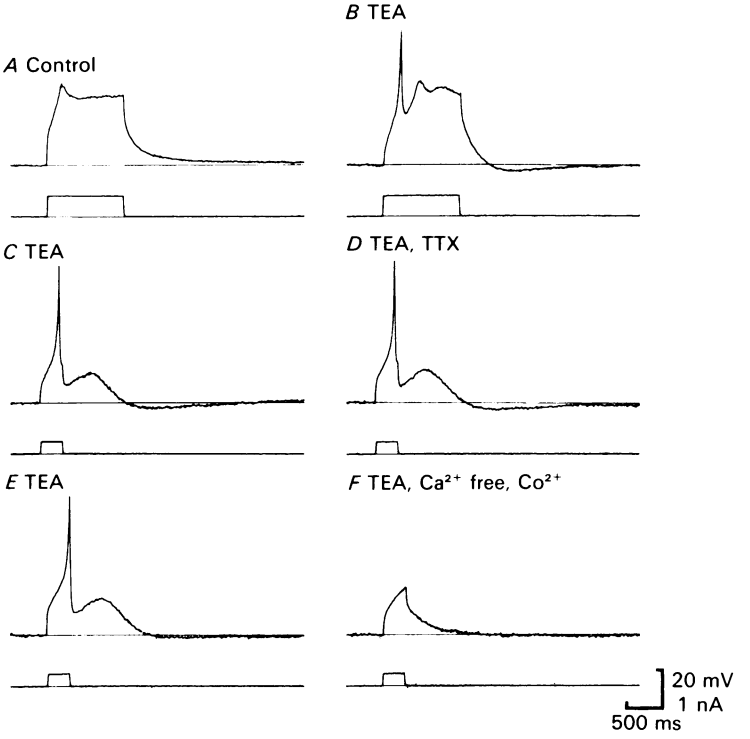

Fig. 2. The effect of TEA, TTX and cobalt on action potentials in a middle cerebral arteriole. *A*, *B*, comparison of voltage responses to the same depolarizing current in control solution (*A*) and after the addition of 10 mM-TEA (*B*). *C*, *D*, comparison of responses with 10 mM-TEA added before (*C*) and in the presence of 300 mM-TTX (*D*). *E*, *F*, comparison of responses with 10 mM-TEA present before (*E*) and after calcium removal with substitution by 2 mM-cobalt chloride (*F*).

When TEA (10 mM) was added to the perfusion fluid, the membrane potential depolarized by some 10–15 mV and a spontaneous discharge of action potentials associated with rhythmic arteriolar constrictions was observed (Haesler & Thorens, 1975; Droogmans *et al.* 1977). Both the mechanical movements and the discharge of action potentials were prevented by hyperpolarizing the membrane potential back to its previous resting value (−60 mV). When action potentials were initiated in the presence of TEA, it was apparent that their peak amplitudes and rates of rise had increased (Fig. 2*B*, *C*, *E*). In addition, after the peak of the action potential, the membrane potential rapidly repolarized but was interrupted by an after-depolarizing potential lasting for some 300 to 1000 ms. Visual inspection of the preparation indicated that in the presence of TEA, a given depolarizing current gave a more intense constriction than in the absence of TEA. In most preparations, the action potential plateau was followed by a slow after-hyperpolarization which lasted a further 1 to 3 s (Fig. 2*B*, *C*, *D*, *E*). The after-hyperpolarization varied in amplitude, from preparation to preparation, being barely detected in some preparations and having peak values of up to 12 mV in others (membrane potential −60 mV). In ten segments, each from a different animal, exposed to 10 mM-TEA, values for peak amplitude of the rapid component, the after-depolarization and the slow after-hyperpolarization were 56 ± 4, 13 ± 2 and 3 ± 2 mV respectively; the threshold membrane potential was -41 ± 2 mV; the maximum rate of rise of the action potential was $1{\cdot}4 \pm 0{\cdot}2$ V/s; the time to peak of the after-depolarization and the slow after-hyperpolarization relative to the commencement of the action potential was 570 ± 60 and 1500 ± 150 ms respectively and the half duration of the action potential, after-depolarization and slow after-hyperpolarization were 28 ± 4, 390 ± 40 and 700 ± 80 ms respectively (all values expressed as the mean ± 1 S.E. of mean).

In the preparations where a large slow after-hyperpolarization was detected, the amplitude of the hyperpolarization was altered by altering the arteriolar membrane potential. When the membrane potential was depolarized, the slow after-hyperpolarization increased in amplitude. Conversely, when hyperpolarized a decrease in amplitude was observed (Fig. 3). In each of three experiments where the membrane potential was varied, no slow after-hyperpolarization could be detected at potentials in the range −75 to −90 mV. These values are close to that predicted for equilibrium potential for potassium ions in arterial smooth muscle (Casteels, 1981; Hirst & van Helden, 1982); presumably the slow after-hyperpolarization reflects an increase in potassium permeability.

Neither the rapid component nor the after-depolarizing component of the action potential recorded in TEA were affected by the addition of TTX (300 nM) to the perfusion fluid (Fig. 2*C*, *D*). In contrast, when calcium ions were omitted from the perfusion fluid both components were abolished. However, these observations were difficult to interpret as calcium removal caused a concomitant fall in both membrane potential and input resistance. When cobalt (2 mM) was added to perfusion fluid either in the presence or absence of calcium (Fig. 2*E*, *F*), again both components of the action potential as well as the associated after-hyperpolarization were abolished without an associated fall in input resistance. This change was readily reversed by the readmission of calcium ions along with removal of cobalt ions.

The observations suggest that calcium ions are necessary for the generation of both

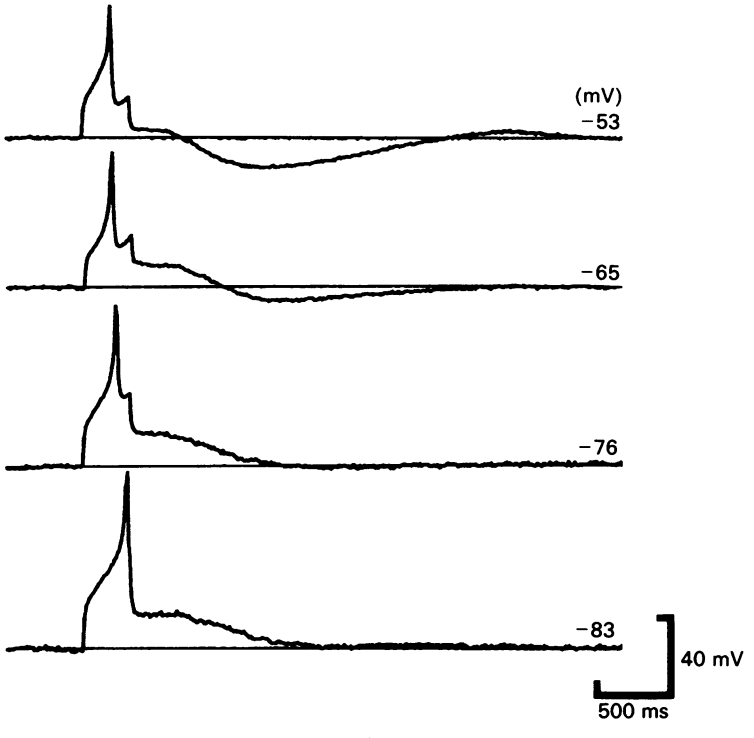

Fig. 3. The effect of membrane potential on the slow after-hyperpolarization recorded from an arteriolar segment. Action potentials elicited by the same depolarizing current (0·5 nA, 300 ms) were recorded at holding potentials of −53, −65, −76, and −83 mV (top to bottom) respectively.

components of the action potential observed in TEA. However, in the absence of TEA membrane inward currents activated by depolarization are largely obscured by the presence of a TEA-sensitive rectifier (Mekata, 1976).

Membrane currents recorded from short segments of middle cerebral arterioles, effect of TEA

When the membrane currents were recorded under voltage clamp from segments of arteriole during the application of either hyperpolarizing or depolarizing voltage command steps, no active inward or outward currents were detected for membrane potentials in the range −80 mV to −50 mV. At membrane potentials more negative than −80 mV, membrane rectification occurred which was reflected as a net increase in inward current. At membrane potentials more positive than −50 mV, voltage dependent currents were observed. These took the form of an initial inward current followed by a persistent outward current (see insets, Fig. 4). In the presence of TEA (10 mM), depolarizing the membrane potential to between −50 and −40 mV gave rise to a net active inward current which showed no inactivation. At more depolarized membrane potentials, the inward current consisted of a rapidly inactivating component and a plateau component (see insets, Fig. 4). These observations have been plotted graphically in Fig. 4 where both the peak current, and the plateau current

(obtained by averaging the current record over the last 500 ms of its duration) have been plotted against membrane potential.

Thus it appears that in arterioles, as in many excitable cells, membrane depolarization activates an outward rectifier which is abolished by TEA. This rectifier would explain the failure of arterioles in control solutions to generate large overshooting action potentials.

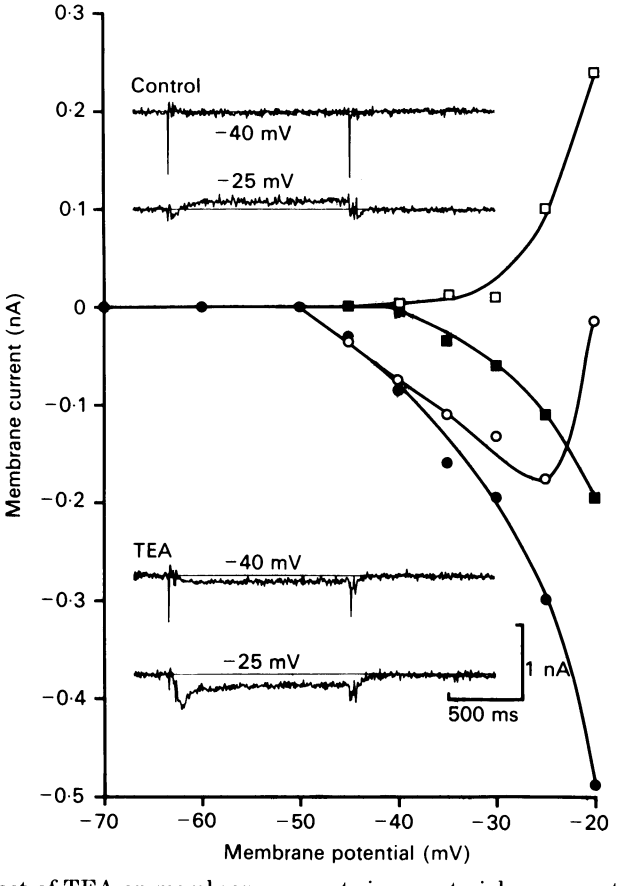

Fig. 4. The effect of TEA on membrane currents in an arteriolar segment. Peak (circles) and plateau (squares) current amplitudes have been plotted as a function of membrane potential in control (open symbols) and in the presence of 10 mM-TEA (filled symbols). Current records for 1 s depolarizations to −40 and −25 mV in control (upper pair) and with TEA present (lower pair) have been included as insets. The membrane potential was held at −60 mV. Lines have been fitted by eye.

Effects of divalent cations and calcium antagonists

In each experiment where membrane currents were recorded over the membrane potential range −60 to −20 mV in the presence of TEA (10 mM) at threshold potentials between −55 and −47·5 mV a small but maintained inward current was detected after subtraction of the resistive leak component (Fig. 5*A*). This current was not detected after replacing calcium in the extracellular fluid with cobalt (Fig. 5*B*). The

current was unlikely to be artifactual (for example, being apparent as a result of non-linearities of the membrane conductance in the presence of calcium) because when segments of arteriole were depolarized to these membrane potentials (~ -50 mV) for periods of 3–10 s, the segments of arteriole gave small constrictions. This would result if calcium entry were occurring. It is to be noted that this current alone was never of sufficient intensity to generate a net inward current but an inward

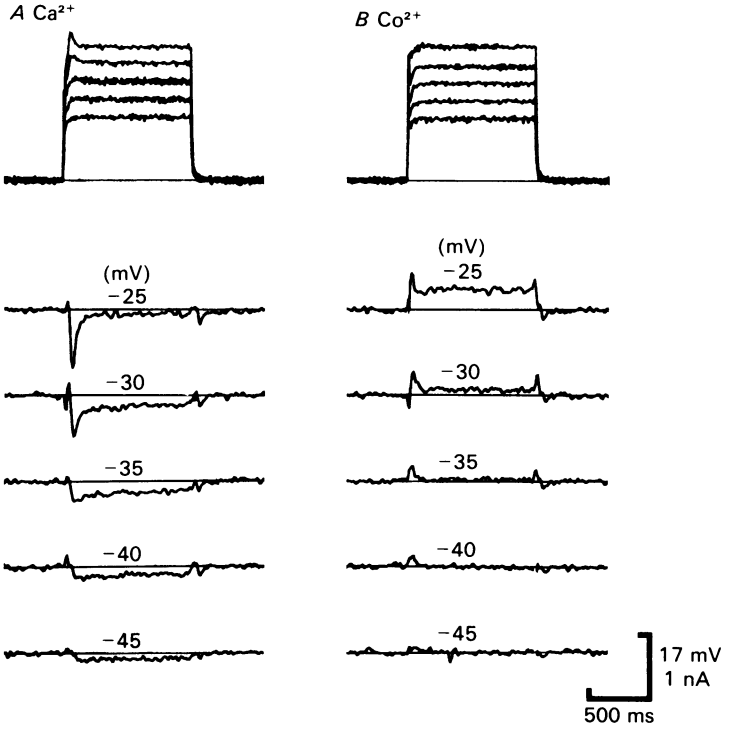

Fig. 5. Comparison of membrane currents in control and cobalt-substituted solutions. The voltage command sequence (upper records) and corresponding currents (lower records) marked according to the membrane potential to which the voltage was stepped are shown in control (*A*) and calcium-free solution with 2 mM-cobalt chloride added (*B*). Records were obtained in the same cell exposed throughout to 10 mM-TEA and in all cases held at -60 mV. It should be noted that in this experiment the voltage clamp failed to control completely the membrane potential at the onset of large depolarizing steps. This caused some peaking of the voltage records in *A* for the longer depolarization and hence caused the fast current to be underestimated by up to 20%. Poor initial voltage control also produced the artifactual outward transients in *B* which result from the subtraction procedure used to correct for membrane charging transients (see Methods).

current was only detected after subtraction of the resistive leak component. The plateau current increased in amplitude with progressive reductions in membrane potential but in the absence of further correction procedures appeared to decrease beyond membrane potentials of about -30 mV (Fig. 5*A*). As is considered below, this decrease was primarily due to residual voltage dependent rectification.

At membrane potentials in the range -40 to -30 mV (mean -34 mV, S.E. of mean

± 1, $n = 9$) the voltage dependent inward current became more complex. The current associated with the onset of depolarization had a rapidly inactivating component. Again the peak amplitude of this current and also its rate of decay increased progressively with changes in membrane potential (Fig. 5*A*). The transient component decayed approximately exponentially and the time constant of decay, determined

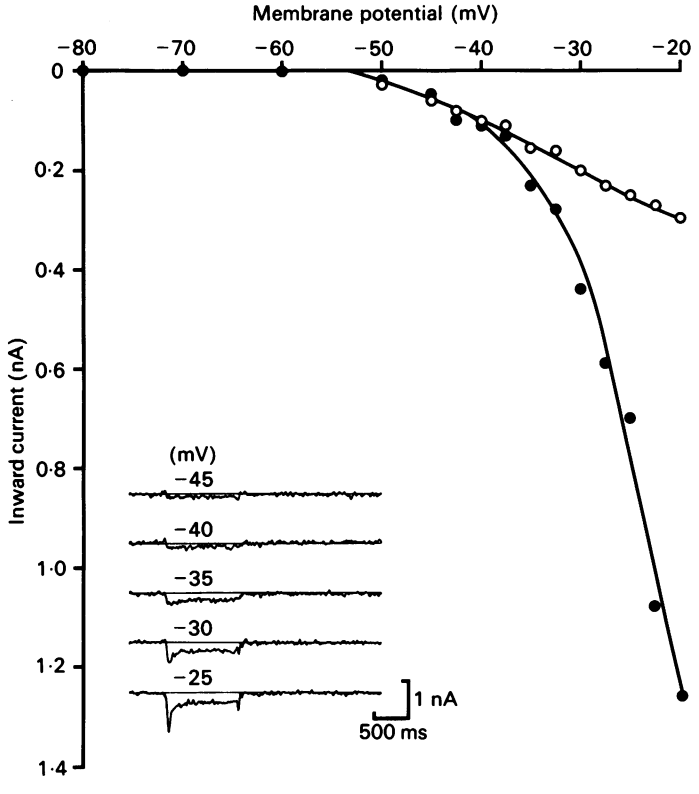

Fig. 6. Voltage dependence of the peak (filled circles) and plateau (open circles) calcium currents. The data are derived from the raw data presented in part in Fig. 5 but now after correction for residual rectification (see text). Lines have been fitted by eye. The inset shows examples of records after the correction procedure for 1 s depolarizing voltages.

at -25 mV was 53 ± 8 ms (mean ± 1 S.E. of mean, $n = 9$). Unlike the maintained current, the transient current was of sufficient intensity at membrane potentials more depolarized than about -35 mV to generate a transient net inward current without resistive correction. Both the plateau and transient currents were abolished by replacing calcium in the extracellular fluid with cobalt. It became apparent that in the presence of cobalt at membrane potentials more positive than -30 mV, the arteriolar membrane showed rectification despite the presence of TEA (Fig. 5*B*). To obtain an estimate of the total current flowing as a result of the presence of calcium the differences between the membrane currents obtained in calcium and in cobalt were taken. Plotted in Fig. 6 is the peak amplitude of the inward current and its mean amplitude over the last 500 ms of the depolarizing step. It can be seen that after this

correction procedure neither the peak of the transient current nor the non-inactivating current showed evidence of a voltage-dependent reduction in the range of membrane potentials examined (< -20 mV). We were unable to examine the membrane currents obtained over more positive voltage ranges than those illustrated; in most experiments rectification of the recording electrodes precluded accurate voltage

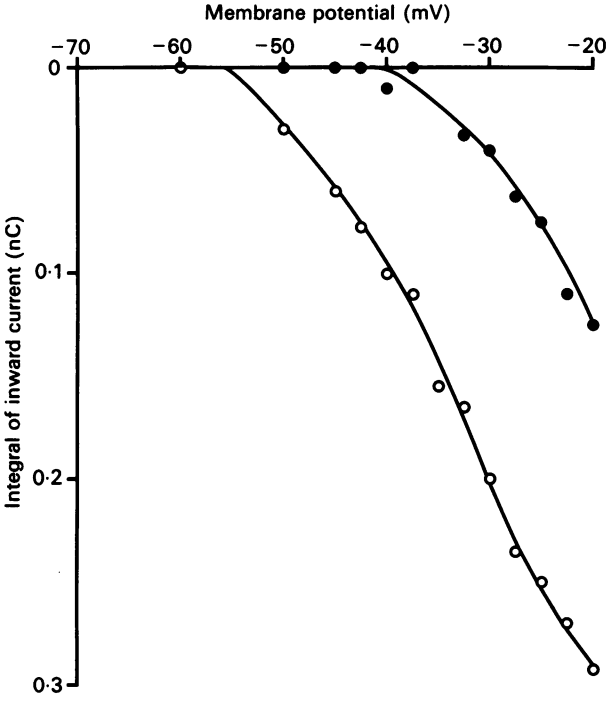

Fig. 7. Voltage dependence of the integrals of the transient (filled circles) and plateau calcium currents (open circles). Integrals have been determined from the same data as that for Fig. 6. Lines have been fitted by eye.

control. Thus although these calcium currents may well show the voltage dependent reduction frequently displayed by similar currents in other tissues (for reviews see Hagiwara & Byerley, 1981), in the membrane potential ranges that are likely to occur in these arterioles under physiological conditions (Fig. 2*A*) this does not occur. It will be appreciated that despite the much greater peak amplitude of the inactivating current, the integral of the plateau current for depolarizations of a second or more are always greater than those of the transient current (Fig. 7)

Additional confirmation of the identity of the currents was sought by substituting barium for calcium and by examining the actions of nifedipine or verapamil. Both the transient current and the plateau current were recorded in calcium-free barium solutions. Verapamil (2 μM) or nifedipine (3 μM) suppressed both the rapid and plateau phases of the action potential observed in the presence of 10 mM-TEA, the effects of nifedipine at this concentration being more dramatic than those of verapamil. Only the action of nifedipine was examined using voltage-clamp studies.

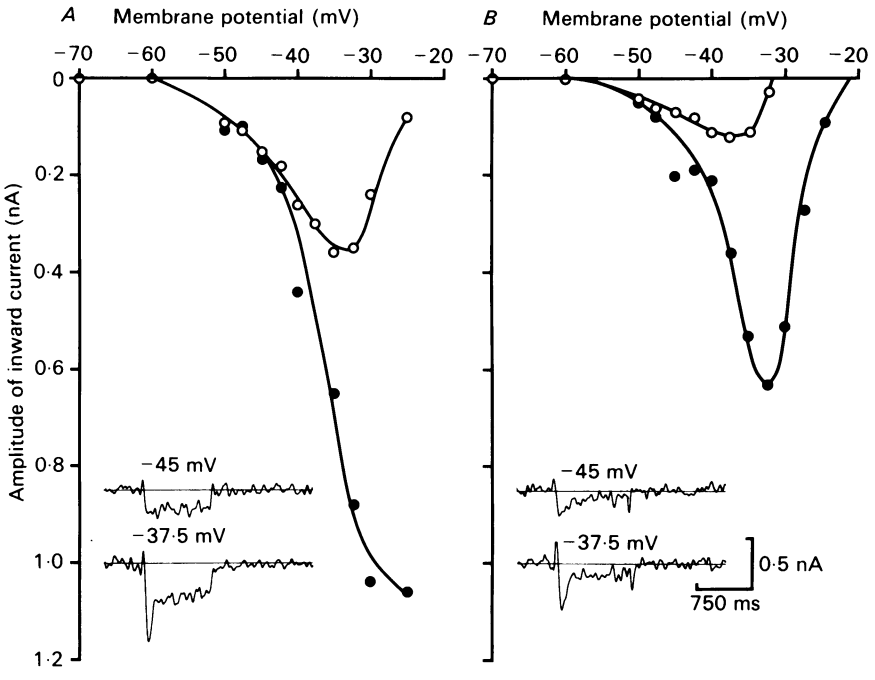

Fig. 8. Effect of nifedipine on peak and plateau calcium currents in an arteriolar segment. Plotted as a function of membrane potential are peak (filled circles) and plateau (open circles) currents in control (*A*) and in the presence of 3 μm-nifedipine (*B*) (10 mM-TEA present in both *A* and *B*). The insets show current records for step depolarizations applied for 1 s to membrane potentials of −45 mV (upper) and −37·5 mV (lower) in control (*A*) and nifedipine (*B*). Scale bars apply to all current records.

The results of an experiment are shown (Fig. 8). It can be seen that nifedipine reduced both the transient current and the plateau but had little or no effect on the TEA resistant rectification.

Properties of the slow after-hyperpolarization – effect of substituting barium for calcium

As has been pointed out, action potentials recorded from middle cerebral arterioles were often followed by a slow after-hyperpolarization which was abolished by calcium removal and not detected at membrane potentials some 20–30 mV negative of rest (see also Keatinge, 1978). In voltage clamp experiments a small amplitude persistent outward current was occasionally detected following the larger membrane depolarizations (Fig. 9). These currents were of small amplitude. In five arteriolar segments the peak of this outward current (recorded back at −60 mV and following a membrane depolarization of 40 mV for 1 s) was 70 ± 30 pA and it reached a peak in $1{\cdot}5 \pm 0{\cdot}3$ s (mean ± 1 S.E. of mean). This outward current was only initiated after the application of somewhat larger voltage steps than those required to initiate inward calcium movement (Fig. 9). This is shown graphically in Fig. 10 where calcium entry (approximated by the integral of the calcium current) is compared to the resultant after-hyperpolarizing current. A corresponding requirement for 'calcium priming' has been reported for other excitable cells (Hirst, Johnson & van Helden, 1985).

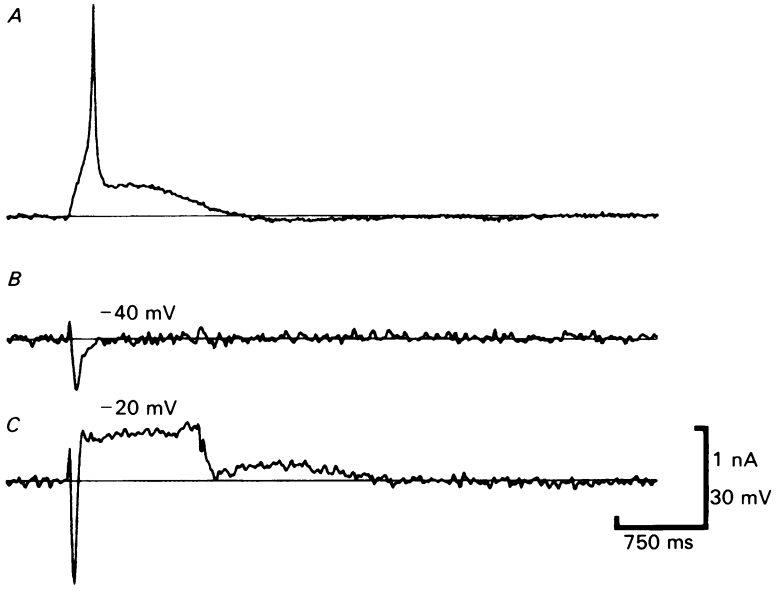

Fig. 9. Action potential and currents in an arteriolar segment. *A*, action potential initiated from a resting potential of −63 mV by a 0·5 nA constant current applied for 50 ms (note small after-hyperpolarization). *B*, current record from the same segment now held under voltage clamp at −60 mV and stepped to −40 mV for 1 s. *C*, as for *B* but voltage stepped to −20 mV (note outward current after cessation of voltage step). All data were obtained with 10 mM-TEA added to bathing solution. No correction for TEA resistant rectification has been made.

Although both the inactivating and non-inactivating inward currents were detected the outward current was not detected after barium had been substituted for calcium (Fig. 10) nor after replacement of external calcium with either manganese or cobalt ions. Thus, this current shares many of the properties of the slow calcium activated potassium conductance detected in many tissues (see Petersen & Maruyama, 1984; Hirst *et al.* 1985). The time course and relatively small amplitude of this calcium-dependent conductance change suggests that it would be unlikely to cause a large contamination of the inward calcium currents measured over time periods of 1 s or less described above. Activation of this calcium-dependent current would explain the curtailment of the after-depolarizing phase of the action potential, which even in the presence of TEA did not last more than 2 s (eg. see Figs. 2, 3 and 9).

DISCUSSION

Membrane depolarization initiated regenerative membrane potential changes in segments of the arterioles originating from the middle cerebral artery of the rat. These potential changes were of small amplitude and usually did not overshoot zero potential. As with other smooth muscle preparations after the addition of TEA to the perfusion fluid, large regenerative action potentials with rapid rising and falling phases followed by an after-depolarization, and in many cases an even later after-hyperpolarization, were detected. This observation suggests that the re-

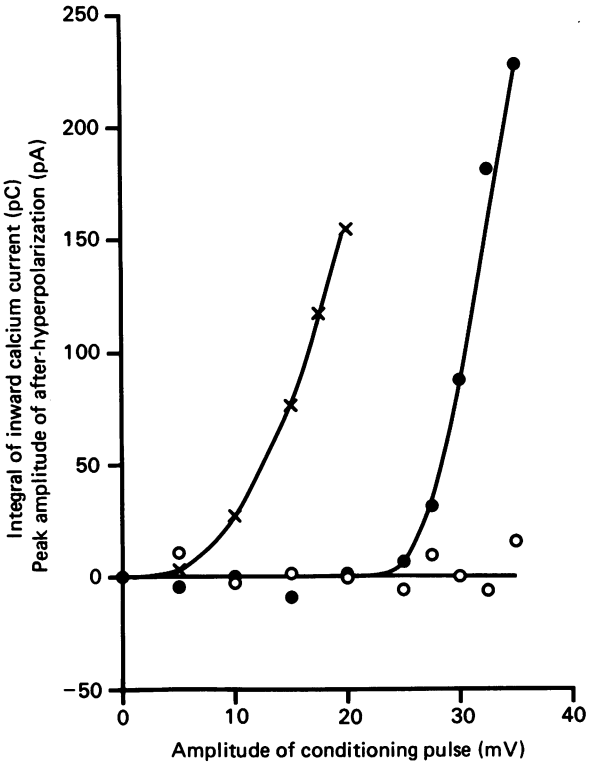

Fig. 10. Comparison of the integral of the calcium current and resultant slow after-hyperpolarizing current. Shown are the integral of the calcium current (crosses) plotted as a function of the depolarizing step (for the limited range of 0 to 20 mV before substantial rectification) and the resultant after-hyperpolarizing current (filled circles) recorded back at the holding potential (−60 mV). The open circles are the current amplitudes measured at the previously determined peak of the after-hyperpolarizing current but now with 2·5 mM-barium substituted for calcium in the bathing solution. Lines have been fitted by eye. All data were obtained with 10 mM-TEA in bathing solution.

generative membrane potential changes occurring in the absence of TEA are heavily damped by this rectifier.

Both the rapid component and the after-depolarizing component of the action potential recorded in TEA are resistant to blockade by TTX, are present after substitution of barium for calcium but are reduced by manganese, cobalt, verapamil and nifedipine. This would suggest that both components result from calcium influx (Baker, Hodgkin & Ridgway, 1971).

After suppression of the voltage sensitive outward rectifier by TEA, voltage-clamp studies revealed two apparently distinct inward currents which were calcium dependent. The currents were distinguishable in that one current commenced activation at low membrane depolarizations, whilst the other was activated at higher membrane depolarizations. The low threshold current did not inactivate substantially over a period of 1 s whereas the high threshold current rapidly inactivated (< 200 ms). Over a period of depolarization of 1 s, the majority of calcium entry occurred via the

non-inactivating current and this led to vessel constriction. Since these arterioles have relatively long membrane time constants (>200 ms) and most arterioles are subject to excitation by tonic sympathetic activity this 'plateau' component of the calcium current will provide the principal pathway for transmembrane calcium entry. If the inward currents are due solely to calcium entry then the integral of the total current during such a depolarization may be used to estimate the increase in calcium concentration in the smooth muscle. The net inward charge resulting from a 35 mV depolarization applied for 1 s is about 0·3 nC (Fig. 6). Taking the average length of the arteriole segments to be 200 μm, with a diameter of 50 μm, and wall thickness of 5 mm, this charge will lead to approximately a 10 μM increase in internal calcium concentration. Such a concentration is very similar to that required to cause constriction of skinned smooth muscle fibres (Endo, Kitazawa, Yagi, Iino & Kakuta, 1977) and suggests that both inward currents, if carried by calcium ions, will supply sufficient calcium for constriction without the need of internal release.

The presence of two distinct inward currents confers a degree of flexibility on the mechanism by which the neural control of arterioles is achieved. During maintained low frequency discharge of sympathetic nerve activity, moderate depolarizations will only activate the non-inactivating current. Since this current never generates a net inward current, calcium inflow will be restricted to areas of depolarization. Little or no propagation will occur. The level of calcium entry will rise and fall smoothly with small changes in membrane potential. Higher frequency sympathetic nerve activity would be expected to result in activation of the higher threshold current. This in turn would lead to a propagating action potential causing constriction of an entire length of arteriole.

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