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# AN α-ADRENOCEPTOR-MEDIATED CHLORIDE CONDUCTANCE IN MESENTERIC VEINS OF THE GUINEA-PIG

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SUMMARY

1. The ionic basis of the depolarizing responses resulting from ionophoresis of noradrenaline onto the smooth muscle of mesenteric veins has been investigated using electrically short segments of vessel.

2. Isolated cut segments of vein were effectively isopotential as assessed by the voltage response to a step change in current. The mean input resistance and time constant of the smooth muscle were 24 MΩ and 131 ms respectively.

3. Data on the noradrenaline-induced slow depolarization indicated that it resulted from a decrease in conductance to potassium ions consistent with the finding of Suzuki (1981).

4. The fast noradrenaline-induced depolarization was found to have a reversal potential of about −22 mV.

5. Exposure to low-chloride solution caused greater than 90% suppression of this fast response with a 50% reduction occurring in less than 2 min. This suppression was not due to a negative shift in reversal potential.

6. The fast response underwent a large positive shift in reversal potential directly after changeover to low-chloride solution at times when any inactivation of the response was minimal. By contrast the fast response showed no evidence implicating either sodium or calcium as charge-carrying ions.

7. It is concluded the fast depolarization is carried by chloride ions.

## INTRODUCTION

Noradrenaline-mediated depolarization of the smooth muscle of mesenteric veins involves a fast component (Van Helden, 1988) and a slow depolarizing response (Suzuki, 1981). The ionic mechanism underlying the fast component of depolarization, previously unknown in this tissue, has been studied using electrically short segments of vein, with recordings made from the smooth muscle using the single-electrode voltage clamp. The accessibility of the preparation to the external perfusate has allowed studies involving rapid changes of external ionic environment. In this way complications such as solution-mediated inactivation of the conductance

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mechanism have been minimized and an ionic mechanism consistent with an increased conductance to chloride ions has been shown. Conductance properties of the slow depolarization have also been studied using segments of vein, and data consistent with the finding that this mechanism results from a decrease in potassium conductance (Suzuki, 1981) are presented. A preliminary account of some of this work has been reported (Van Helden, 1986).

## METHODS

The methods are outlined in a previous report (Van Helden, 1988). Experiments performed on segments of vein in which currents were recorded used the single-electrode voltage clamp procedure (see Finkel, Hirst & Van Helden, 1984). Intracellular measurements were made using electrodes filled with 0·5–1 M-potassium methyl sulphate. Segments were studied in one of two baths, a larger chamber which was perfused at a rate of 3–5 bath volumes/min or a smaller chamber which was perfused at about 30 bath volumes/min. The effectiveness of the latter perfusion rate in altering solution composition around the smooth muscle was assessed by monitoring smooth muscle membrane potential on exposure to a solution of increased potassium concentration (15 mM). Changeover rates similar to that for arterioles were found (see Hirst & Van Helden, 1982), with better than 90% achievement of the final steady-state potential in less than 10 s.

Special modifications of the control solution (Van Helden, 1988) used in this study were: low-chloride solution (NaCl replaced with 120 mM-sodium isethionate); low-sodium solution (all 120 mM-NaCl replaced with 120 mM-Tris chloride); low-calcium solution ($CaCl_2$ removed) and low-calcium cobalt solution ($CaCl_2$ replaced with 2 mM-$CoCl_2$).

All mean values presented are expressed as the mean $\pm$ 1 standard error of the mean with $n$ the sample size.

## RESULTS

### *Passive electrical properties*

The electrical properties of the smooth muscle of segments of small mesenteric veins were studied using intracellular microelectrode techniques. The mean membrane potential from segments of length 150–200 $\mu$m and diameter 50–130 $\mu$m was $-65{\cdot}3 \pm 1{\cdot}1$ mV ($n = 55$ from thirty-eight animals). This value was not significantly different from the value of $-64{\cdot}1 \pm 0{\cdot}7$ mV ($n = 95$) obtained in intact veins (Van Helden, 1988). The smooth muscle of these segments responded to injection of a current pulse with an exponential voltage change (see Fig. 1). This finding is consistent with segments being approximately isopotential with the equivalent circuit of a simple spherical cell. It is also predicted from the length constant ($\lambda$) of the smooth muscle in these small veins. The length constant, as determined in intact veins using extracellular polarization, was $1{\cdot}3 \pm 0{\cdot}3$ mm ($n = 4$). Thus segments of length less than 200 $\mu$m with sealed ends would, on the assumption of a linear cable, be effectively isopotential (with not more than 4% difference in potential between current injection point and ends) during point injection of a constant current, as their cable length was less than $0{\cdot}25\lambda$ (Jack, Noble & Tsien, 1975). These observations are in agreement with those on arterioles. As for the small veins, arterioles have walls containing a monolayer of smooth muscle cells and when cut to lengths less than $0{\cdot}25\lambda$ are effectively isopotential (Hirst & Neild, 1978, 1980).

The electrical resistance and time constant of vein segments were $24 \pm 3$ MΩ and $131 \pm 9$ ms respectively ($n = 55$). Histological measurements showed that the small

veins had walls which were composed of a monolayer of smooth muscle cells of thickness 3–5 μm. An estimate of the specific membrane properties was made on the assumption that the cells form a uniform equivalent cylindrical structure. The membrane resistance, internal resistivity and membrane capacitance calculated on this basis for a mean segment length, diameter and wall thickness of 180, 100 and 4 μm respectively were 27 kΩ cm², 620 Ω cm and 4·9 μF/cm². The values for

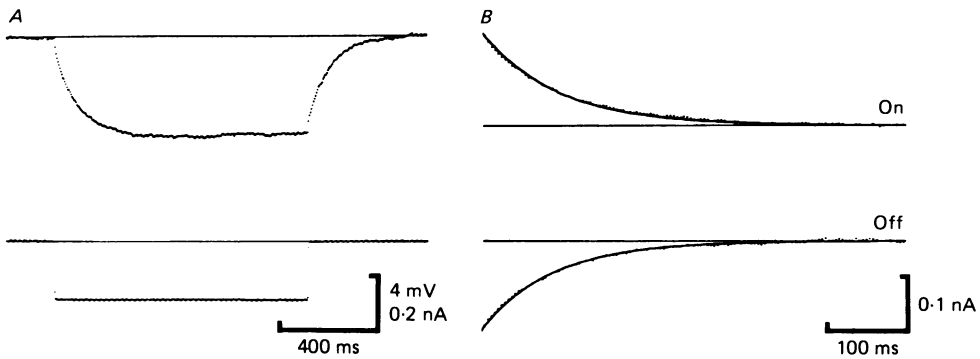


Fig. 1. The passive electrical properties of a short segment of mesenteric vein. This segment (diameter 115 μm; length 185 μm) had smooth muscle with a membrane potential of −62 mV and input resistance of 33 MΩ. The membrane potential could be altered by injection of current; make or break of a small constant current applied with an amplifier in the current clamp mode resulted in a transient voltage change (*A*). The on- or off-transients were well fitted by an exponential with time constant of 84 ms (*B*).

resistivity and capacitance are similar to those determined in arterioles (Hirst & Neild, 1978). However, the value for membrane resistance is about 50% that of arterioles, consistent with the shorter length constant and faster time constant of these small veins.

*Noradrenaline-induced currents*

Noradrenaline-induced depolarizations in the smooth muscle of venous segments were voltage clamped using the single-electrode procedure as previously applied to arteriolar segments (Finkel *et al.* 1984). Examples of current records so obtained are presented in Fig. 2 for single responses (*A*) and for the average of six responses (*B*). Upper records show the normal response superimposed on the response under voltage clamp. Voltage deflections were effectively reduced to about 10% of control. Corresponding currents are presented in the lower records. The residual voltage deflection could be improved further by increasing the gain of the clamp. However, this limited the stability of voltage control, making experiments of longer duration difficult.

*Ionic basis of the fast component*

The properties of the fast component resulting from ionophoresis of noradrenaline were studied with minimal interference from the slow depolarization by superfusing the preparation at a high flow rate. The mechanism by which a high flow rate reduced

the slow depolarizing response is unknown but could relate to either the reduced sensitivity of the underlying receptors and hence a need for longer exposure time to the noradrenaline and/or the distribution of the different receptors (Van Helden, 1988). The reversal potential of the fast inward current was then determined by holding the smooth muscle at different membrane potentials under voltage clamp.

The fast response to ionophoretically applied noradrenaline decreased in amplitude with depolarization and showed reversal at about −30 to −15 mV. Examples are

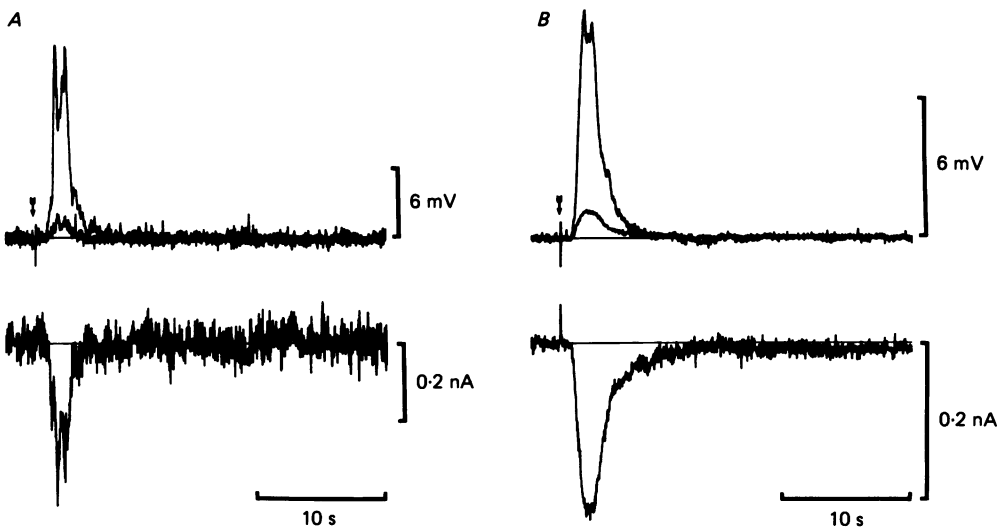


Fig. 2. Currents underlying the fast response to ionophoretically applied noradrenaline in the smooth muscle of a short segment of vein. The potential changes recorded intracellularly to either single application (*A*) or the average of six responses (*B*) were suppressed by about 90% during recording with the single-electrode voltage clamp at moderate but stable gain. Resulting currents for single stimulation or an average response ($n = 6$) are shown. The segment of diameter 105 $\mu$m and length 190 $\mu$m had a membrane potential of −64 mV and an input resistance of 28 MΩ. Arrows indicate brief ionophoretic application of noradrenaline (10 nC used in all cases).

presented in Fig. 3 for two segments. The inset in Fig. 3*A* shows examples of currents recorded at −25 and −15 mV before and after reversal. It is to be noted that the spontaneous currents observed after cessation of the evoked currents also reversed. The mean reversal potential for the ionophoretically induced currents was $-22 \pm 3$ mV ($n = 7$). This value was in agreement with corresponding measurements made with segments held under current clamp with a value of $-25 \pm 4$ mV ($n = 4$). A further observation was made when segments were hyperpolarized to potentials more negative than about −90 mV. Under these conditions there was a marked decrease in amplitude of the fast component.

The finding of a reversal potential of about −22 mV is consistent with data obtained on an $\alpha_1$-adrenoceptor-mediated response observed in the rat anococcygeus muscle (Byrne & Large, 1985), implicating an ionic mechanism involving chloride ions. This was further investigated by experiments involving ionic substitution.

However, suppression of the fast response by exposure to low-chloride solution, which occurs in both preparations, complicated such studies (see Large, 1984). The problem was resolved by first studying the kinetics of the effect of low-chloride solution on the conductance change and then using this information to study reversal potentials in solutions of different ionic content. The dependence of the fast conductance change on the duration of exposure to low-chloride solution was studied in four segments, again using the rapid rate of perfusion. All responses were increased

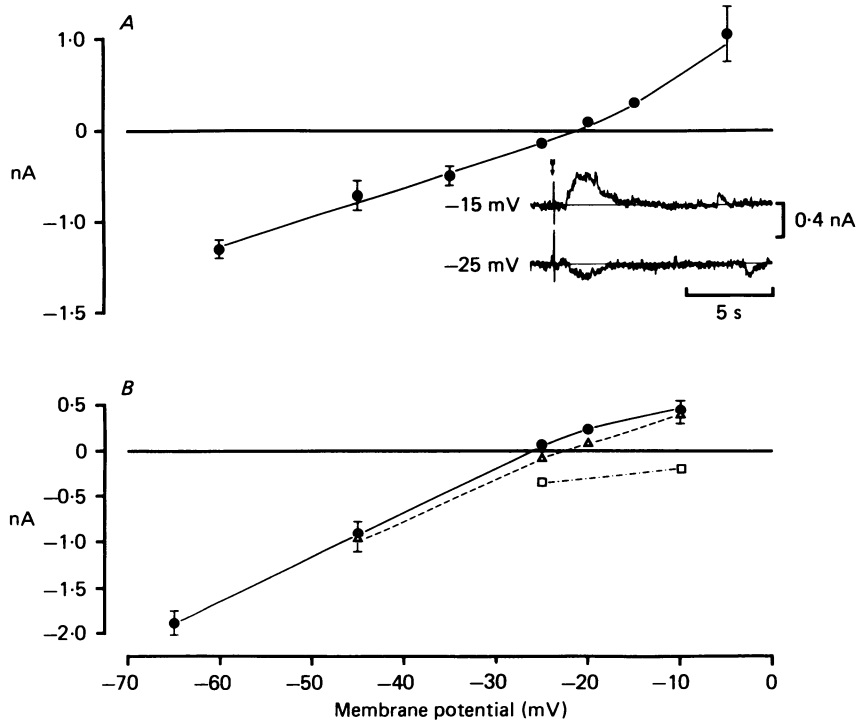


Fig. 3. Voltage dependence of the fast current induced by brief ionophoresis of noradrenaline in the smooth muscle of segments of vein. Shown in *A* and *B* are the peak amplitudes of currents recorded under single-electrode voltage clamp in different segments and plotted as a function of membrane potential. The inset in *A* shows records obtained near the reversal potential with the arrow indicating ionophoresis of noradrenaline (20 nC used throughout). The different symbols in *B* denote measurements obtained in control solution (●), 30 s after changeover to low-sodium solution (△) and 30 s after changeover to low-chloride solution (□). The bars denote the standard error of the mean from three to five repeat measurements. Lines have been fitted by eye.

in amplitude during about the first 40 s exposure to low-chloride solution, after which responses decreased in amplitude to less than 50% of the control in 2 min. An example is presented in Fig. 4. Complete suppression was not always observed even after 15 min exposure to low-chloride solution. However, in these cases the amplitude of the response was still less than 20% that of control values. The relevant finding of these experiments is the initial increase in amplitude of the fast current, which is consistent with an increase in driving force for chloride ions. It is then postulated

that the decrease in the ensuing response occurs through inactivation of this conductance or through a decrease in internal chloride ion content.

An experiment performed to identify the nature of the ionic species responsible for the fast component is presented in Fig. 5 (see also Fig. 3*B*). This experiment utilized the finding that inactivation by low chloride was not substantial within the first

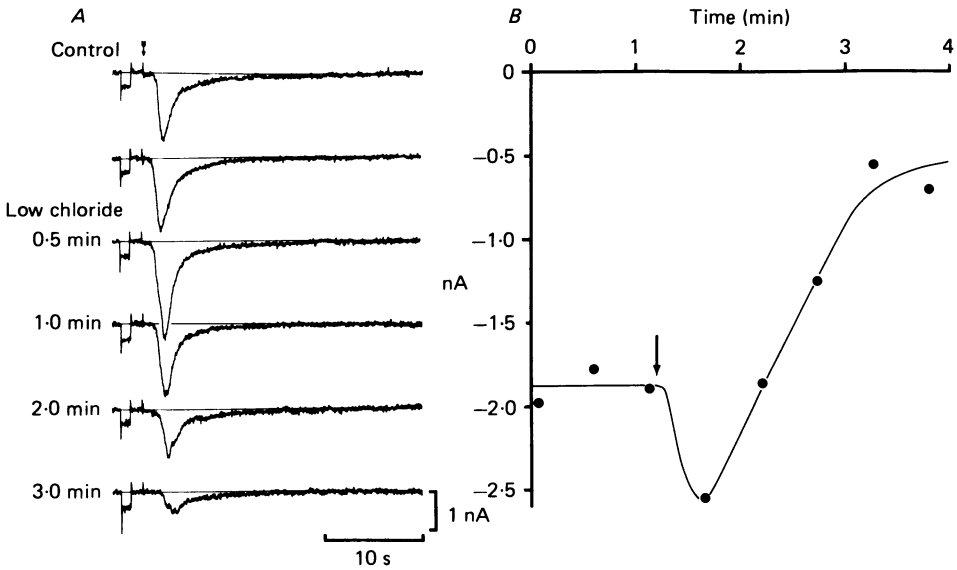


Fig. 4. Time-dependent suppression of the fast current induced by ionophoresis of noradrenaline in the smooth muscle of a segment of vein held at −65 mV. Shown in *A* are currents recorded every 30 s in response to the same ionophoretic pulse (200 ms, 100 nA) applied as indicated (arrow). These were enhanced then suppressed upon exposure to low-chloride solution (rapid perfusion maintained throughout). The current step evident before application of noradrenaline is that resulting from a hyperpolarizing pulse (amplitude 10 mV, duration 1 s). Shown in *B* is a plot of the peak amplitude of the inward current produced by noradrenaline and plotted as a function of time. The arrow denotes the time of changeover from control to low-chloride solution. The line has been drawn by eye.

40 s after solution changeover. The protocol adopted was to compare fast ionophoretically induced currents in control and 30 s later in low-chloride solution, with the membrane held at different potentials; the experiment was then repeated in low-sodium solution. An example of the results of this experiment is presented in Fig. 5*A*. At potentials negative to reversal there was an increase in the magnitude of the inward current. At holding potentials of −25 mV (near reversal) and at −10 mV, outward currents became clearly inward upon exposure to the low-chloride solution. By comparison, substitution with low-sodium solution (Fig. 5*B*) had no substantial effects on the fast currents. The data from this experiment and from similar observations made on three other segments indicate that the fast depolarization is carried by chloride ions.

Some comment can also be made on the inactivation process caused by low-

chloride solution. It was observed that the reversal potential, as measured over the first 2–5 min exposure to low-chloride solution, remained at least 20 mV more positive than the control values. While it was not possible to measure the absolute values, which required large depolarization beyond the capacity of the current-passing electrode, the data indicate that suppression of the fast depolarization in low-

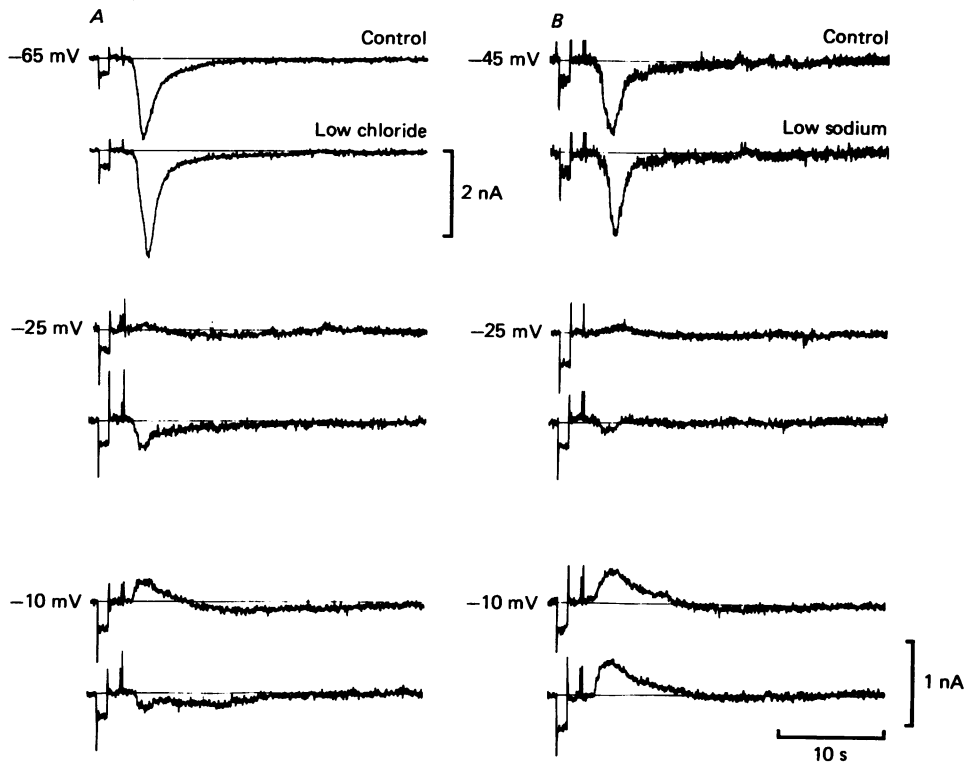


Fig. 5. The effect of ionic substitution on the fast noradrenaline-induced current in the smooth muscle of a segment of vein. Shown in *A* are records obtained to the same ionophoretic pulses of noradrenaline in control (upper record) and 30 s later in low-chloride solution at three membrane potentials. Shown in *B* are records obtained for ionophoretic pulses of the same size in control and 30 s later in low-sodium solution at three membrane potentials. The current on the start of each trace is that resulting from a 10 mV hyperpolarizing pulse of 1 s duration. The arrow denotes ionophoretic application of noradrenaline (duration 100 ms, amplitude 200 nA). The lower current scale bar is applicable to all records in *A* and *B* except the records in *A* obtained at −65 mV.

chloride solution cannot be explained on the basis of altered reversal potential. Rather, the effect must be related to an inactivation of the chloride conductance mechanism itself.

*Ionic basis of the slow response*

The ionic basis of the slow depolarization to ionophoresis of noradrenaline was studied in six segments perfused at a lower rate, in which the slow depolarization was clearly discernible at the resting potential. The slow depolarization was voltage

dependent and at hyperpolarized potentials (e.g. −80 mV) caused little if any depolarization, while an increase in membrane resistance after ionophoresis of noradrenaline was evident (Fig. 6). However, clear reversal of the depolarization could not be obtained as large hyperpolarizations caused large increases in resting membrane conductance, thus markedly reducing any potential changes due to an agonist-induced increase in membrane resistance. Experiments were therefore made using ionic substitution.

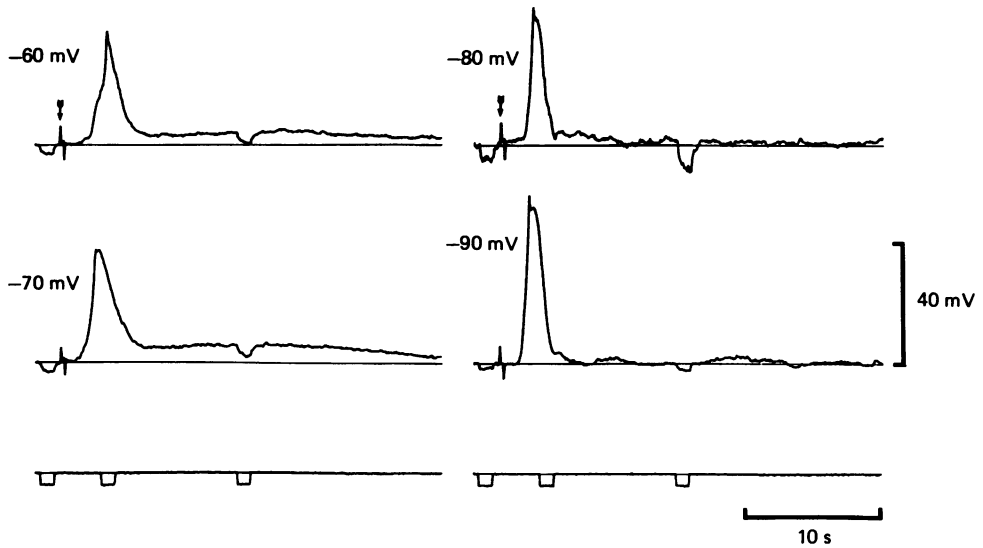


Fig. 6. The effect of membrane potential on the slow depolarization induced by ionophoresis of noradrenaline in the smooth muscle of a segment of vein. Records obtained at hyperpolarized potentials (−80 and −90 mV) had smaller slow depolarizations than those obtained at −60 or −70 mV. The lower records are currents which were injected to monitor membrane resistance with current pulses of 0·1 nA injected as shown except for the −80 mV record where the pulses were increased to 0·2 nA. The arrows indicate application of noradrenaline (ionophoretic duration 300 ms, amplitude 200 nA for all responses).

Further evidence implicating potassium ions as responsible for the slow depolarization is presented in Fig. 7*A* and *B*. In Fig. 7*A*, for a segment held under current clamp, it can be observed that increasing the external potassium concentration from 5 to 15 mM caused the ionophoretically induced slow depolarization to be lost when the segment was held in the resting state at −65 mV, leaving in isolation the fast depolarization. Again the slow depolarization could not be reversed in high-potassium solutions, presumably due to an increase in membrane conductance with hyperpolarization. Under conditions of increased external potassium, this rectification commenced at more positive potentials than for control solutions (Fig. 7*A*). Also shown in Fig. 7*A* are responses obtained in control and 15 mM-potassium solutions at starting potentials of −45 and −15 mV. The slow component in 15 mM-potassium is smaller at the −45 mV level but similar in size at the −15 mV level. The difference in amplitude of the slow component at the −45 mV

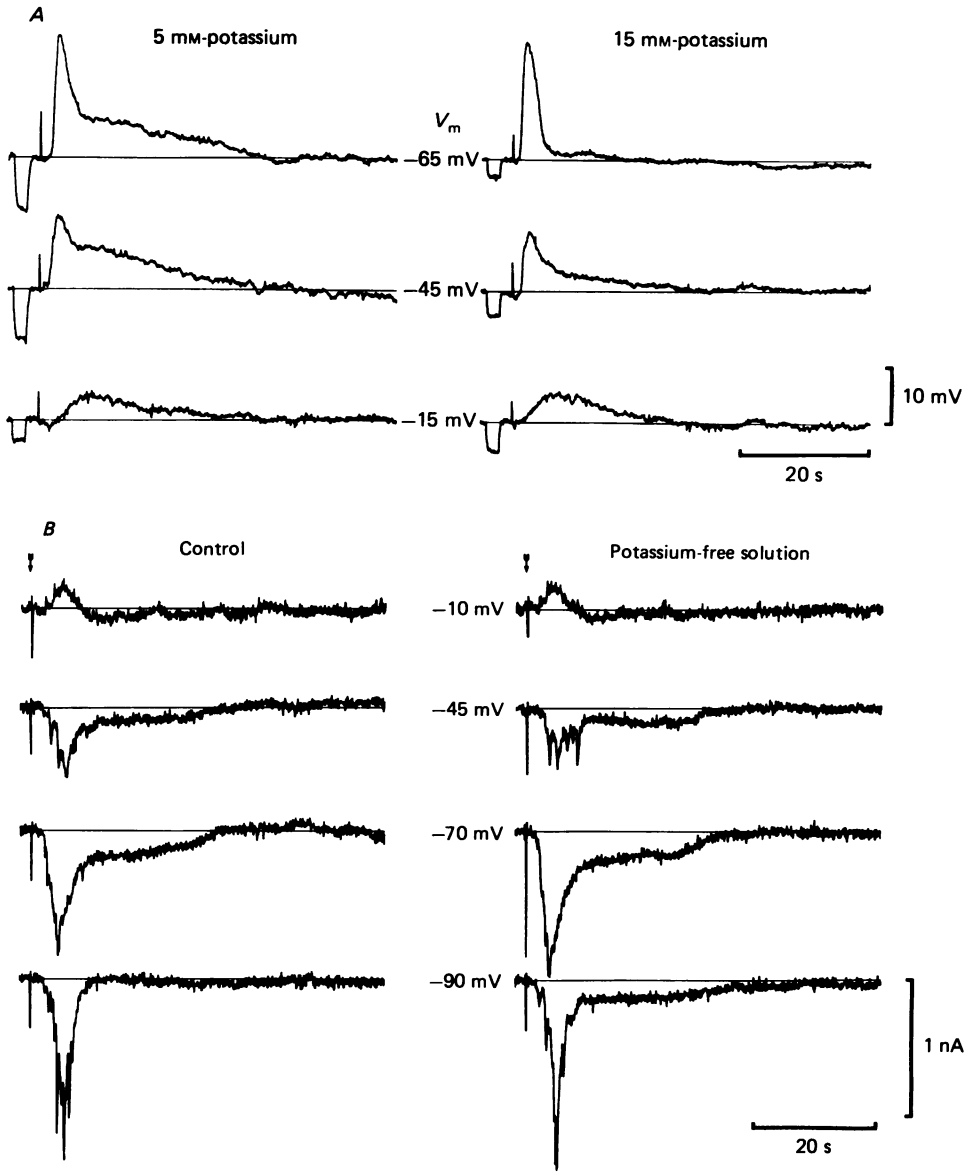


Fig. 7. *A*, potassium dependence of the slow depolarization induced by ionophoresis of noradrenaline in the smooth muscle of a segment of vein. Shown in control and 15 mM-potassium solutions are fast and slow potentials recorded at different pre-stimulus holding potentials for the same ionophoretic pulse of noradrenaline (100 ms, 100 nA). The slow depolarization is not evident at the −65 mV pre-stimulus potential and considerably smaller at the −45 mV level in the test solution. *B*, potassium dependence of the slow current induced by ionophoresis of noradrenaline in the smooth muscle of a segment of vein. Shown in control and potassium-free solutions are fast and slow currents recorded at different membrane potential to the same ionophoretic pulse of noradrenaline (100 ms, 100 nA). The main difference in the responses is the reappearance of the slow component at −90 mV in potassium-free solution. Arrows indicate the application of noradrenaline.

level cannot be interpreted simply as a decreased driving force for potassium ions as there is about a 50% decrease in membrane resistance compared to control.

The effects of reducing the external potassium concentration are shown in Fig. 7*B* for a segment held under voltage clamp. Here a comparison is shown for current records obtained at a series of holding potentials in control (5 mM-potassium) and potassium-free solutions. The slow inward current which virtually disappeared at

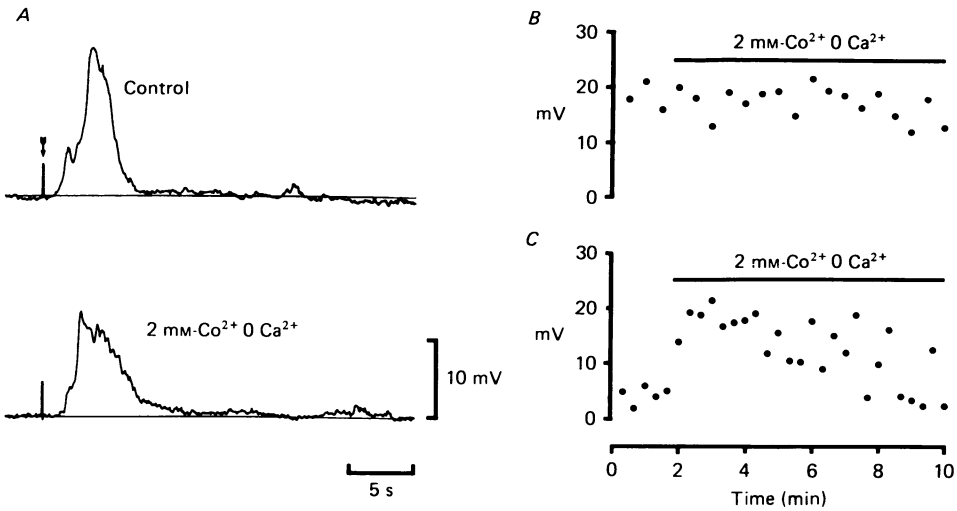


Fig. 8. The effect of low-calcium cobalt solution on the response to ionophoresis of noradrenaline on the smooth muscle of segments of vein. Shown in *A* are records obtained in a segment in control and after 10 min exposure to low-calcium cobalt solution held at a pre-stimulus potential of −80 mV throughout. The arrow denotes the application of ionophoretic noradrenaline (duration 100 ms, amplitude 150 nA). The plots (*B* and *C*) show the amplitude of the fast depolarization with exposure to the cobalt solution for two different segments, with membrane potentials maintained at pre-stimulus levels of −80 mV for both segments. Responses were elicited every 30 s for the same ionophoretic pulse. A high perfusion rate was maintained throughout.

−90 mV in control solution remained inward in potassium-free solution, while there was no obvious effect on the fast component. At other potentials records in the two solutions were similar, with reversal of the fast component evident at −10 mV.

It is also to be noted that the slow component was markedly reduced at −10 mV, and even at −45 mV was smaller than at −70 mV. This does not fit with the assumption of a mechanism involving a decreased potassium conductance ($g_K$). One possibility is that the slow component is contaminated by a slowly inactivating phase of the fast component. While this has not been disproved, it is believed unlikely because of the clear isolation of the fast component in control at −90 mV where it is seen to be a rapid transient inward current (see also Fig. 7*A*) and also because events during the fast component show considerable fluctuation in amplitude which is not observed during the slow depolarization. A second possibility is that the slow component shows voltage dependence of the $g_K$ mechanism so that the response is decreased at depolarized potentials. Further studies on this issue have not been

made; however, it is to be noted that the relative suppression of the slow response with depolarization is somewhat variable, as is evident by comparison with Fig. 7*A* where the slow component showed relatively less voltage dependence with depolarization.

*Calcium removal*

The role of calcium ions in mediating the noradrenaline-induced fast and slow depolarizations was examined by comparison of responses before and after exposure to low-calcium solution. Both responses persisted upon exposure to low-calcium solution for at least 10 min. However, as this solution also caused run-down of the membrane potential (a phenomenon also observed in arteriolar smooth muscle; see Hirst & Van Helden, 1982), more detailed experiments were performed using low-calcium solution with cobalt ions used as substitute. The membrane potential remained stable in this solution, and in three segments which responded to ionophoretic noradrenaline with both fast and slow depolarizations the responses persisted even after 10 min exposure.

Time-dependent actions that this solution had on the fast depolarization were studied in segments exposed to high flow rates of the bathing solution. This protocol allowed repetitive generation of the fast depolarization with minimal desensitization or slow depolarization. The rapid flow allowed effective changeover of solutions in less than 10 s, as assessed by measuring membrane potential and resistance changes into or from high-potassium-containing solutions. Fast responses were either little altered (Fig. 8*A* and *B*) or markedly enhanced (Fig. 8*C*) upon exposure to the low-calcium cobalt solution. Responses over the next 5–10 min were then either minimally affected (Fig. 8*B*) or there was a gradual diminution (Fig. 8*C*) with time. The data indicate that the noradrenaline-induced depolarizations are not carried by calcium ions. The effects of longer term exposure ($>$ 10 min) to calcium-free solutions caused a run-down in the size of the responses.

## DISCUSSION

The procedure of cutting small blood vessels into short segments has been applied to determine properties of the conductance changes induced by noradrenaline in the smooth muscle of mesenteric veins. As for arteriolar smooth muscle (Hirst & Neild, 1978, 1980), these short segments are effectively isopotential, exhibit similar passive electrical properties and can be voltage clamped using the single-electrode recording procedure. It has been shown in the smooth muscle of mesenteric veins that the responses produced by exogenous noradrenaline are the same as those produced by noradrenaline released through neural stimulation (Suzuki, 1981; Van Helden, 1988). Furthermore, it is clear that in addition to the slow depolarizing response (Suzuki, 1981), noradrenaline also produces a fast depolarization which is distinct, not only in its pharmacology and stimulus dependence (Van Helden, 1988) but in its ionic mechanism.

Evidence that the fast depolarization results from an increase in conductance to chloride ions has been presented through the use of small venous segments which have walls comprising only a single layer of smooth muscle and about which solution

composition can be altered rapidly. This has allowed examination of ionic mechanisms, with tests for the possibility that the fast depolarization resulted from an increase in permeability to sodium, calcium or chloride ions. The response just after exposure to test solution was not decreased by reduction in sodium or calcium ion content but was increased by reduction in chloride ion content of the bathing solution due to a positive shift in reversal potential. This is consistent with the hypothesis that chloride ions are the predominant charge carriers underlying the fast response. The normal reversal potential of the response is similar to that for the fast noradrenaline-induced depolarization recorded in the rat anococcygeus muscle (Byrne & Large, 1985) with values between $-15$ and $-30$ mV, values consistent with the chloride reversal potential of the vas deferens measured directly with intracellular ion-selective electrodes (Aickin & Brading, 1982). A mechanism involving an increase in chloride conductance is also consistent with ion flux studies on other vascular smooth muscle where a noradrenaline-induced efflux of chloride ions has been recorded in both the rat portal vein (Wahlstrom, 1973) and the rabbit ear artery (Droogmans, Raeymaekers & Casteels, 1977).

Some comment can also be made about the chloride-dependent inactivation of the fast response. This phenomenon has been observed in other tissues such as guinea-pig myometrium (Bülbring & Szurszewski, 1974) and the rat anococcygeus (Large, 1984). The finding that in low-chloride solution there was suppression of this response, with reversal potential maintained positive to that under control conditions, is consistent with positively shifted reversal potentials measured directly in the vas deferens under similar conditions using ion-sensitive electrodes (Aickin & Brading, 1982). The data indicate that suppression of the response in venous smooth muscle does not occur because of changes in reversal potential but results from inactivation of the permeation mechanism (see also Large, 1984; Byrne & Large, 1985). The effect is unlikely to be specifically due to the isethionate ions used to substitute for chloride ions as it has been observed to occur in other tissues where different anion substitutes have been used (see Bülbring & Szurszewski, 1974; Large, 1984). Reduction of external chloride ions has been shown to affect other intracellular parameters in smooth muscle (Aickin & Brading, 1983) and it may be that suppression results from these or related phenomena (see also Large, 1984). The action is unlikely to be an antagonism to $\alpha$-adrenoceptors as both the slow depolarizing response and constriction are still produced in response to noradrenaline in low-chloride solution (Van Helden, 1988).

The finding that the depolarizing responses to noradrenaline were maintained for at least 10 min exposure to low-calcium solution with cobalt substitution indicates that large entry of calcium ions is not a prerequisite for activating responses. This is consistent with the view that responses are mediated by intracellular events activated by receptor stimulation. Whether this chloride conductance results from specific activation of a specialized subset of chloride channels or the channels belong to a more general class such as the calcium-activated chloride channels has yet to be determined.

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REFERENCES

AICKIN, C. C. & BRADING, A. F. (1982). Measurement of intracellular chloride in guinea-pig vas deferens by ion analysis, $^{36}$chloride efflux and micro-electrodes. *Journal of Physiology* **326**, 139–154.

AICKIN, C. C. & BRADING, A. F. (1983). Towards an estimate of chloride permeability in the smooth muscle of guinea-pig vas deferens. *Journal of Physiology* **336**, 179–197.

BÜLBRING, E. & SZURSZEWSKI, J. H. (1974). The stimulant action of noradrenaline (α-action) on guinea-pig myometrium compared with that of acetylcholine. *Proceedings of the Royal Society* B **185**, 225–262.

BYRNE, N. G. & LARGE, W. A. (1985). Evidence for two mechanisms of depolarization associated with $\alpha_1$-adrenoceptor activation in the rat anococcygeus muscle. *British Journal of Pharmacology* **86**, 711–721.

DROOGMANS, G., RAEYMAEKERS, L. & CASTEELS, R. (1977). Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. *Journal of General Physiology* **70**, 129–148.

FINKEL, A. S., HIRST, G. D. S. & VAN HELDEN, D. F. (1984). Some properties of excitatory junction currents recorded from submucosal arterioles of guinea-pig ileum. *Journal of Physiology* **351**, 87–98.

HIRST, G. D. S. & NEILD, T. O. (1978). An analysis of excitatory junctional potentials recorded from arterioles. *Journal of Physiology* **280**, 87–104.

HIRST, G. D. S. & NEILD, T. O. (1980). Some properties of excitatory junction potentials recorded from arterioles of guinea-pigs. *Journal of Physiology* **303**, 43–60.

HIRST, G. D. S. & VAN HELDEN, D. F. (1982). Ionic basis of the resting potential of submucosal arterioles in the ileum of the guinea-pig. *Journal of Physiology* **333**, 53–67.

JACK, J. J. B., NOBLE, D. & TSIEN, R. W. (1975). *Electric Current Flow in Excitable Cells*. Oxford: Oxford University Press.

LARGE, W. A. (1984). The effect of chloride removal on the responses of the isolated rat anococcygeus muscle to $\alpha_1$-adrenoceptor stimulation. *Journal of Physiology* **352**, 17–29.

SUZUKI, H. (1981). Effects of endogenous and exogenous noradrenaline on the smooth muscle of guinea-pig mesenteric vein. *Journal of Physiology* **321**, 495–512.

VAN HELDEN, D. F. (1986). A paradox resolved – transient noradrenaline induced chloride conductance generates action potentials in guinea-pig mesenteric veins. *Proceedings of the Australian Physiological and Pharmacological Society* **17**, 49*P*.

VAN HELDEN, D. F. (1988). Electrophysiology of neuromuscular transmission in guinea-pig mesenteric veins. *Journal of Physiology* **401**, 469–488.

WAHLSTROM, B. A. (1973). A study on the action of noradrenaline on ionic content and sodium, potassium effluxes in the rat portal vein. *Acta physiologica scandinavica* **89**, 522–530.