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Microelectrode study on the ionic mechanisms which contribute to the noradrenaline-induced depolarization in isolated cells of the rabbit portal vein

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1 Experiments were carried out to determine the identity of the ionic mechanisms which contribute to the noradrenaline-evoked depolarization recorded with microelectrodes in freshly dispersed rabbit portal vein cells.

2 In normal physiological salt solution with microelectrodes containing 1 M NaCl the reversal potential (E_r) of the noradrenaline-induced response was -7.6 ± 2.9 mV. When the external NaCl was replaced by equipmolar concentrations of NaI, NaBr and $NaNO_3$, E_r was -33 ± 3.5 mV, -29.1 ± 5.2 mV and -18.4 ± 1.1 mV, respectively.

3 In physiological salt solution E_r of noradrenaline-evoked responses recorded with electrodes filled with 1 M NaI or 1 M $NaNO_3$ was $+16.3 \pm 3.9$ mV and $+10.0 \pm 7.6$ mV, respectively. These results suggest that an increase in anion conductance contributes to the depolarization to noradrenaline.

4 Data from experiments with organic anions indicated that glutamate behaves as a less permeant anion but that benzenesulphonate blocks the anion conductance to unmask another conductance mechanism activated by noradrenaline.

5 When external NaCl was substituted by choline Cl and Tris Cl E_r was -21.3 ± 3.7 mV and -20.5 ± 2.8 mV, respectively. These results suggest that noradrenaline also activates a cation conductance mechanism in freshly dispersed rabbit portal vein cells. It is concluded that the depolarization to noradrenaline recorded with a microelectrode is produced by the simultaneous activation of an anion channel and a separate cation channel.

Introduction

In a previous study with patch pipette techniques we provided evidence in isolated cells of the rabbit portal vein that noradrenaline produces an increase in membrane conductance to chloride ions and to cations (Byrne & Large, 1988b). There appeared to be a curious interaction between these two separate conductance mechanisms. When sodium chloride was the major salt in the patch pipette (experiments were carried out in potassium-free conditions to remove a prominent calcium-activated potassium conductance increase) the response to noradrenaline could be described mainly by an increase in chloride conductance. However, when sodium glutamate was the major constituent of the patch pipette the inward current evoked by noradrenaline appeared to be carried by cations. Moreover the reversal potential of the noradrenaline-induced response was about 70 mV more positive than the chloride equilibrium potential. Thus in these conditions it appeared that noradrenaline produced little or no increase in chloride conductance. It was observed that the amplitude of the responses was similar whether the current was carried by either chloride or cations. It would seem that the contents of the patch pipettes and subsequent dialysis of the cell interior have a profound influence on the qualitative nature of the conductance mechanisms evoked by noradrenaline in freshly dispersed rabbit portal vein cells. These results raise doubts concerning the physiological implications of the ionic mechanisms evoked by noradrenaline which are identified with patch pipette techniques. It is possible that membrane mechanisms may be obscured or exaggerated in patch pipette studies. For example the most prominent response to noradrenaline recorded with patch pipettes in freshly dispersed rabbit ear artery cells is hyperpolarization

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(or outward current) produced by an increase in potassium conductance (Benham, Bolton, Byrne and Large, unpublished). In whole tissue experiments α-adrenoceptor activation by exogenous noradrenaline and nerve stimulation evokes only depolarization (Suzuki & Kou, 1983). In the present study we have carried out experiments to ascertain whether the depolarization recorded with microelectrodes is produced by an increase in chloride and/or cation conductance in cells isolated from the rabbit portal vein. It is evident that in isolated smooth muscle cells microelectrodes produce a much smaller disturbance of the intracellular milieu than is found with patch pipettes. For example, with microelectrodes it is possible to obtain many reproducible responses to noradrenaline in freshly dispersed cells. This is not the case with patch pipettes and in many cells only a single response to noradrenaline can be obtained (see Byrne & Large, 1987a; 1988b). In this paper we provide evidence which suggests that the noradrenaline-induced depolarization recorded with a microelectrode is mediated by an increase in both chloride and cation membrane conductance and therefore supports a physiological role for both ionic mechanisms.

Methods

Rabbits (2–2.5 kg) of either sex were killed by overdose of i.v. sodium pentobarbitone. Isolated cells were obtained from rabbit portal vein by digestion with papain according to a method described in detail previously (Byrne & Large, 1988a,b). Cells were stored on cover slips at 4°C and were used on the same day as dispersion.

A single microelectrode was used for recording membrane potential and passing current with an Axoclamp-2A amplifier. Depolarizations or hyperpolarizations to noradrenaline were recorded under current clamp with the Bridge circuit of the Axoclamp-2A amplifier. Under voltage clamp, membrane currents were measured in the discontinuous single-electrode voltage clamp mode. In this mode, the function of the microelectrode alternates between current passing and voltage recording at a high frequency. The voltage is measured only when the discontinuous current is zero, allowing a precise measurement of membrane potential. The duty cycle used was current passing for 30%, and voltage recording for 70% of each cycle. The procedure was to select a sampling frequency at which the head-stage voltage after current passing returned to true baseline. The sampling frequency was between 1.0 and 2.0 kHz. Microelectrodes had resistance of 100–200 MΩ when filled with either 1 M NaCl or 1 M KCl.

The normal bathing solution contained (mM): NaCl 126, KCl 6, $MgCl_2$ 1.2, $CaCl_2$ 1.5, HEPES 10 and glucose 11, buffered to pH 7.2 with NaOH and experiments were carried out at room temperature (20–24°C). In external substitution experiments 126 mM NaCl was replaced with equimolar concentration of choline Cl, Tris Cl, NaI, NaBr, $NaNO_3$, Na glutamate or Na benzenesulphonate. The external solution always contained 10^{-6} M propranolol to block any β-adrenoceptor-mediated hyperpolarization (Byrne & Large, 1988a).

Junction potentials produced by anion substitution in the external solution were minimized by placing an agar bridge between the recording bath and the chamber with the indifferent electrode. With this configuration junction potentials were small (<5 mV) and corrections were made as described previously (Byrne & Large, 1987a).

In a few experiments 1 M NaCl in the microelectrode was replaced by either 1 M NaI or $NaNO_3$. Noradrenaline was applied by ionophoresis.

The values given in the text are the mean $\pm$ s.e.mean. Statistical significance of changes in the reversal potential from the normal value (1 M NaCl electrode and 126 mM NaCl external solution) was estimated with Student's *t* test.

Drugs used were: atropine sulphate, bovine albumin (fatty acid-free), dithiothreitol, noradrenaline bitartrate, papain type IV (all Sigma), and ($\pm$)-propranolol hydrochloride (ICI).

Results

General observations

In the present experiments microelectrodes filled with NaCl were used in order to remove the calcium-activated potassium current evoked by noradrenaline in these cells which would allow more accurate measurement of the reversal potential of the depolarization to noradrenaline. There is evidence to indicate that with NaCl-filled microelectrodes, substantial amounts of intracellular K^+ had been replaced with Na^+. Firstly, an outward current to depolarizing voltage steps could not be recorded if NaCl was used as the electrolyte in the microelectrodes. Figure 1 illustrates currents evoked by depolarizing steps to -30 mV and $+30$ mV from a holding potential of -50 mV when either a KCl (Figure 1a) or a NaCl (Figure 1b) electrode was used. The currents at -30 mV are similar with both KCl and NaCl electrodes (upper records Figure 1a and b) and essentially reflect the input resistance of the cell. A hyperpolarizing 20 mV step produced a current response of similar amplitude. However a command step to $+30$ mV evokes a large outward current with

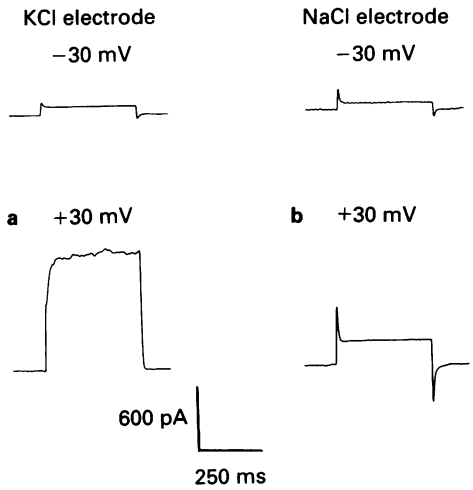

Figure 1 Evidence for the lack of a voltage-dependent outward current with the use of a NaCl-filled microelectrode. The records are current responses to depolarizing voltage commands to -30 mV (upper traces) and $+30$ mV (lower traces) with the use of a microelectrode filled with either 1 M KCl or 1 M NaCl. Holding potential: -50 mV. Comparison of the lower traces illustrate that the step to $+30$ mV evokes a large outward (potassium) current with a KCl microelectrode (a) but there is no apparent voltage-dependent current with a NaCl microelectrode (b) and the amplitude of the current response in (b) reflects the input resistance of the cell.

a KCl electrode (lower record Figure 1a). In contrast, with a NaCl electrode there was no evidence of a voltage-dependent response at $+30$ mV and the amplitude of the current at the end of the command pulse (lower record of Figure 1b) is about 4 times larger than the corresponding value at -30 mV (i.e. an indication of the input resistance). These data suggest that with NaCl-filled microelectrodes there is little (or no) voltage-dependent potassium current.

A second line of evidence which suggests that potassium had been largely replaced by sodium is that there was no evidence of a calcium-activated increase in potassium conductance to noradrenaline which has been demonstrated if the intracellular electrode is filled with KCl (Byrne & Large, 1988a,b). Although these results indicate that use of microelectrodes filled with NaCl eliminate potassium currents we have no data on the intracellular concentration of sodium achieved in our experiments. It is possible that there may be substantial amounts of potassium remaining in the cells and that the intracellular concentration of sodium was sufficiently high to block the potassium currents. For example, it has been shown that internal sodium ions block large unitary calcium-dependent potassium currents in bovine adrenal chromaffin cells (Marty, 1983). An estimate of the internal sodium activity could be obtained in experiments with a Na-sensitive microelectrode which would also yield interesting information regarding the change in intracellular sodium concentration with time. However, technical difficulties of impaling an isolated smooth muscle cell with both a recording and a Na-sensitive microelectrode preclude such observations at the present time.

It is possible that the increased intracellular concentration of Na^+ would stimulate the electrogenic sodium/potassium ATPase which might in turn influence the observed membrane potential changes to noradrenaline. However it seems unlikely that this occurred in the present experiments as the resting membrane potential was about 0 mV when recorded with NaCl-filled electrodes. If the electrogenic sodium/potassium ATPase was active it might be expected that the resting membrane potential would be at more hyperpolarized values. In addition the amplitude and time course of the noradrenaline-evoked depolarizations recorded in the present experiments were similar to the characteristics of the responses recorded with microelectrodes filled with 1 M KCl (Byrne & Large, 1988a).

On most occasions the ionophoretic application of noradrenaline produced a monophasic depolarization when recorded with a NaCl-filled microelectrode (see Figure 2a). Sometimes biphasic depolarizations were recorded but these responses were not reproducible and overall there was no convincing evidence that the response could be differentiated temporally into two components representing two conductance mechanisms. We investigated whether a chloride and/or a cation conductance increase was involved in the noradrenaline-evoked depolarization by altering independently the cation and anion gradients. A change in the reversal potential (E_r) by these experimental manipulations would implicate the appropriate conductance mechanism in the response to noradrenaline.

Responses to noradrenaline in different external anion solutions

Figure 2a illustrates the measurement of the reversal potential (E_r) of the noradrenaline-induced response in a cell with a NaCl-filled microelectrode in normal bathing solution (126 mM NaCl). In these conditions the resting membrane potential is close to 0 mV and small amounts of inward and outward current were passed through the recording electrode to obtain the holding membrane potentials indicated. The ionophoretic application of noradrenaline-evoked depolarization at a membrane potential of -52 mV and the amplitude of the response declined as the membrane

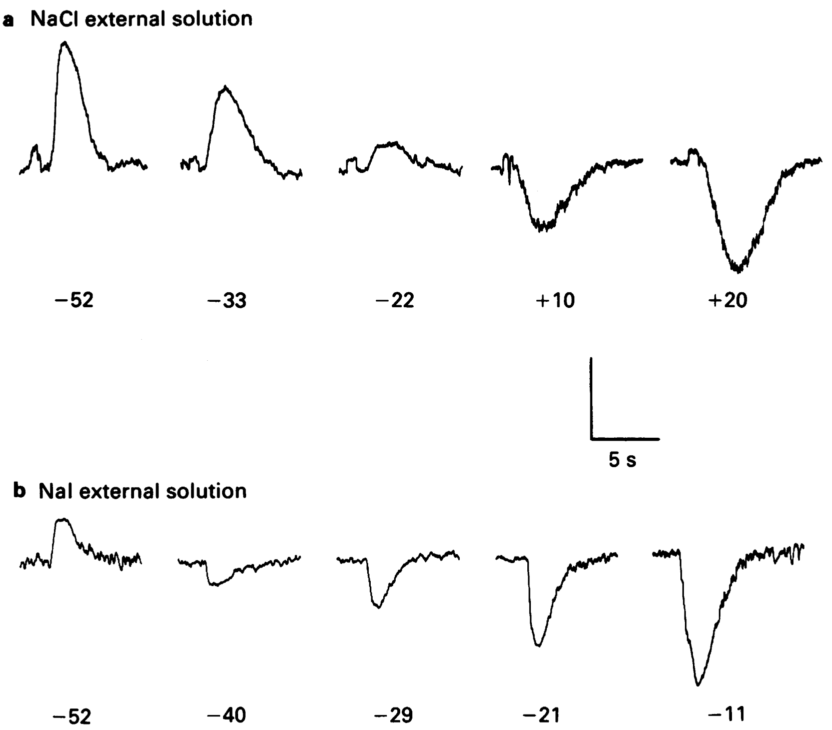

Figure 2 Responses to noradrenaline in either external 126 mM NaCl (a) or 126 mM NaI (b) recorded with a NaCl-filled electrode. Membrane potentials are indicated under each trace. Noradrenaline was applied by ionophoresis: 10 nA for 500 ms in (a) or 200 ms in (b). A small ionophoretic artefact (small upward deflection) can be seen just prior to the responses in (a). Vertical calibration bar: 20 mV in (a) and 10 mV in (b).

potential was reduced and reversed to hyperpolarization at between −22 and +10 mV.

The amplitude of the responses are plotted as a function of holding potential in Figure 3 (circles) and E_r was −9 mV. In eight experiments with a NaCl-filled electrode with 126 mM NaCl bathing solution the mean reversal potential was -7.6 ± 2.9 mV (Table 1). In the conditions used, this value of the reversal potential may be close to both the chloride and sodium equilibrium potentials although we do not have independent estimates of E_{Cl} and E_{Na}.

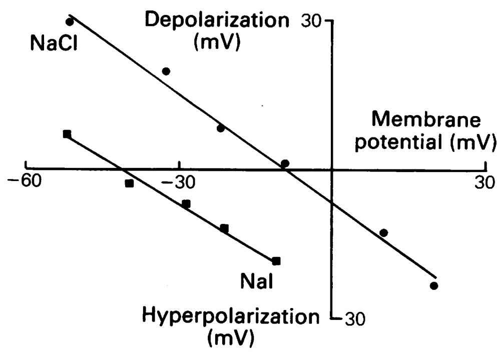

Figure 3 Relationship between the amplitude of the noradrenaline-evoked response and membrane potential in either external 126 mM NaCl (●) or 126 mM NaI (■). Data taken from responses shown in Figure 2.

Figure 2b shows responses to noradrenaline when 126 mM NaCl in the bathing solution was replaced by an equimolar concentration of NaI. It is evident that the reversal potential was between −52 mV and −40 mV. The results are plotted in Figure 3 (squares) and the interpolated equilibrium potential is −42 mV. The mean E_r in external NaI solution was -33.0 ± 3.5 mV (Table 1) and is statistically significant from the value found in external NaCl solution ($P < 0.001$). Similar experiments were carried out with 126 mM NaBr and $NaNO_3$ and the E_rs were respectively -29.1 ± 5.2 mV and -18.4 ± 1.1 mV (Table 1). The change in the reversal potential of the noradrenaline-evoked responses produced by substitution of external Cl^- with I^-, Br^- and NO_3^- suggests that an anion conductance increase at least partially underlies the depolarization to noradrenaline.

Table 1 Reversal potential of the noradrenaline-induced response in various conditions

External solution†	*Electrode solution*††	*Reversal potential* (mV)	(n)
NaCl	NaCl	-7.6 ± 2.9	(8)
NaI	NaCl	-33.0 ± 3.5*	(5)
NaBr	NaCl	-29.1 ± 5.2*	(8)
$NaNO_3$	NaCl	-18.4 ± 1.1**	(8)
Na glutamate	NaCl	$+19.3 \pm 5.7$*	(6)
Na benzenesulphonate	NaCl	-8.7 ± 4.5	(7)
NaCl	NaI	$+16.3 \pm 3.9$**	(3)
NaCl	$NaNO_3$	$+10.0 \pm 7.6$***	(3)
Choline Cl	NaCl	-21.3 ± 3.7**	(6)
Tris Cl	NaCl	-20.5 ± 2.8**	(4)

Statistically different from control value with external NaCl and NaCl electrode: *$P < 0.001$, **$P < 0.005$ and ***$P < 0.05$.
† All salts at a concentration of 126 mM and †† electrode solutions at 1 M.

Other experiments were carried out with relatively large organic anions which normally do not permeate chloride channels very well. Figure 4 illustrates the results of two experiments in which external NaCl was replaced either by Na glutamate (squares) or Na benzenesulphonate (circles) and the respective E_r values were +25 mV and −10 mV. The overall mean values were $+19.3 \pm 5.7$ mV and -8.7 ± 4.5 mV (Table 1) respectively for Na glutamate and Na benzenesulphonate. The statistically significant shift of the reversal potential of the noradrenaline-induced response to a more positive potential than the control value (−7.6 mV) is consistent with the idea that glutamate acts simply as a less permeant anion. This is patently not so for benzenesulphonate as E_r with this anion was similar to the control E_r. It seems extremely unlikely that benzenesulphonate is as permeable as chloride and we feel that benzenesulphonate does not simply act as an impermeant anion but has some other action. We would like to suggest that benzenesulphonate somehow blocks the anion response which unmasks the cation conductance mechanism (see Discussion for details).

Replacement of Cl^- in the microelectrode with other anions

In a few experiments the recording electrodes were filled with 1 M NaI and $NaNO_3$ (rather than NaCl) with 126 mM external NaCl. With NaI and $NaNO_3$ electrodes the E_r of the noradrenaline-induced response was respectively $+16.3 \pm 3.9$ mV (Table 1) and $+10.0 \pm 7.6$ mV (Table 1). The shift of E_r to positive potentials with NaI and $NaNO_3$ electrodes support the above data that an anion current contributes to the noradrenaline-evoked depolarization and that I^- and NO_3^- permeate the anion channel more readily than Cl^- ions.

Substitution of sodium with other cations in the external solution

Experiments were carried out in which most of the external sodium was replaced by cations that normally permeate cation channels rather poorly. In these conditions the E_r of a cation conductance would move to more negative potentials. Figure 5

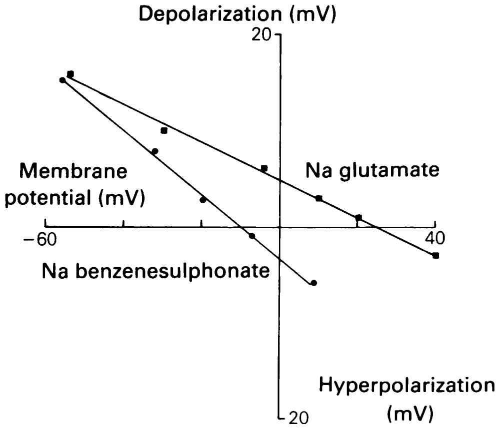

Figure 4 Relationships between noradrenaline-evoked responses and membrane potential when the major external anion was either benzenesulphonate (●) or glutamate (■). See text for further details.

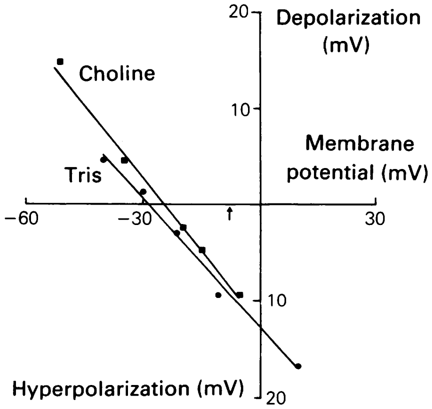

Figure 5 Effect of membrane potential on the responses to noradrenaline when either 126 mM Tris Cl (●) or choline Cl (■) was present in the bathing solution. The arrow indicates the mean E_r with NaCl in the external solution.

shows the results from two experiments in which external 126 mM NaCl was replaced by equimolar concentrations of either choline chloride (squares) or Tris chloride (circles). It can be seen that in these experiments E_r of the noradrenaline-induced depolarization was -25 mV (choline) and -28 mV (Tris). The mean E_r values for external choline and Tris were -21.3 ± 3.7 mV and -20.5 ± 2.8 mV (Table 1) respectively. The shift of the reversal potential to more negative potentials in the presence of external choline and Tris indicates that a cation conductance mechanism also contributes to the noradrenaline-evoked depolarization in freshly dispersed rabbit portal vein cells.

Discussion

The present experiments were undertaken to see if anionic and cationic mechanisms identified with patch pipette techniques contributed to the noradrenaline-induced depolarization observed with microelectrodes. In experiments where external Cl^- was largely replaced by more permeant anions (I^-, Br^- and NO_3^-) the reversal potential of the noradrenaline-induced responses was shifted to more negative potentials as predicted if an anion conductance increase contributed to the noradrenaline-induced depolarization. This postulate was supported by the results from studies where NaI and $NaNO_3$ were used as the microelectrode filling solution in which the reversal potential was shifted to positive membrane potentials. Thus the overall evidence supports strongly the idea that an increase in chloride conductance subscribes to the noradrenaline-evoked depolarization. Experiments with less permeant anions produced apparently conflicting data. Whereas glutamate appeared to behave simply as a less permeant substitute the results with benzenesulphonate were not as straightforward. When external NaCl was replaced by Na benzenesulphonate there was no change in E_r of the response to noradrenaline. This suggests that either benzenesulphonate and chloride are similarly permeable (extremely unlikely) or that the presence of benzenesulphonate reveals another conductance mechanism. We feel that the latter proposal is more likely to be valid since previously it has been suggested that benzenesulphonate blocks a chloride channel in the rat anococcygeus muscle (Large, 1984). Van Helden (1988) has claimed that another organic anion, isethionate, may also block a chloride conductance mechanism in the guinea-pig mesenteric vein. Thus in our experiments it is possible that the depolarization to noradrenaline recorded in Na benzenesulphonate is brought about by an increase in membrane cation conductance. Experiments in which external Na was replaced by choline and Tris moved the reversal potential of the noradrenaline-induced response to more negative membrane potentials. The only reasonable explanation for these results is that an increase in cation conductance contributes to the depolarization to noradrenaline. Thus, in conclusion, it would appear that the depolarization to noradrenaline is mediated by an efflux of chloride through an anion channel and an influx of cations through a separate cation-selective channel. The fact that both cationic and anionic conductance mechanisms to noradrenaline can be observed with microelectrode recording is evidence that both membrane mechanisms may be activated by α-adrenoceptor stimulation in physiological conditions.

If it is assumed that the relative contribution to the cation conductance mechanisms does not alter in the different anion solutions, then the values of the reversal potentials to noradrenaline suggest the following permeability sequence through the anion channel: $I^- > Br^- > NO_3^- > Cl^- >$ glutamate. This interpretation would be incorrect if variable amounts of the anions used entered the cells through resting leak anion channels. If this occurred the value of the equilibrium potential of the anions would vary from anion to anion depending on the concentration gradient established across the cell membrane. However, the shift of the reversal potential of the noradrenaline-induced responses to positive potentials when NaI and $NaNO_3$ were used in the microelectrodes indicates that our conclusions are likely to be valid. The order of halide permeability ($I^- > Br^- > Cl^-$) has also been found in rat lacrimal glands cells (Evans & Marty, 1986), cultured Schwann cells (Gray *et al.*, 1984) and in *Xenopus laevis* oocyte membranes reconstituted into planar bilayers (Young *et al.*, 1984). Moreover the same sequence is found for the channel opened by glycine and γ-aminobutyric acid (GABA) in mouse cultured spinal neurones (Bormann *et al.*, 1987). In a previous publication we noted the similarity between the calcium-activated chloride conductance increase in rat lacrimal glands (Marty *et al.*, 1984) and the rat anococcygeus muscle (Byrne & Large, 1987b). Interestingly in rat lacrimal gland cells the chloride channel appears to be more permeable to NO_3^- than to Br^- (Evans & Marty, 1986) whereas the reverse order was found in our experiments. There is no obvious explanation for this discrepancy unless the structures of the channels are different. Further experiments with patch pipettes are needed to substantiate this point.

Finally, it is becoming increasingly evident that chloride currents may have an important physiological role in smooth muscle. Evidence for a chloride current has been presented in cells from rat common carotid artery (Shoemaker *et al.*, 1985), in the ctenophore *Mnemiopsis* (Stein *et al.*, 1985), rat anococcygeus muscle (Byrne & Large, 1987a), guinea-pig uterus (Coleman & Parkington, 1987), rabbit portal vein (Byrne & Large, 1988b) and the guinea-pig mesenteric vein (van Helden, 1988). In the guinea-pig vas deferens it has been demonstrated that the chloride equilibrium potential is about −25 mV (Aickin & Brading, 1982). Therefore any transmitter or local mediator which increases chloride conductance will evoke depolarization and consequent contraction if the voltage threshold for opening of voltage-dependent calcium channels is reached.

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