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THE EFFECT OF CHLORIDE REMOVAL ON THE RESPONSES OF THE ISOLATED RAT ANOCOCCYGEUS MUSCLE TO α_1-ADRENOCEPTOR STIMULATION

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SUMMARY

1. The effect of Cl removal on the depolarizations and contractions produced by activation of the α_1-adrenoceptor in the isolated rat anococcygeus was studied.

2. In normal Krebs solution the ionophoretic application of noradrenaline and phenylephrine elicited large depolarizations; when NaCl was replaced with Na benzenesulphonate application of the agonists usually evoked no membrane response but sometimes a hyperpolarization or a biphasic membrane potential change was observed. Graded replacement of Cl by benzenesulphonate produced a progressive reduction in the depolarizing ability of noradrenaline and phenylephrine.

3. When NaCl was replaced with Na isethionate or glucuronate, noradrenaline evoked small depolarizations in most of the cells but the sensitivity to noradrenaline (mV/nC) was reduced by over 80%. In other cells either no potential change or hyperpolarization was observed.

4. In solutions containing low Cl the resting membrane potential was slightly depolarized by a mean value of 5–10 mV (depending on the anion substitute) and the membrane resistance was unaltered.

5. In normal Krebs solution noradrenaline and phenylephrine evoked biphasic contractile responses but when NaCl was replaced by Na benzenesulphonate the initial rapid contraction was diminished greatly or abolished. However, the maximum tension produced by any concentration of noradrenaline was unaltered in low Cl solution although the contractions produced by phenylephrine were depressed in low Cl media.

6. These data are further evidence that stimulation of the α_1-adrenoceptor in smooth muscle can produce contraction which is not mediated by a change in membrane potential.

INTRODUCTION

The rat anococcygeus is innervated by the sympathetic nervous system (Gillespie, 1972) and the addition of noradrenaline produces contraction which is mediated by α_1-adrenoceptors (Doxey, Smith & Walker, 1977; Docherty & Starke, 1981). It has

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been shown that the noradrenaline-induced contraction in the rat anococcygeus is accompanied by depolarization and a reduction in membrane resistance (Creed, 1975; Creed, Gillespie & Muir, 1975). The results of electrophysiological experiments in guinea-pig myometrium (Bülbring & Szurszewski, 1974), sheep carotid artery (Mekata & Niu, 1972) and rat portal vein (Shuba, Gurkovskaya, Klevetz, Kochemasova & Tarenenko, 1976) suggest that the depolarization produced by noradrenaline in smooth muscle involves an increase in chloride (Cl) permeability. Also in ion flux studies noradrenaline increased Cl efflux from the rat portal vein (Wahlström, 1973) and rabbit ear artery (Droogmans, Raeymaekers & Casteels, 1977). The present experiments were undertaken to elucidate the ionic mechanism underlying the noradrenaline-induced depolarization in the rat anococcygeus and in this paper the effects of reducing the extracellular Cl concentration ($[Cl]_o$) on the electrical and mechanical responses produced by noradrenaline and phenylephrine are described.

In the anococcygeus the ionophoretic application of noradrenaline produces prazosin-sensitive depolarizations in over 90 % of the cells tested (Large, 1982, 1983) which suggests that the α_1-adrenoceptors are distributed fairly uniformly over the muscle surface. The ease with which depolarizations are obtained using the technique of ionophoresis has been utilized in the present study and the results show that a decrease in $[Cl]_o$ reduces or abolishes the amplitude of noradrenaline-induced depolarizations whereas the maximum mechanical tension evoked by α_1-adrenoceptor activation is affected to a lesser extent.

A preliminary account of some of this work has been published (Large & Wilsoncroft, 1984).

METHODS

In electrophysiological experiments the rat anococcygeus was set up in the same manner as described elsewhere for the mouse tissue (Large, 1982). Briefly, the muscle was attached to a slide mounted on the stage of a Zeiss Nomarski microscope and was superfused continuously with Krebs solution. Normal Krebs solution contained (mM): NaCl, 119; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4$, 1·2; KH_2PO_4, 1·2; $NaHCO_3$, 25; and glucose, 11 and was bubbled with 5 % CO_2–95 % O_2. Reduction of $[Cl]_o$ was achieved by substitution of NaCl with equimolar amounts of Na benzenesulphonate, isethionate or glucuronate. In some experiments KCl was replaced by K gluconate.

Membrane potentials were recorded with intracellular micro-electrodes filled with K acetate with resistances of 80–150 MΩ. Noradrenaline and phenylephrine were applied by ionophoresis from similar micropipettes filled with the appropriate drug solution (0·5 M). When applying agonists the tip of the ionophoretic electrode was placed within 100 μm of the recording electrode. In these experiments the amplitude of the ionophoretic pulse was kept constant at 10 nA and the amount of drug applied was altered by varying the width of the ionophoretic pulse. In a few experiments membrane resistance was measured using a partition chamber (Abe & Tomita, 1968). For these studies current was applied to the tissue by external plate electrodes placed 1·0 cm apart and the electrotonic potential was recorded with an intracellular micro-electrode positioned within 0·1 mm of the leading electrode.

In parallel experiments individual muscles were set up in a 10 ml chamber and noradrenaline-induced contractions were recorded using an isometric strain gauge transducer. All experiments were carried out at room temperature (20–24 °C) as muscles contracted spontaneously at temperatures higher than about 30 °C making electrophysiological experiments impossible.

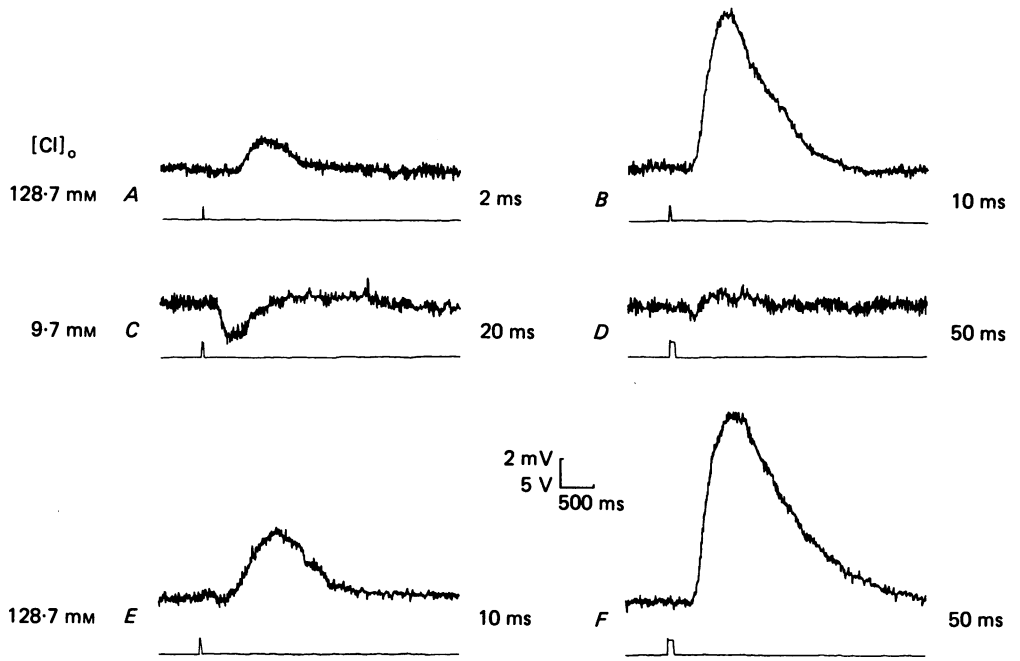

Fig. 1. Effect of replacing NaCl with Na benzenesulphonate on the depolarizations produced by the ionophoretic application of noradrenaline. In each pair of traces the upper record is the membrane potential and the lower record monitors the voltage applied to the ionophoretic micro-electrode. The latter record appears triangular sometimes because the ionophoretic pulses are brief compared to the total sweep time and are limited by the frequency response of the *X–Y* recorder used to obtain hard copies. The figures to the right of the records refer to the length of the ionophoretic pulse. These records are from three different cells. *A* and *B* were recorded from one cell in normal Krebs solution, $E_m = -60$ mV; *C* and *D* from another cell 45 min after NaCl was replaced with Na benzenesulphonate, $E_m = -51$ mV; *E* and *F* after 35 min wash in normal Krebs solution, $E_m = -61$ mV.

RESULTS

In normal Krebs solution the ionophoretic application of noradrenaline to the rat anococcygeus produces charge-dependent depolarizations (Fig. 1 *A* and *B*) which can be recorded over the entire muscle surface as is found in the mouse anococcygeus. The sensitivity of preparations to noradrenaline, expressed as mV depolarization per nanocoulomb of charge passed through the ionophoretic pipettes, was usually in the range of 15–30 mV/nC but could be as high as 100–200 mV/nC (see, for example, control values in Fig. 2) and thus in all experiments agonist-induced depolarizations were recorded in at least six cells in normal Krebs solution before changing to a solution of a different ionic composition.

Effect of reducing the extracellular Cl concentration on the depolarizations produced by noradrenaline

Replacement of all the NaCl with Na benzenesulphonate reduced the depolarizing ability of noradrenaline within 5 min and no simple depolarizations could be recorded after 20 min exposure to 119 mM-Na benzenesulphonate even if ionophoretic pulses 100 times larger than normal were used. Usually no membrane response was obtained but on a few occasions the application of noradrenaline hyperpolarized the membrane (Fig. 1*C*). These responses were not always reproducible and sometimes were

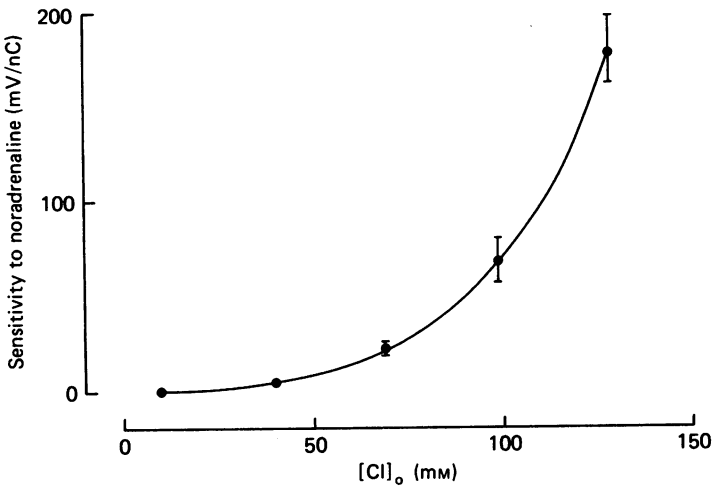

Fig. 2. Relationship between $[Cl]_o$ and the sensitivity to noradrenaline. Each point is the mean of eight to twenty-four cells and the vertical bars represent the standard error (S.E.) of the mean except where the S.E. of the mean is smaller than the point itself. The anion substitute was benzenesulphonate.

converted into biphasic responses as shown in Fig. 1*D*. These effects were reversible and on returning to normal Krebs solution noradrenaline produced dose-dependent depolarizations (Fig. 1*E* and *F*) although there may be a hint of a hyperpolarization preceding the depolarization in Fig. 1*E*.

The depolarizing ability of noradrenaline was estimated in muscles bathed in solutions of various $[Cl]_o$ and those data are summarized in Fig. 2. For the purpose of these experiments the sensitivity (mV/nC) refers only to depolarizations and hyperpolarizations do not enter the analysis. A relatively small reduction (30 mM) in $[Cl]_o$ markedly reduced the sensitivity to noradrenaline from 177 to 67 mV/nC and when the $[Cl]_o$ was 39·7 mM the sensitivity to noradrenaline was decreased to 4·6 mV/nC. In this series of experiments the sensitivity to noradrenaline in normal Krebs solution was unusually high, but in muscles where larger amounts of noradrenaline were necessary to depolarize the cells, reduction in $[Cl]_o$ produced similar results. Thus in three muscles, the control sensitivity to noradrenaline was 19·9 mV/nC but in $[Cl]_o$ = 98·7 mM the sensitivity decreased to 8·5 mV/nC, which

represents a 57% reduction in sensitivity to noradrenaline, whereas the same reduction in $[Cl]_o$ shown in Fig. 2 produced a 62% fall in noradrenaline sensitivity.

In ionic substitution experiments it is important to assess the extent to which altered responses can be attributed to a reduction in the concentration of a given ion (Cl in this case) or if altered responses are caused by additional action(s) of the substitute ion. Thus further experiments were carried out using isethionate or glucuronate as substitutes for Cl.

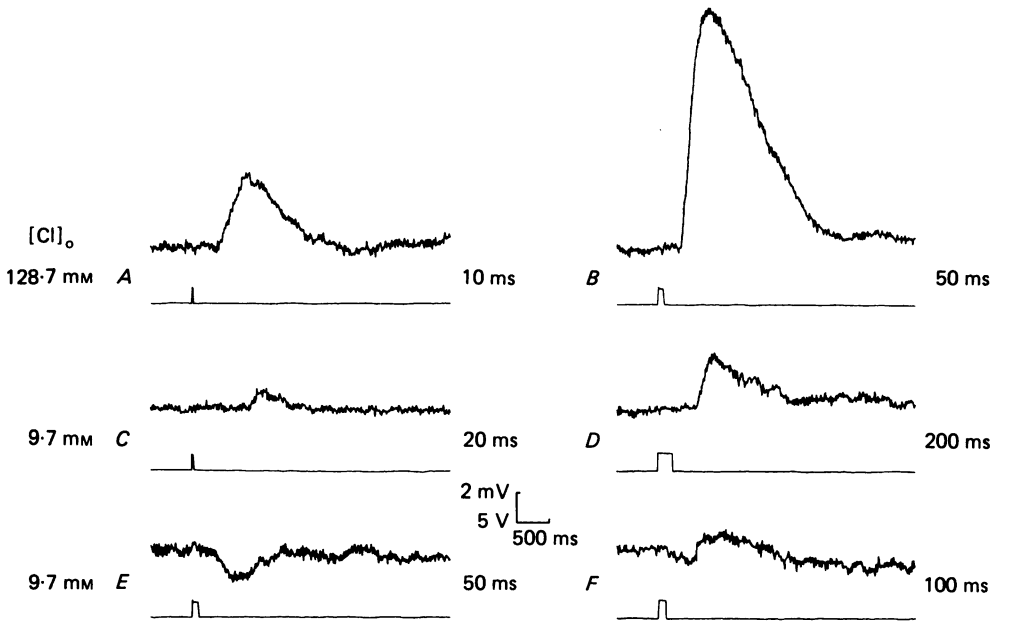

Fig. 3. Effect of replacing NaCl with Na isethionate on the depolarizations produced by noradrenaline. These records are from three cells: *A* and *B* in normal Krebs solution, $E_m = -65$ mV; *C* and *D* ($E_m = -59$ mV) and *E* and *F* ($E_m = -61$ mV) were recorded from two other cells 45–60 min after NaCl was replaced with Na isethionate.

When 119 mM-Na isethionate was used to replace NaCl small depolarizations were observed in about 60% of the cells (Fig. 3*C* and *D*) but much larger ionophoretic pulses were required, indicating a marked reduction in the sensitivity to noradrenaline (Table 1). In the remaining cells usually there was no response or rarely an hyperpolarization (Fig. 3*E*) or even a biphasic response (Fig. 3*F*). These latter membrane responses occurred less frequently than were found with benzenesulphonate as the substitute anion. It can be seen from Table 1 that when $[Cl]_o = 9{\cdot}7$ mM (using isethionate) the sensitivity to noradrenaline was decreased to 18% of the control value and in one experiment when the $[Cl]_o$ was reduced further (to 5·0 mM) by replacing KCl with K gluconate the sensitivity was decreased to 5% of the control value.

Replacement of NaCl with Na glucuronate produced results similar to those found with isethionate. Noradrenaline depolarized most of the cells tested but the sensitivity was greatly decreased; in normal Krebs solution, the sensitivity was $21{\cdot}7 \pm 2{\cdot}7$ mV/nC

($n = 11$) and in 119 mM-Na glucuronate, $3{\cdot}4 \pm 0{\cdot}79$ mV/nC ($n = 9$), representing an 84% reduction in sensitivity to noradrenaline.

An apparent reduction in the sensitivity to noradrenaline might result if the time course of the depolarizations was prolonged. This was not so in low $[Cl]_o$, as can be seen if Fig. 3*A* and *D* are compared. This conclusion is confirmed in Table 1 in which certain characteristics of the time course of noradrenaline-induced depolarizations in

TABLE 1. Characteristics of noradrenaline-induced depolarizations in normal Krebs and low Cl (isethionate substitution) solutions

	Amplitude (mV)	Sensitivity (mV/nC)	Latency (ms)	Rise time (ms)
Normal Krebs ($[Cl]_o = 128{\cdot}7$ mM)	$8{\cdot}03 \pm 0{\cdot}74$ ($n = 32$)	$26{\cdot}0 \pm 3{\cdot}6$	435 ± 24	461 ± 26
60 mM-isethionate ($[Cl]_o = 68{\cdot}7$ mM)	$4{\cdot}12 \pm 0{\cdot}72$ ($n = 19$)	$7{\cdot}4 \pm 1{\cdot}2$	444 ± 18	348 ± 20
119 mM-isethionate ($[Cl]_o = 9{\cdot}7$ mM)	$3{\cdot}60 \pm 0{\cdot}27$ ($n = 19$)	$4{\cdot}6 \pm 1{\cdot}4$	477 ± 30	291 ± 15

The figures are the mean ± S.E. of the mean.

TABLE 2. Comparison of the time course of noradrenaline-induced hyperpolarizations and depolarizations

	Amplitude (mV)	Latency (ms)	Growth time (ms)
Depolarizations ($n = 16$)	$2{\cdot}93 \pm 0{\cdot}27$	503 ± 42	389 ± 42
Hyperpolarizations ($n = 15$)	$2{\cdot}53 \pm 0{\cdot}30$	292 ± 29*	313 ± 33

The depolarizations were recorded from muscles bathed in normal Krebs solution and the hyperpolarizations were recorded from the same tissues bathed in 9·7 mM-Cl (benzenesulphonate or isethionate substitution).

* $P < 0{\cdot}001$.

normal Krebs solution and low $[Cl]_o$ are summarized. In 9·7 mM-external Cl when the sensitivity was reduced to 18% of the control value neither the latency (the time from the start of the ionophoretic pulse to the onset of depolarization) nor the rise time of the depolarization was increased. Thus it can be concluded that the reduced sensitivity in low Cl is not due to a prolongation of the depolarizations.

An interesting observation was that the noradrenaline-induced hyperpolarizations recorded in low $[Cl]_o$ had a shorter latency than occurred with depolarizations. In Table 2 I have calculated some characteristics of depolarizations recorded in normal Krebs solution and hyperpolarizations recorded from the same muscles but in low $[Cl]_o$. The mean latency of the hyperpolarizations was over 200 ms shorter than the equivalent value of the depolarizations but the growth time of both types of membrane response was similar. This unmasking of a rapid hyperpolarization is curious because in normal Krebs solution there is no sign of a hyperpolarization preceding a depolarization, although there is a hint of biphasic response on returning to normal Krebs solution after low $[Cl]_o$ (Fig. 1*E*). At present there is no obvious explanation for this phenomenon and it was not investigated further.

Effect of a reduction in $[Cl]_o$ on resting membrane potential and membrane resistance

The resting membrane potential (E_m) was slightly depolarized in low Cl solutions. For example in one series of experiments E_m in normal Krebs solution was $-59{\cdot}3 \pm 0{\cdot}6$ mV ($n = 39$) and in low (9·7 mM) $[Cl]_o$ $-50{\cdot}7 \pm 1{\cdot}4$ mV (benzenesulphonate, $n = 24$) or $-53{\cdot}2 \pm 1{\cdot}8$ mV (isethionate, $n = 19$). The extent of the depolarization in low Cl solutions is almost certainly underestimated in the above values because these means were calculated from cells in which the action of noradrenaline was investigated and there was a bias to select cells with a relatively high E_m. However, the decrease in the depolarizing ability of noradrenaline cannot be accounted for by a reduction in E_m as large depolarizations can be recorded in normal Krebs solution from cells with an E_m of about -30 mV and, in Krebs solution with no added Ca, noradrenaline produces depolarizations in cells which have an E_m of -20 mV (unpublished data). Equally some of the cells in low Cl solution in which noradrenaline evoked no membrane response or hyperpolarization had an E_m more negative than -60 mV.

Since noradrenaline depolarizes the rat anococcygeus by a mechanism associated with an increase in membrane conductance (Creed, 1975; W. A. Large, unpublished observations), a reduction in the resting membrane resistance (R_m) might decrease the depolarization produced by noradrenaline. In a few experiments R_m was estimated using the partition chamber method of Abe & Tomita (1968) and in low Cl solutions the current–voltage relationship was similar to that obtained in normal Krebs solution which suggests that R_m is not altered in low Cl solution. Another method of estimating R_m is to measure the hyperpolarization produced by activation of the electrogenic Na pump, e.g. by readmitting K to the solution bathing muscles which have been exposed to K-free saline; in this case, the amplitude of the observed hyperpolarization is proportional to the membrane resistance (see Aickin & Brading, 1983) and similar experiments were carried out in the present study. Muscles were exposed to K-free Krebs solution for 15–30 min and readmission of K hyperpolarized the membrane by $19{\cdot}4 \pm 2{\cdot}7$ mV ($n = 7$) in normal Krebs solution compared to $19{\cdot}2 \pm 2{\cdot}8$ mV ($n = 6$) observed in muscles bathed in solution in which NaCl was replaced with Na benzenesulphonate. This result also suggests that R_m is not altered in Cl-deficient solution and this agrees with the finding of Aickin & Brading (1983) who studied the effect of low Cl solution on guinea-pig vas deferens. The anion substitutes used in the present experiments are known to chelate Ca so it is important to investigate whether the reduced depolarizations to noradrenaline are due to low Ca concentration. In Krebs solution with no added Ca the resting membrane potentials were depolarized to less than -30 mV and it was difficult to maintain impalements but in a few cells with a stable E_m application of noradrenaline did depolarize the membrane. Thus, it seems unlikely that the reduced responses in low Cl solution can be attributed to simply a decrease in the Ca concentration.

Contractions produced by noradrenaline in low Cl solutions

A surprising observation in the electrophysiological experiments was that the ionophoretic application of noradrenaline always produced contraction even on those occasions when an hyperpolarization was recorded with the intracellular microelectrode and thus a systematic study of noradrenaline-induced contractions was

made. Contractions produced by two concentrations of noradrenaline in normal Krebs and low Cl solution are illustrated in Fig. 4. The most obvious feature is that the maximum tension achieved by both concentrations of noradrenaline is unaltered when NaCl is replaced by Na benzenesulphonate. This result is confirmed in Fig. 5*A* where the full dose–response relationship in normal Krebs and low Cl solution ($[Cl]_o$ = 9·7 mM using benzenesulphonate as the substitute anion) is illustrated. The

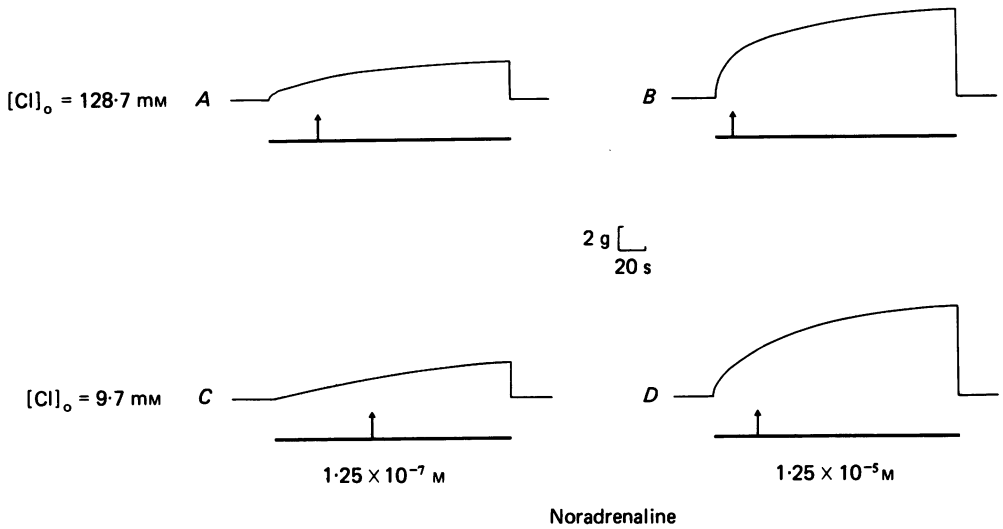

Fig. 4. Contractions produced by two concentrations of noradrenaline in normal Krebs solution (*A* and *B*) and after NaCl was replaced by Na benzenesulphonate (*C* and *D*). Noradrenaline was present for the time represented by the horizontal bars and the vertical arrows indicate the time to half-peak contraction. Note that $t_{\frac{1}{2}p}$ is greater in low Cl solution.

threshold noradrenaline concentration for contraction was not increased and the maximum tension produced by any noradrenaline concentration in low $[Cl]_o$ was not significantly different from the values measured in normal Krebs solution.

However, the time course of the noradrenaline-induced contraction was altered in low $[Cl]_o$. In normal Krebs solution the contraction was biphasic and consisted of an initial fast response followed by a slower increase in tension (Fig. 4). In a $[Cl]_o$ of 9·7 mM the initial rapid contraction was abolished or diminished whereas the relatively slow phase remains unaltered (this effect is even more pronounced with phenylephrine, see Fig. 7). This effect is reflected in the measurement of the over-all time to half-peak contraction ($t_{\frac{1}{2}p}$). In normal Krebs solution an increase in the noradrenaline concentration decreases $t_{\frac{1}{2}p}$; this generalization was true in low $[Cl]_o$ but for any noradrenaline concentration $t_{\frac{1}{2}p}$ was greater in low $[Cl]_o$ compared to normal Krebs solution by a mean value of 1·95.

Similar results on the amplitude and time course of contractions elicited by noradrenaline were obtained if isethionate rather than benzenesulphonate was used as the replacement anion.

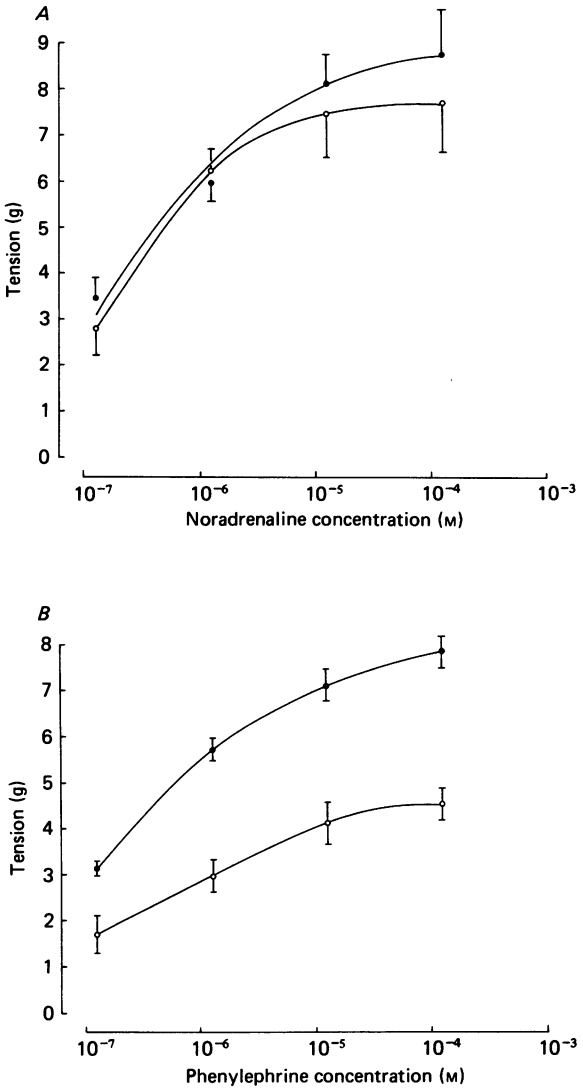

Fig. 5. Dose–response relationship of agonist-induced contractions for noradrenaline (*A*) and phenylephrine (*B*) in normal Krebs solution (●) and low Cl (benzenesulphonate) solution (○). Each point is the mean from six muscles.

Effect of low $[Cl]_o$ *on the electrical and mechanical responses evoked by phenylephrine*

It was of interest to investigate the effect of reducing $[Cl]_o$ on the responses produced by other α-receptor agonists and experiments were carried out using phenylephrine. The ionophoretic application of phenylephrine readily depolarizes the smooth muscle in the rat anococcygeus (Fig. 6*A* and *B*) and replacement of NaCl with Na benzenesulphonate markedly reduces the responses to phenylephrine and when all NaCl had been substituted no depolarizations could be obtained

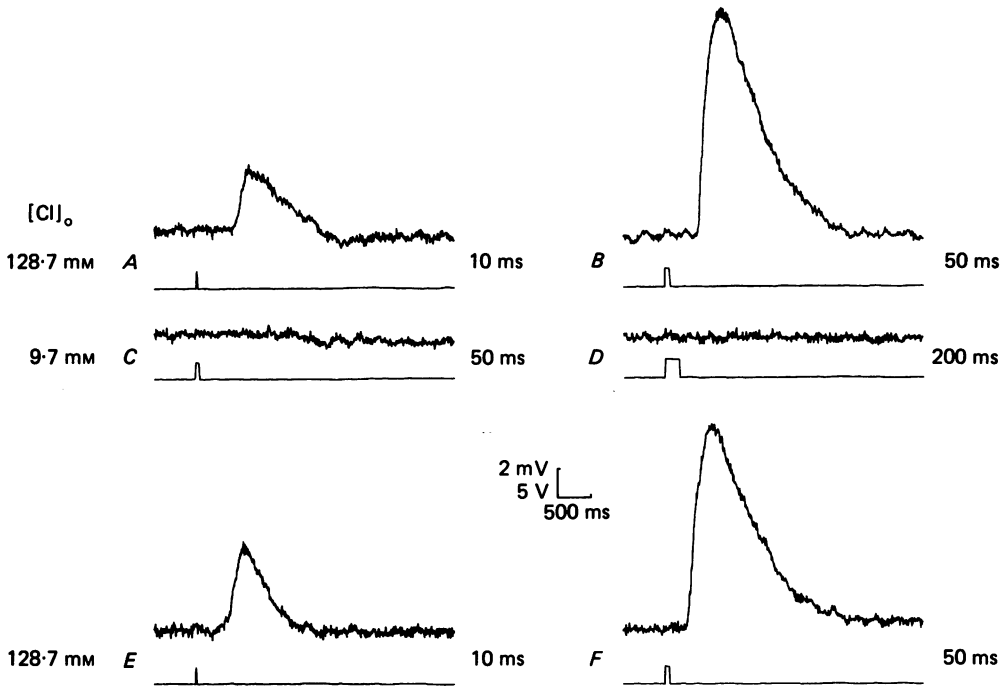

Fig. 6. Effect of replacing NaCl with Na benzenesulphonate on the depolarizations produced by the ionophoretic application of phenylephrine. These records are from three cells: *A* and *B* in normal Krebs solution, $E_m = -64$ mV; *C* and *D* 47 min after replacing NaCl with Na benzenesulphonate, $E_m = -60$ mV; and *E* and *F* after 30 min wash in normal Krebs solution, $E_m = -59$ mV.

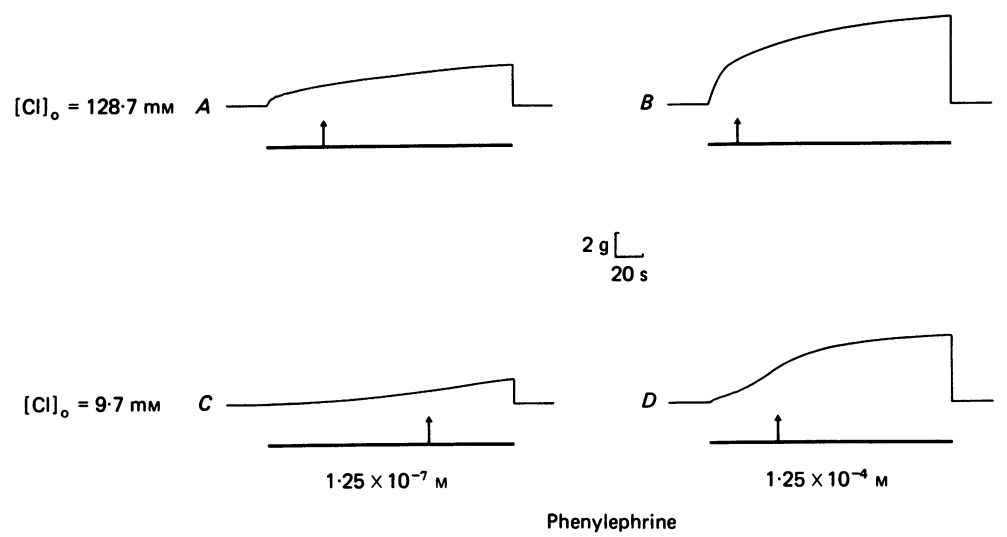

Fig. 7. Contractions produced by two concentrations of phenylephrine in normal Krebs solution (*A* and *B*) and after NaCl was replaced by Na benzenesulphonate (*C* and *D*).

(Fig. 6*C* and *D*). These effects were easily reversible and phenylephrine produced large depolarizations on returning to normal Krebs solution (Fig. 6*E* and *F*).

Contractions evoked by phenylephrine are more obviously biphasic than those elicited by noradrenaline (e.g. see Fig. 7*B*) and reduction of $[Cl]_0$ effectively eliminated the initial fast contraction (compare Fig. 7*C* and *D* with *A* and *B*) leaving the slow component which resulted in an increase of $t_{\frac{1}{2}p}$ (the mean increase was 2·12 times). The effect of low $[Cl]_0$ on the initial rapid phase of contraction is illustrated

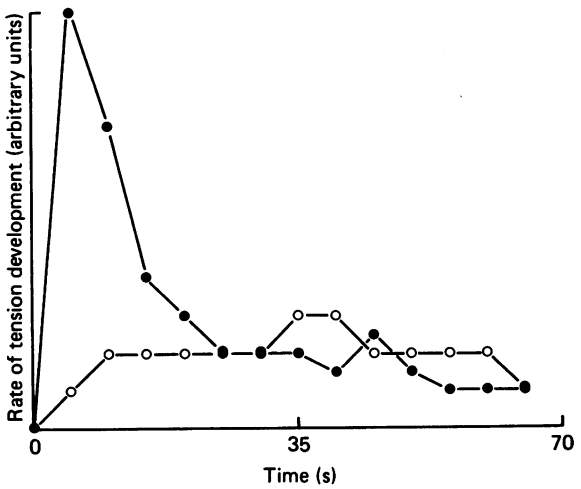

Fig. 8. Rate of tension development of contractions from Fig. 7*B* (●) and 7*D* (○) plotted against time. The rate of tension increase was calculated at 5 s intervals for the first 65 s of the contractions. The vertical scale is in arbitrary units but the peak value represents 1·7 g/5 s.

better in Fig. 8 where the rate of tension development is plotted against time for contractions shown in Fig. 7*B* and *D*. In normal Krebs solution the rate of tension increase reached a peak about 5 s after the onset of contraction and decreased to about 20% of this maximum value in 25 s. In Cl-deficient solution the maximum rate of contraction was only 20–30% of the maximum value in normal Krebs solution. In contrast to noradrenaline the maximum tension produced by all concentrations of phenylephrine was depressed significantly (Fig. 5*B*).

DISCUSSION

The present data provide further evidence that noradrenaline can initiate contraction by a mechanism which is not dependent on a change in membrane potential. Recently it has been shown that some α-adrenoceptor agonists (e.g. naphazoline) contract the mouse anococcygeus muscle by acting on α_1-adrenoceptors (Gibson & Yu, 1983) but do not depolarize this tissue very readily (Large, 1983). In contrast noradrenaline and phenylephrine produce large depolarizations (and contraction) and two subclasses of α_1-adrenoceptor were postulated; stimulation of one type leads to

depolarization and contraction and activation of the other class leads to contraction that is not mediated by a membrane potential change (Large, 1983). Unpublished experiments with different agonists show the rat and mouse anococcygeus to be similar in this respect. In normal Krebs solution the contractions evoked by noradrenaline and phenylephrine are biphasic and in low $[Cl]_o$ the initial fast component is reduced or abolished with little or no effect on the slow phase. It is tempting to speculate that the biphasic nature of the contractions reflect the depolarizing and non-depolarizing mechanisms of eliciting contraction in this tissue, especially as naphazoline (which is not as good as noradrenaline in depolarizing the muscle) produces a slow monophasic contraction (W. A. Large, unpublished observations).

Interpretation of the present results with respect to the ionic mechanism of the noradrenaline-induced depolarization is difficult. The results presented here are similar qualitatively to the findings of Bülbring & Szurszewski (1974) who concluded that noradrenaline increases Cl permeability in guinea-pig myometrium. However, Aickin & Brading (1982, 1983) have demonstrated in the guinea-pig vas deferens that although reducing $[Cl]_o$ decreases the intracellular Cl concentration the Cl equilibrium potential (E_{Cl}) remains at a more depolarized value than E_m. In fact if the same situation occurs in the anococcygeus, replacement of NaCl would change E_{Cl} from about -20 mV in normal Krebs solution to about $+20$ mV in low Cl solution, i.e. the driving force for Cl would increase from about 40 to 70 mV. Thus, if noradrenaline increases Cl permeability in the anococcygeus it might be expected that noradrenaline would elicit large depolarizations in low $[Cl]_o$, especially at short times (say within 10 min) after changing to low Cl solution, but these were not observed. In fact the speed with which the depolarizations were reduced in amplitude suggest that the anions used have actions other than being simple substitutes and therefore it is probable that these compounds have some sort of blocking action. It seems unlikely that the anions inhibit the binding of noradrenaline to the α-adrenoceptor as large contractions were observed but it is possible that they block ion channels opened by the action of noradrenaline. It is interesting that in the guinea-pig vas deferens replacement of Cl with benzenesulphonate increases the depolarization produced by noradrenaline (Magaribuchi, Ito & Kuriyama, 1971) which suggests a different ionic mechanism for noradrenaline in that tissue compared to the anococcygeus. Also it cannot be ruled out that other factors such as intracellular alkalinization (Aickin & Brading, 1983) which result from removal of extracellular Cl might inhibit the noradrenaline-induced depolarizations.

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