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THE CONTRACTURE PRODUCED BY SODIUM REMOVAL IN THE NON-PREGNANT RAT MYOMETRIUM

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SUMMARY

1. Mechanical responses to Na removal were investigated in the circular and longitudinal muscles of the non-pregnant rat myometrium at 35 °C. In both muscles, reduction of the external Na concentration to less than 20 mM produced an initial acceleration of phasic contractions and a sustained tonic contracture. No difference was found with different Na substitutes (Tris-hydroxymethyl aminomethane, choline, dimethyl diethanol ammonium). However, when Mg was substituted for Na, only the tonic contracture was produced without the phasic contractions.

2. Readmission of 5–10 mM-Na, after exposure to Na-free solution, relaxed the contracture produced by Na removal. The degree of relaxation was dependent on the Na concentration readmitted and on the period of pre-treatment with Na-free solution, being stronger with longer pre-treatment.

3. In the presence of Na, excess Ca failed to increase the muscle tone. In the absence of Na, the tension development was closely related to the external Ca concentration up to 20 mM. In the absence of both Ca and Na, some tension remained. Even after pre-treatment with Ca-free solution containing 0·1–0·5 mM EGTA, removal of Na caused some mechanical response. A similar small tension development was observed when Na removal was repeated during prolonged absence of external Ca for more than 3 h.

4. Verapamil (2×10^{-4} M) markedly suppressed the response to Na removal, but it did not block it, either in the presence or in the absence of Ca. Ouabain (10^{-3} M) in the presence of verapamil potentiated the early phasic component of the response to Na removal, but the tonic component was little affected or even slightly reduced.

5. The results indicate that there are three components in the mechanical response to Na removal: the phasic and tonic components, which are highly Ca-dependent, and the third small tonic component, which is independent of external Ca. Most of the phasic and tonic responses seem to be due to an increase in Ca permeability, but this may be secondary to membrane depolarization. A Na–Ca exchange mechanism is also considered to contribute to the transient phase of the response to Na removal and to Na readmission.

INTRODUCTION

It has been reported that reduction of the external Na concentration ($[Na]_o$) produces an increase in tension in the longitudinal muscle of the myometrium of the rat (parturient or 21 days pregnant) (Marshall, 1963) and of the mouse (near term) (Osa, 1971, 1973). In the mouse a large depolarization (more than 20 mV) is observed in a solution containing less than 16·8 mM-Na (Tris substitution), while in the rat, little change in membrane potential is noticed in 7·1 mM-Na (Li substitution). The membrane depolarization and the contracture produced by Na removal have also been reported for the guinea-pig stomach muscle (Ohba, Sakamoto & Tomita, 1977; Sakamoto & Tomita, 1982). Since the tension development caused by Na removal in the mouse myometrium is abolished by removing the external Ca (Osa, 1971) or by adding Mn (1·2 mM) (Osa, 1973), an increase in Ca influx is considered to be responsible for the contraction in Na-deficient solution.

The contraction in Na-deficient solution can also be explained by a Na–Ca exchange mechanism, originally proposed for the regulation of intracellular Ca in cardiac muscle (Reuter & Seitz, 1968) and squid axon (Baker, Blaustein, Hodgkin & Steinhardt, 1969). The possibility that Na–Ca exchange may be involved in the tension development in myometrium in Na-deficient solution has also been considered (Osa, 1971; Angles d'Auriac & Worcel, 1976; Ma & Bose, 1977), as it has in other smooth muscles (cf. Brading, 1981; Van Breeman, Aaronson & Loutzenhiser, 1979) including stomach muscle (Sakamoto & Tomita, 1982).

However, no careful studies on the effects of Na removal on tension development have been done in the myometrium, which is one of the most sensitive smooth muscles in producing the mechanical response on Na removal. In the present experiments, the myometrium of non-pregnant rats was used and the responses of the longitudinal and circular muscles were compared. It was found that there are at least two different components in the tension development in Na-deficient solution, one being dependent on, and the other independent of, extracellular Ca.

METHODS

Non-pregnant Wistar rats (200–250 g) were stunned and bled. After carefully removing the endometrium, muscle strips, about 1 mm wide and 7 mm long, were cut in the longitudinal or in the circular direction of the uterine horn, and the circular or longitudinal muscle layer, respectively, was removed as completely as possible under a dissection microscope. The hormonal state was checked by examining the vaginal smear. However, in the present experiments, no difference in the effects of Na removal was detected between oestrus and dioestrus stages.

The longitudinal and circular muscle strips were mounted in the same organ bath (1 ml in volume), through which solution flowed at a constant rate of 1·5 ml/min. The tension was isometrically recorded using a strain gauge and a potentiometric pen-recorder. The preparations were equilibrated in normal Krebs solution for at least 30 min and the resting tension was adjusted to 0·05 g in Ca-free solution. The final size of the preparations in the organ bath was about 700 μm in diameter and about 8 mm in length. Normal Krebs solution was applied for a further 30 min before a test solution was applied. The normal Krebs solution had the following composition (mM): NaCl, 121·5; $KHCO_3$, 5·9; $CaCl_2$, 2·4; $MgCl_2$, 1·2; glucose, 11·8; Tris-hydroxymethyl aminomethane (Tris) 15·3; pH was adjusted to 7·4 at 35 °C by HCl. When the concentrations of NaCl and $CaCl_2$ were changed, they were replaced usually with Tris on an equiosmolar basis. In some experiments, choline, dimethyl diethanol ammonium (DDA) or Mg was also used for Na substitution. When Mg was used, the Cl

concentration was kept constant by partial replacement with sucrose (66·6 mM), the Mg concentration being 62 mM. For complete Ca removal, 0·1–0·5 mM-EGTA was added to Ca-free solution, Ca contamination of which was found to be $0·5–1·5 \times 10^{-6}$ M when determined by atomic absorption analysis. The experiments were carried out at 35 °C.

RESULTS

Effects of Na removal

Both circular and longitudinal muscle strips of the non-pregnant rat myometrium produced spontaneous contractions. Although the frequency and duration of the phasic contractions varied from preparation to preparation, there was a general tendency that, in preparations of similar size, the force of tension of the circular muscle was less than that of the longitudinal muscle.

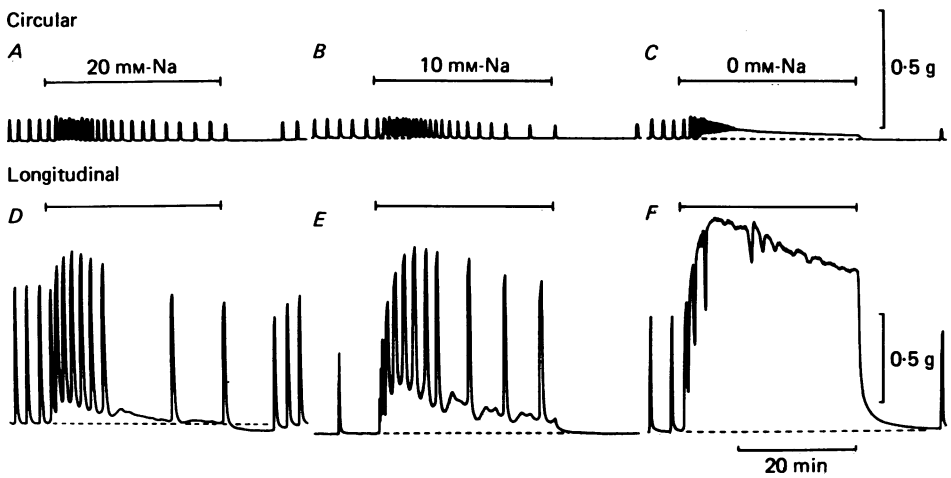

Fig. 1. Effects of reducing the external Na concentration on the mechanical activity of non-pregnant rat myometrium. Simultaneous records of isometric tension from circular (upper) and longitudinal (lower) muscles mounted in the same organ bath. (This also applies to all following Figures.) Na was isosmotically replaced with Tris leaving 20 mM (*A*, *D*), 10 mM (*B*, *E*) or 0 mM (*C*, *F*) solution for 30 min, as indicated by horizontal bars on top of the records. The interval between each response is 30 min.

When the external Na concentration ($[Na]_o$) was reduced to 80 mM by replacing it with Tris, no effect was observed, but in 40 mM-Na the frequency of spontaneous activity was slightly and transiently increased without increase in resting tone. When $[Na]_o$ was decreased below 20 mM, the mechanical activity was clearly accelerated.

Fig. 1 shows that the size of spontaneous contractions of the circular muscle was smaller than that of the longitudinal muscle. In the circular muscle reduction of $[Na]_o$ initially increased, but subsequently gradually reduced, the amplitude of phasic contractions which, at high frequency, fused into a relatively small tonic contraction. The peak tension produced in response to Na removal was only slightly (about 20 %) larger than that of the original spontaneous contractions, but declined and slowed during continued exposure to low $[Na]_o$. In the complete absence of Na, spontaneous contractions ceased within 10 min and only a tonic contracture remained. In some

preparations, phasic contractions continued for more than 20 min, superimposed on the raised muscle tone, although this was not fully maintained.

In the longitudinal muscle the increase in peak tension caused by Na removal was more marked than in the circular muscle, the increase being 50–100 %. The maximum tension was reached 5–10 min after reduction of $[Na]_o$ and then the phasic contractions gradually decreased in size and in frequency. However, this decline of the phasic

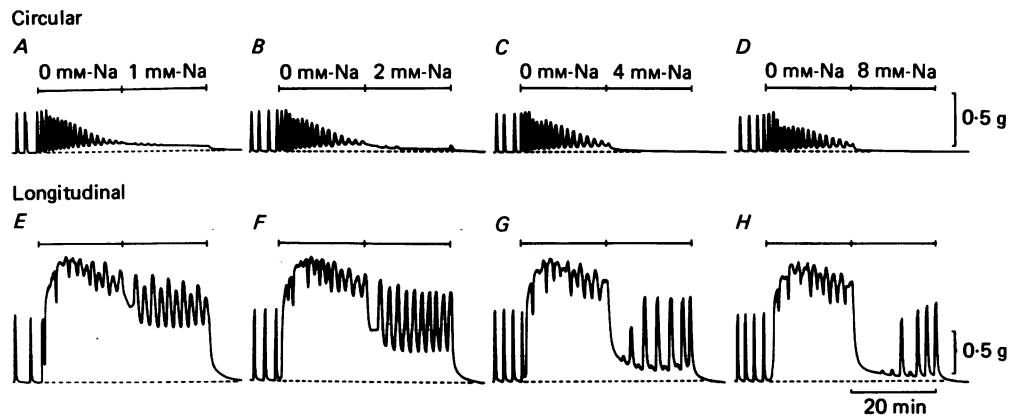

Fig. 2. Effects of readmission of low concentrations of Na to Na-free (Tris) solution. Following exposure to Na-free solution for 20 min, Na (1–8 mM) was added, also for 20 min, and then the solution changed to normal Krebs solution for 40 min before the next Na removal. For explanation see text.

contractions was slow and with reduction to 10 mM-Na a tonic contracture developed which was maintained. The relatively slow time course of the effects does not seem to be due to slow exchange of ions in the extacellular space, because the contracture produced by excess K or by Na removal in the presence of verapamil reaches a peak within 2 min (see Fig. 7). During exposure to Na-free solution for more than 30 min, phasic activity was usually detectable during the early phase, followed by irregular fluctuations of tension superimposed on a large elevated tonic contracture.

The response to Na removal was essentially the same when substitutes other than Tris, such as choline or DDA, were used. When Na was replaced by Mg, the phasic response was not observed and only a smooth increase in tonic tension was produced. The effect of Na removal was not affected by atropine (3×10^{-6} M), tetrodotoxin (10^{-6} M) or phentolamine (2×10^{-6} M).

The response to Na removal usually became greater when it was repeated at 30–40 min intervals for up to three trials, after which it remained roughly the same. Therefore, in the following experiments to be described, observations were made after the response to the third application of Na-free solution had been recorded.

Effects of Na readmission

When the $[Na]_o$ was returned to normal, the muscle was quickly and completely relaxed. The duration of the quiescent period was proportional to the degree of $[Na]_o$ reduction and to the time of exposure to a low concentration of Na. Fig. 2 shows effects of readmission of 1, 2, 4 and 8 mM-Na after complete removal of Na for 20 min. The

tonic part of the response was concentration-dependently suppressed by Na, although the effect of readmitting 1 mM-Na was scarely detectable in the circular muscle, due to the small magnitude of sustained tension. The phasic contractions of the longitudinal muscle were decreased in frequency and increased in magnitude since the sustained tension was decreased by increasing the $[Na]_o$ readmitted. There was a transient suppression of the phasic contractions just after readmission of Na and

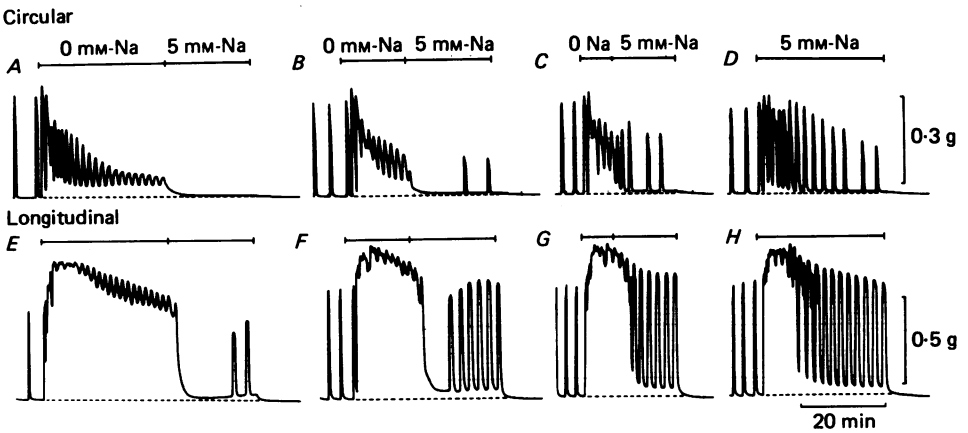

Fig. 3. Effects of readmission of 5 mM-Na following exposure to Na-free (Tris) solution for different periods. The Na was readmitted 30 min (*A*, *E*), 15 min (*B*, *F*) and 7·5 min (*C*, *G*) after its removal, as indicated by horizontal bars on top of each record. In *D* and *H* reduction of Na from normal (121·5 mM) to 5 mM is shown. The interval between each response is 40 min. For further explanation see text.

this was stronger with higher Na concentration. In the preparation shown in Fig. 2, some tonic tension still remained in the longitudinal muscle following application of 8 mM-Na, but in other preparations it was abolished by Na at this concentration.

The suppression of the spontaneous phasic contractions by Na readmission depended not only on the Na concentration readmitted but also on the duration of the preceding exposure to Na-free solution, as shown in Fig. 3. Reducing the Na concentration from 121·5 mM to 5 mM caused a large rise in tension, as expected from the results shown in Fig. 1 and also illustrated in Fig. 3 (*D* and *H*). However, the same Na concentration (5 mM) given after exposure to Na-free solution inhibited the mechanical response. A strong suppression of the phasic contractions was produced by 5 mM-Na following 30 min of exposure to Na-free solution (*A* and *E*), but the suppression became weaker when exposure to Na-free solution was shortened. Similarly, the relaxation of the tonic contracture by Na readmission was also stronger with longer pre-treatment with 0 mM-Na solution. After a short exposure to Na-free solution the relaxation produced by 5 mM-Na was incomplete.

Responses to Ca removal and readmission

When Na and Ca were simultaneously removed, the phasic activity quickly disappeared, but a small sustained tension usually remained (Fig. 4). This remaining tension in Na-free and Ca-free solution will be described later. Readmission of only Ca produced an increase in tension whose amplitude was dependent on the Ca

concentration. The response was very much clearer in the longitudinal than in the circular muscle, particularly when the Ca concentration was less than 2 mM. When the same Ca concentration was repeatedly applied in Na-free solution, the response became gradually smaller. Consequently, the Ca concentration-dependence of the response is presumably stronger than that shown in Fig. 4. The rate of relaxation when Ca was again removed varied in different preparations, but it always became

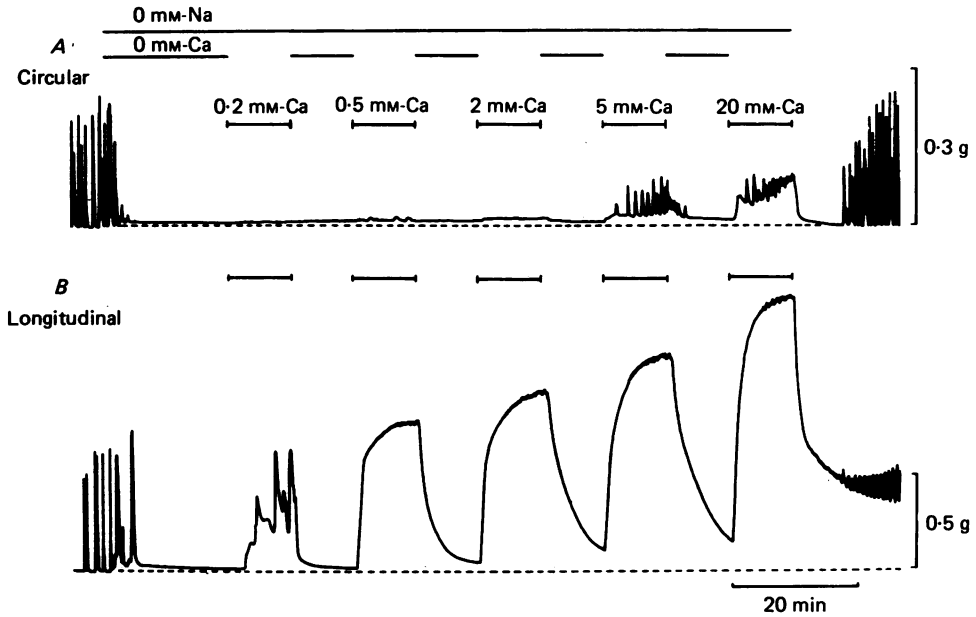

Fig. 4. Effects of removing Na and Ca simultaneously, and responses to Ca readmission in the continued absence of Na. The first solution containing 0 mM-Na (Tris), 0 mM-Ca, 0·1 mM-EGTA was applied for 20 min, and then different Ca concentrations were readmitted for 10 min at intervals of 10 min, as indicated on top of the records. Note that some tension was maintained in the absence of Ca.

slower with longer exposure to Na-free solution and with higher concentrations of Ca readmitted.

In the circular muscle, the response to Ca in Na-free solution was weaker during dioestrus and it increased during oestrus. However, no clear difference was found in the longitudinal muscle between oestrus and dioestrus periods.

The response to readmission of Ca was also studied in the presence of 95 mM-Na. When Ca concentration was increased, the osmolarity was adjusted by changing the Tris concentration, keeping the Na concentration constant. In the solution containing 95 mM-Na, removal of Ca caused complete relaxation, and readmission of 2–5 mM-Ca produced only the phasic contractions, the pattern of which was indistinguishable from that of spontaneous contractions in normal solutions. When Ca concentration was increased to 20 mM, the size of phasic contractions was suppressed.

Responses to Na removal in the absence of Ca

Since some tension remained in Ca-free and Na-free solution, as shown in Fig. 4, the effects of Na removal were further studied following pre-incubation in Ca-free solution. As shown in Fig. 5, after observing the control response to Na-free solution containing the normal Ca concentration, the preparation was first exposed to Ca-free Krebs solution for various periods (1–20 min) and then Na was also removed. This

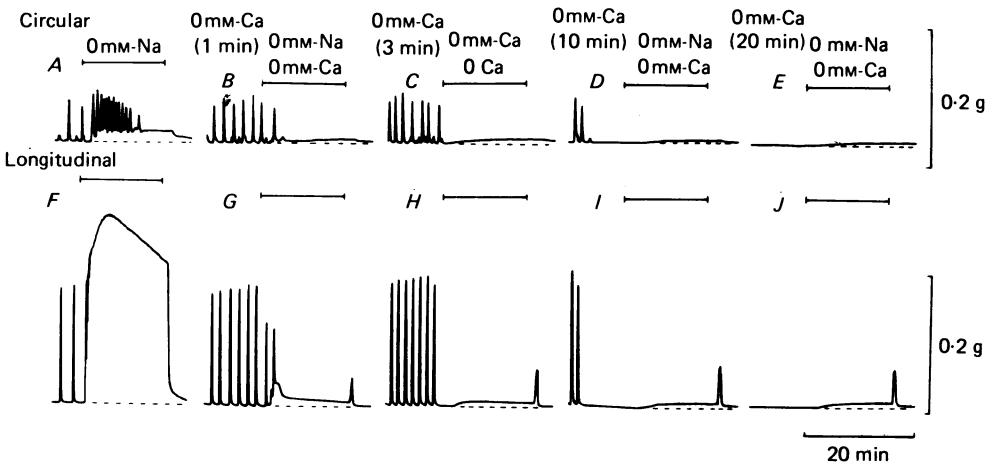

Fig. 5. Responses to Na removal in the absence of Ca. The control responses in the presence of Ca are shown in *A* and *F*. Before removing Na (Tris substitution) for 15 min, the preparations were pre-incubated in Ca-free solution containing 0·1 mM-EGTA, for different lengths of time (1–20 min) as indicated in the upper part. The interval between records is 30 min.

caused every time a small but definite increase in tension. The magnitude of the tension response to Na removal was independent of the period of pre-treatment with Ca-free solution, and a small sustained tension persisted throughout the absence of Na. The response was clearly observed even if the preparation had been previously exposed to Ca-free solution for more than 10 min, and had been completely relaxed. This response produced by Na removal in Ca-free solution was not affected when EGTA was increased from 0·1 to 0·5 mM.

When Na removal was repeated at intervals of 30 min in the continuous absence of Ca, the small steady-state tension of successive responses was very similar to that of the first response.

Effects of verapamil

Since the tension developed after removing Na in Ca-free solution, the response to Na removal was also studied in the presence of verapamil or D-600, which are supposed to block the influx of Ca (Fleckenstein, Grün, Tritthart & Byon, 1971; Mironneau, 1973; Vassort, 1975). Fig. 6 shows the effects of verapamil on the contracture produced by Na removal. The preparations were pre-incubated for 15 min in the solution containing verapamil before Na was removed in the presence of

verapamil. Spontaneous activity in the longitudinal muscle was more susceptible to verapamil, but the maximum inhibition of the Na-free contracture was achieved at a concentration of about 2×10^{-4} M for both circular and longitudinal muscle. The Na-free contracture was not completely suppressed even when verapamil concentration was increased to more than 2×10^{-4} M. Effects of verapamil on the contracture produced by excess K solution (87·4 mM) containing 40 mM-Na were also observed.

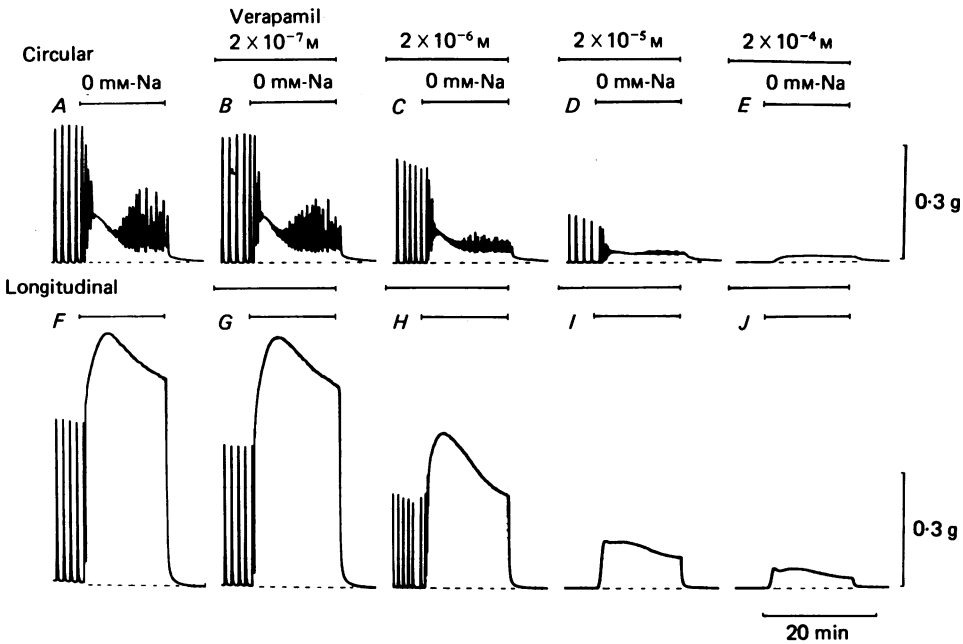

Fig. 6. Effects of verapamil (2×10^{-7} M to 2×10^{-4} M) on responses to Na removal. The preparations were pre-treated with verapamil at each concentration for 15 min and then Na was replaced by Tris in the presence of verapamil of the same concentration, as indicated on top of the records. Removal of Na was followed by normal Krebs solution without verapamil for 15 min.

In the preparations shown in Fig 7, the K contracture consisted of an early phasic and a late tonic component (*A*) and the tension development was smaller than that produced by Na removal (*B*).

In the presence of verapamil (2×10^{-4} M) the spontaneous activity was abolished, but application of excess K and removal of Na both produced mechanical response. The response to excess K was smaller than that to Na removal also in the presence of verapamil, although they were both significantly reduced compared with the control. The contracture produced by excess K was completely abolished by removing the external Ca. On the other hand, when Ca was removed during the contracture caused by Na removal in the presence of verapamil (*D* and *I* in Fig. 7), the tension decreased slightly, particularly in the longitudinal muscle, but it was not abolished. Moreover, the mechanical response was clearly produced again by removal of both Na and Ca in the presence of verapamil, and addition of Ca (2·4 mM) to this solution now produced some increase in tension in the longitudinal muscle, but

scarcely affected the circular muscle. Thus, verapamil does not seem to block Ca influx completely, at least not in the longitudinal muscle at this concentration.

The magnitude and the pattern of the response to excess K, Na-free solution, or Na-free and Ca-free solution varied in different preparations, but the effects of verapamil on these responses were essentially the same in all preparations examined. The action of D-600 (a methoxy derivative of verapamil, at 2×10^{-5} M) was similar to that of verapamil.

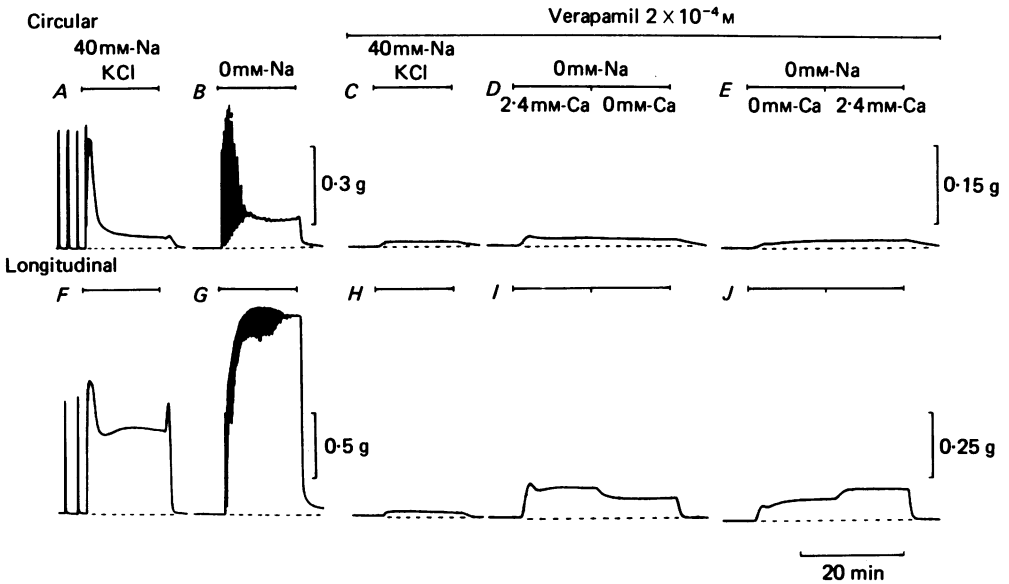

Fig. 7. Effects of verapamil on mechanical responses produced by excess K (87·4 mM) and Na removal (Tris substitution). After control responses to K (*A*, *F*) and to Na-free solution (*B*, *G*) had been observed, verapamil (2×10^{-4} M) was applied and the responses to K and to Na-free solution observed at 30 min intervals in the continuous presence of verapamil (*C*, *H* and *D*, *I*). In *D* and *I*, Ca was removed from the Na-free solution and in *E* and *J* Ca was readmitted after first producing the response to Na-free solution in the absence of Ca.

Effects of ouabain

Treatment with ouabain (10^{-4}–10^{-3} M) slighly potentiated the early phase of the response to Na removal from normal Krebs solution. However, the effect was not so marked, particularly in the longitudinal muscle, due to the fact that the control response was already quite large.

Fig. 8 shows the effects of ouabain (10^{-3} M) on the response to Na removal in the presence of verapamil (2×10^{-5} M). After repeating Na removal twice at an interval of 40 min in the presence of verapamil, ouabain was added to the Krebs solution containing verapamil. This caused the gradual development of small spontaneous mechanical activity. Ouabain potentiated the early part of the tension response to Na removal, and in the longitudinal muscle this effect became larger with the time

of exposure to ouabain. The steady level of tonic tension response to Na removal was not potentiated but usually slightly reduced.

DISCUSSION

Both the circular and longitudinal muscles of the non-pregnant rat myometrium produce a contractile response on Na removal. The response is fundamentally the same when either Tris, choline, DDA or Mg is used for substitution of Na, suggesting

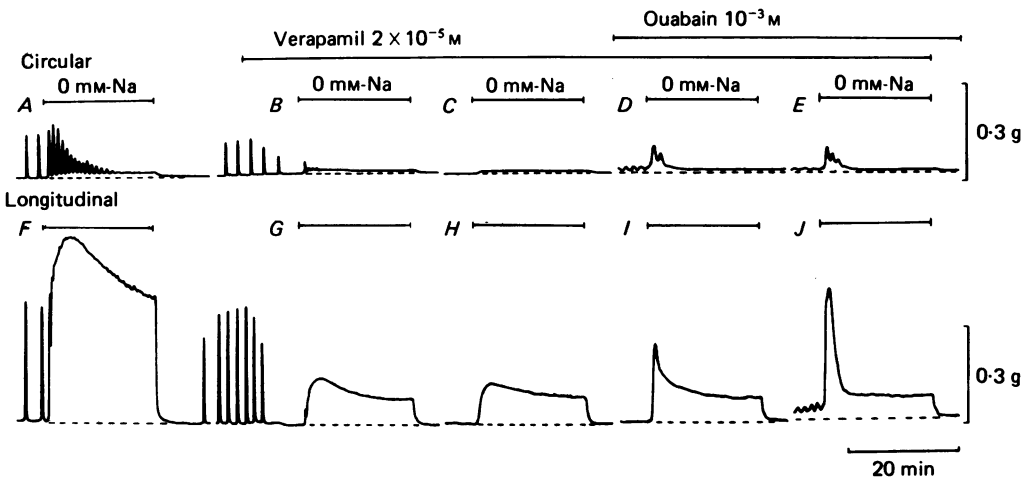

Fig. 8. Effects of verapamil, and of ouabain in the presence of verapamil. *A* and *F*: control responses to Na removal (Tris substitution). Verapamil (2×10^{-5} M) was applied 10 min before Na removal in *B* and *G* and ouabain (10^{-3} M) was applied 10 min before Na removal in *D* and *I*. The interval between Na removal was 30 min.

that the lack of Na is responsible. The tension development is smaller in the circular muscle than in the longitudinal muscle and there is a clear difference between them in the pattern of the response to Na removal. In the circular muscle the tonic contracture was much smaller than the phasic contractions, while in the longitudinal muscle the sustained increase in tension was dominant in Na-free solution. However, the reason for this has not been investigated in the present experiments.

In the myometrium of pregnant rat the sustained contraction produced by reduction of the external Na concentration (7·1–35·8 mM) is accompanied by a continuous discharge of action potential (Marshall, 1963), although depolarization of the membrane has not been noticed. In the pregnant mouse, exposure to 0–5 mM-Na causes membrane depolarization from −55·6 mV to between −25 and −35 mV, with continuous spike activity which is gradually reduced in amplitude and eventually disappears (Osa, 1971, 1973). A similar depolarization on Na removal has been reported for the circular muscle of the guinea-pig stomach (Ohba *et al.* 1977; Sakamoto & Tomita, 1982). Both the longitudinal and circular muscles of the non-pregnant myometrium of the rat, used in the present experiments, were also found to be depolarized and the depolarization was not prevented by verapamil

(2×10^{-4} M) (T. Masahashi & T. Tomita, unpublished observations). Continuous spike activity occurs during the depolarization in the longitudinal muscle, while spike activity often ceases after about 10 min in the circular muscle. It is likely that the phasic contractions and small fluctuation of tension in Na-free solution are related to the spike activity. In addition to spike activity, the sustained depolarization is probably responsible for the tonic contracture, particularly in the circular muscle.

It has been shown that the contraction produced by Na removal in the pregnant mouse myometrium is abolished by Ca removal and is inhibited by Mn, suggesting that an increase in Ca influx is responsible for the mechanical response (Osa, 1971, 1973). The tension development in Na-free solution observed in the rat myometrium is also strongly suppressed by removal of Ca and by Ca antagonists such as verapamil. It is, therefore, concluded that most of the contractile response is dependent on the presence of extracellular Ca. Moreover, when Ca is readmitted to Na-free solution, the tension increases concentration-dependently, while a similar readmission of Ca to Ca-free Krebs solution containing the high concentration (95 mM) of Na produces only a phasic contraction without significant increase in muscle tone. This may also indicate that the membrane is significantly permeable to Ca in the absence of Na.

Thus, the easiest explanation for the mechanical response produced by Na removal would be an increase in Ca conductance, as suggested by Osa (1971, 1973). However, the relationship between the depolarization of the membrane and the increase in Ca conductance in Na-deficient solution is not clear. Na removal may produce primarily an increase in Ca conductance, leading to the membrane depolarization. Alternatively, some other mechanism, such as changes in an electrogenic ionic transport, may depolarize the membrane and this may in turn increase the Ca conductance, as proposed for the circular muscle of the guinea-pig stomach (Sakamoto & Tomita, 1982). Since the depolarization due to Na-removal can be induced in the presence of verapamil, the second possibility seems more likely.

The relaxation produced by readmission of a small amount of Na to Na-free solution could be explained by the Na–Ca exchange mechanism (Reuter & Seitz, 1968; Baker *et al.* 1969; for smooth muscle, see Brading, 1981; Van Breeman *et al.* 1979), i.e. Ca being transported out of the cell by an influx of Na, since the relaxation seems to be dependent on the Na concentration gradient which drives Na into the cell. When the $[Na]_o$ is reduced from normal (121·5 mM) to 5 mM, a contraction is produced, while after exposure to Na-free solution for 30 min, readmission of 5 mM-Na completely abolishes the mechanical response. The time of 30 min is thought to be enough to deplete almost all free intracellular Na in the guinea-pig taenia coli (Brading, Burnett & Sneddon, 1980), and the degree of relaxation when 5 mM-Na is readmitted depends on the time of exposure to Na-free solution: the shorter the time the smaller is the suppression.

In the presence of verapamil, ouabain treatment markedly potentiates the early phasic contraction in response to Na removal. In order to demontrate the potentiating effect of ouabain it seems necessary to suppress with verapamil the large contraction due to spike activity. The ouabain effect can also be explained by a similar idea: i.e. an outflux of Na, intracellularly accumulated by ouabain, drives Ca into the cell. However, this response is only transient, probably due to quick reduction of intracellular Na concentration in Na-free solution, and the tonic response in Na-free

solution is not affected or even reduced by ouabain. Furthermore, in the absence of Na, a quick and large relaxation can be produced when Ca is removed. Thus, significant contribution of the Na–Ca exchange to the tonic contracture on Na removal is doubtful, although this mechanism may be involved in the initial transient phase of the resonse to Na removal and readmission.

It was found that in Ca-free solution or in the presence of verapamil, Na removal can still produce some mechanical response. The magnitude of this response remains nearly constant when Na removal is repeated in Ca-free solution containing 0·1–0·5 mM-EGTA, and it is independent of the duration of pre-treatment with Ca-free solution. It has been reported that in pregnant mouse myometrium (longitudinal muscle) the removal of Na does not produce any tension when Ca is also omitted (Osa, 1971). Similarly in the longitudinal muscle of the rat myometrium, the increase in tension observed in Ca-free and Na-free solution disappeared when the pregnancy reached the middle stage, although the Na-free contracture was still clearly produced at this stage (T. Masahashi & T. Tomita, unpublished observation). Thus, there seems to be no direct causal relationship between Ca-dependent and Ca-independent contracture.

It is considered that the contracture produced by Na removal is largely Ca-dependent, particularly in the longitudinal muscle, and that an increase in Ca conductance and Na–Ca exchange mechanism are both involved in the Ca-dependent contracture.

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