Effects of Na Removal and Readmission on the Mechanical Response in the Guinea-pig Tracheal Smooth Muscle

Minoru Kawanishi, Kenji Baba,* and Tadao Tomita**

Department of Anaesthesiology, *2nd Department of Internal Medicine, and **Department of Physiology, Nagoya University, Nagoya, Aichi, 466 Japan

Abstract In the guinea-pig tracheal smooth muscle, tonic contraction, maintained in normal medium, was abolished by Ca removal or by indomethacin (10⁻⁶ M). Removal of Na produced a transient contraction followed by a gradual decrease in tension to a level lower than the control. When Na was readmitted, a rapid relaxation was observed. The degree of the relaxation was enhanced with the exposure time to Na-free solution, and with the concentration of Na readmitted. At 5.9 mM K, the contraction produced by Ca readmission with simultaneous Na removal became smaller and slower as the external Na was reduced before Ca readmission, while at 40 mM K, the Ca-induced contraction was less dependent on the Na concentration. The rate of relaxation on Ca removal was slower when the Na concentration was reduced both at 5.9 and 40 mM K. Verapamil (10⁻⁵ M) had a weak suppressing effect in normal solution, but suppressed markedly in 40 mM K medium. In the presence of verapamil, the difference between the effects of Na removal and of Na readmission on Ca-induced contraction was markedly potentiated, particularly at 40 mM K. It was concluded that the Na-Ca exchange mechanism may contribute to the transient response on Na removal and to the relaxation phase following Na readmission. At 5.9 mM K, removal of Na may increase the Ca conductance and also reduce the contribution of prostaglandins. On the other hand, when depolarized by excess K, the effect of Na is less significant; probably due to a high Ca conductance.

Key Words: tracheal smooth muscle, Na removal, Na-Ca exchange, smooth muscle tone.

It has been reported that Na removal from the external medium has a marked influence on tone, tension development, relaxation, and responses to drugs in many types of smooth muscle (cf. Van Breemen et al., 1979), which suggests that
Na is involved in the regulation of Ca movement through the cell membrane. One of the mechanisms which affects the tension development when the Na concentration is modified may be a Na-Ca exchange process, originally proposed for the Ca flux in cardiac muscle (Reuter and Seitz, 1968) and squid axon (Baker et al., 1969). In this mechanism, the transport of Ca is coupled with that of Na in an opposite direction, and it is driven by a transmembrane gradient of Na concentration. There are many studies concerning the effects of changing the external Na concentration on the mechanical response in smooth muscles. However, the involvement of the Na-Ca exchange mechanism in Na-Ca interactions has been controversial (Van Breemen et al., 1979).

In the bovine tracheal smooth muscle it has been shown that contraction develops when the external Na concentration is reduced or when the intracellular Na is increased by ouabain or K-free solution, and that the presence of external Na is necessary for complete relaxation (Bullock et al., 1981). These observations are interpreted to support the Na-Ca exchange hypothesis presented for the vascular smooth muscle (Reuter et al., 1973). The guinea-pig tracheal smooth muscle has a sustained muscle tone which seems to be related to a continuous rhythmic electrical activity or a nearly fused state of the activity (Small, 1982). Since the effect of Na has not been investigated on this muscle, we have studied a possible Na-Ca interaction in the mechanical activity of the guinea-pig tracheal muscle in normal K (5.9 mM) and excess (40 mM) K solutions. The excess K medium was used to reduce changes in membrane potential with modification of the external Na concentration and to increase the mechanical response by high Ca permeability.

METHODS

Guinea-pigs (250–350 g) were stunned and bled. The trachea consisting of 36 to 38 tracheal rings was carefully removed. Two muscle strips of one ring width (about 1.5 mm wide) were isolated with the attached cartilage, one from an upper (between the 5th and 10th ring from the top) and the other from a lower part (between the 25th and 30th) of the trachea, and both strips were suspended in the same organ bath (capacity 1 ml), through which test solutions flowed continuously at a speed of 1.5 ml/min. The tension development of the two preparations was simultaneously, but independently, recorded isometrically with two strain gauges. The responses to Na removal were fundamentally the same in the two preparations taken from different regions of the trachea.

When spontaneous tension had gradually developed, isoprenaline (10⁻⁶ M) was applied to produce complete relaxation. During the relaxation, the strip was stretched to give a resting tension of 250 mg and then the isoprenaline was washed out. Following this procedure the preparation developed a resting tone of various degrees, which was stabilized at a more or less constant level within 1 hr.
before starting the experiments. The results were confirmed with at least three different preparations by repeating the same experiment.

The normal Krebs solution had the following composition (mM): NaCl, 137; KHCO₃, 5.9; CaCl₂, 2.4; MgCl₂, 1.2; glucose, 11.8. Choline was usually used as a Na substitute, and atropine sulphate (5×10⁻⁵M) was added to all the solutions throughout the experiments. In some experiments, Tris-hydroxymethyl aminomethane (Tris), or sucrose were used as Na substitutes. The ionic composition was isosmotically modified by supplementing it with an appropriate ion, as described in the text. When Ca was removed, 0.5 mM ethyleneglycol-bis-(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA) was added. The experiments were carried out at 35°C.

RESULTS

The tension gradually increased during the first 30 min after mounting the preparation in the organ bath and thereafter it was maintained at a more or less steady level, with some irregular fluctuations. The tonic contraction was abolished
by removing the extracellular Ca or by application of indomethacin (10^-6 M), a blocking agent of prostaglandin synthesis.

When the external Na was completely replaced with choline, Tris or sucrose, the tension was transiently increased and then decreased, at various rates, to a level lower than that before Na removal. Figure 1 shows typical examples of the responses to Na removal for about 2 hr with various Na substitutes. Substitution with Tris often produced a second sharp decline of tension 30–50 min after removal of Na, which was followed by a partial recovery, as shown in Fig. 1b. The reason for this phenomenon is not clear.

The muscles were relaxed completely on readmission of normal Krebs solution, and after more than 10 min the spontaneous tension gradually developed again.

Thus, although there were some differences in response to various substitutes for Na, the general pattern in Na-free solution was the same; following a transient contraction the tension decreased.

In the following experiments, the relaxation produced by Na readmission was more carefully investigated. When Na removal was shorter than 5 min, the initial contraction due to Na removal was immediately followed by the relaxing effects of Na (137 mM) readmission. As shown in Fig. 2, the degree of relaxation caused by Na readmission was increased by increasing the exposure time to 0 Na (choline substitute) solution, and the relaxation became complete after 40 min removal of Na. Although the amplitude of the relaxation produced by Na readmission was greater after longer removal of Na, the rate of relaxation on Na readmission was nearly the same.

Figure 3a shows effects of readmitting various concentrations of Na. After 60 min in 0 Na solution, Na was readmitted from 5 to 137 mM stepwise, each time for 30 min, alternately with a treatment of Na-free solution for 30 min.
application of Na, even at 5 mM, produced a large relaxation at 5.9 mM K (a), and a level of peak relaxation was concentration-dependently lowered with increasing Na concentrations. The tension started to increase in the solution containing Na, and this increase was greater and faster with higher Na concentrations. When Na was removed from the solution containing more than 20 mM Na, a transient increase in tension was produced before reduction of tension.

When the external K concentration was maintained at 40 mM, and Na concentrations were altered isosmotically by replacing with choline, the effect of Na readmission was different from that observed at 5.9 mM K. An example of such an experiment is shown in Fig. 3b. The tension was well maintained in high K, 0 Na (40 mM K, 103 mM choline) solution (b) compared with that in normal K, 0 Na (5.9 mM K, 137 mM choline) solution (a). In other similar experiments, it was shown that when 0 Na solution containing 40 mM K was applied for 2 hr without readmission of Na, the rate of reduction of the tension with time was small, and the tension at 2 hr was still higher than the control level.

The effect of Na readmission was weak at 5 mM, but marked relaxation was produced by Na of more than 10 mM, although no complete relaxation was produced in the depolarized preparation by 40 mM K. The recovery of tension during Na readmission was very poor, even in the solution containing more than

Fig. 3. Effects of readmission of various concentrations of Na at 5.9 mM (a) and 40 mM K (b) in two different preparations. After equilibrating in normal Krebs solution for about 1 hr, 137 mM Na was completely replaced with equimolar choline (a) or with 103 mM choline and 34.1 mM K, increasing K concentration to 40 mM (b). The first Na readmission was 5 mM after 60 min in 0 Na solution, then Na concentration was increased stepwise by substituting choline, as indicated. Between Na applications, the preparation was treated with 0 Na solution for 30 min. For further explanation see text.
The rate of relaxation clearly depended on the Na concentrations, both at normal and excess K medium.

The tension development produced by Ca readmission after exposure to Ca-free solution was also studied in relation to the external Na concentrations (Fig. 4). Following exposure to Ca-free Krebs solution for 30 min, 2.4 mM Ca was applied for 15 min with simultaneous removal of Na by completely replacing it with choline, as indicated at the beginning of the records. Na concentration in Ca-free solution was successively reduced before Ca readmission and Na removal. For further explanation see text.

When the Na removal preceded the Ca readmission, the contraction became smaller and slower with longer pretreatment with Na-free (choline substitute) solution in the presence of normal K concentration (5.9 mM) (Fig. 5a). On the other hand, in the presence of 40 mM K, pretreatment with Na-free solution had almost no effect on the maximum tension development, although the rate of con-
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Fig. 5. Effects of pretreatment with Na-free (choline) solution on the rate of contraction produced by Ca readmission at 5.9 mM (a) and 40 mM K (b). Preparations were treated in Ca-free solution containing 0.5 mM EGTA for 30 min, then 2.4 mM Ca was readmitted in the absence of Na. Before readmission of Ca, the preparation was treated with 0 Na solution for various durations (0, 5, 10, 20, 30 min) and the contraction produced by Ca was traced.

Fig. 6. Effects of Na removal (a, c) and Na readmission (b, d) on Ca-induced contraction at 5.9 mM (a, b) and 40 mM K (c, d). Following incubation for 30 min in Ca-free solution (0.5 mM EGTA), 2.4 mM Ca was readmitted with simultaneous removal of Na for 18 min. Twenty min later, Na was removed for 30 min and then Ca was applied again with simultaneous readmission of Na. (a) and (b) were obtained from the same, and (c) and (d) from another preparation. See text for further explanation.

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traction was slightly slowed with prolonged removal of Na (Fig. 5b). Thus, the effects of changing the exposure time to 0 Na solution before Ca application were similar to those of changing the Na concentrations in the preincubation medium.

In Fig. 6, Ca (2.4 mM) was readmitted with simultaneous removal of Na (a, c) or with simultaneous readmission of Na (b, d). The tension development by Ca readmission was similar when Na was removed by replacement with choline at 5.9 mM K (a), or at 40 mM K (c). Slight tension remained during the pretreatment with Ca-free, Na-free solution, and this tension disappeared by readmitting Na, even when Ca was also readmitted (b, d), before the tension started to increase. The duration of this mechanical suppression with Na was longer at 5.9 mM K (b) than at 40 mM K (d). When Na was readmitted simultaneously with Ca, the rate of tension development was markedly slowed (b, d) compared with that caused by Ca with simultaneous Na removal (a, c), although the final level of tension reached after 18 min was nearly the same under both conditions. When Na and Ca were simultaneously removed, there was a transient increase in tension (b, d), as observed with Na removal in the presence of Ca (Fig. 1).

Fig. 7. Effects of verapamil on Ca-induced contraction at 5.9 mM (open circle) and 40 mM K (filled circle). Ca (2.4 mM) was applied for 20 min at intervals of 25 min, during which Ca-free (0.5 mM EGTA) solution containing various concentrations of verapamil was superfused, and verapamil concentration was cumulatively increased. The curve was the average of 4 different experiments, expressed as percentage of the contraction induced by 2.4 mM Ca in the absence of verapamil. The standard deviation was within 10%.
When Ca was readmitted without modifying Na concentration either in the presence of Na or in the absence of Na, the rate of tension development was midway between those with simultaneous Na removal and Na readmission.

In order to find the mechanism for the difference between Ca-induced contractions in normal and excess K solutions, the effects of verapamil, a Ca-channel blocker, were studied. In the experiments shown in Fig. 7, Ca was readmitted for 20 min at 25 min intervals at 5.9 mM K and 40 mM K, and their peak tensions were plotted against verapamil concentrations. Verapamil up to $10^{-5}$ M had a
very weak effect at 5.9 mM K. On the other hand, in the presence of 40 mM K, the tension was markedly reduced by verapamil at a concentration higher than $10^{-6}$ M, although some tension still remained even with $10^{-5}$ M verapamil for 25 min. This suggests that the contraction in excess K solution is mainly due to an increase in Ca conductance and that a different mechanism may be responsible for the Ca-induced contraction in normal Krebs solution.

The increased susceptibility to verapamil in excess K solution was also demonstrated in the mechanical response induced by cumulative Ca readmission (Fig. 8). The concentration-tension curve for Ca was slightly shifted to the right by verapamil ($10^{-5}$ M) at 5.9 mM K (a). In the presence of 40 mM K (b), the tension response appeared at a lower concentration of Ca and the suppression of the Ca-induced contraction by verapamil ($10^{-5}$ M) was much greater compared with that at 5.9 mM K.

Figure 9 shows the same experiments as shown in Fig. 6, but in the presence of $10^{-5}$ M verapamil. The mechanical response produced by Ca at 5.9 mM K was not fundamentally different before and after treatment with verapamil, both with Na removal (a) and with Na readmission (b). On the other hand, verapamil suppressed the Ca-induced response more significantly when the K concentration was increased to 40 mM (c, d) from 5.9 mM (a, b), and the difference between the effects of Na removal (a, c) and Na readmission (b, d) on the Ca-induced contraction became much clearer. In the Na-free solution containing 40 mM K, the...
suppressing effect of Na was so strong that readmission of Na (103 mM) and Ca (2.4 mM) abolished the small remaining tension and no mechanical response appeared during the exposure to this solution for 18 min (d).

The transient contraction that appeared when Na and Ca were simultaneously removed (Fig. 6b, d) was blocked by verapamil only in excess K solution (Fig. 9d), but not in normal K solution (Fig. 9b).

DISCUSSION

It is known that removal of the external Na affects the tension development in many smooth muscles (cf. VAN BREEMEN et al., 1979). In general, the effect is to produce or increase the mechanical response, but the suppressing effect of complete removal of Na has also been reported. For example, in the guinea-pig taenia coli, the tonic phase of K contracture is smaller in the absence than in the presence of Na (KARAKI et al., 1982), and the carbachol-induced response is diminished in Na-free solution (BRADING et al., 1980).

In guinea-pig tracheal smooth muscle, which has a high resting tone in the normal Krebs solution, the effects of Na removal and Na readmission are complicated. In Na-free solution, the resting tension is gradually reduced following a transient increase. When Na is readmitted, marked relaxation is produced and then the tension slowly returns. Since the muscle tone is abolished by indomethacin (OREHEK et al., 1975), or by aspirin (ONO et al., 1979), prostaglandins are probably involved in the generation of the sustained tension in the normal medium. Thus, it is possible that the decrease in muscle tone in Na-free solution is a result of reduction of prostaglandin synthesis or of the responsiveness to prostaglandins. In the present experiments, however, this possibility has not been investigated.

Another factor which might be responsible for the decrease in muscle tone is metabolic disturbance caused by Na removal, as suggested for the K contracture in the guinea-pig taenia coli (KARAKI et al., 1982). However, since the tension in Na-free solution can be maintained at a level higher than in the normal Krebs solution when Na is completely replaced with choline in the presence of 40 mM K, the metabolic effect may not be a major factor in the guinea-pig tracheal muscle.

In some tissues such as cardiac muscle and squid giant axon, it has been demonstrated that the movement of Na across the plasma membrane is coupled with the opposite movement of Ca, and the existence of such a Na-Ca exchange mechanism has been considered in the vascular smooth muscles (cf. BLAUSTEIN, 1977). However, in most smooth muscles, strong evidence for this mechanism is still lacking (cf. VAN BREEMEN et al., 1979).

If the Na-Ca exchange process is involved in the response, one would expect potentiation of tension development by removal of the external Na, a gradual decline of tension as the intracellular Na concentration is reduced, and relaxation on readmission of Na, as observed in the present experiments. Furthermore, the
degree of relaxation caused by Na readmission in the preparation pretreated with Na-free solution is dependent on the duration of Na removal and on the concentration of Na readmitted. This response is very similar to that observed in the non-pregnant rat myometrium (Masahashi and Tomita, 1983). These observations suggest that an outward movement of Ca is driven by a Na-influx through the Na-Ca exchange process. The presence of the Na-Ca exchange mechanism has also been postulated in the bovine tracheal muscle in bicarbonate-buffered, but not in Tris-buffered, solution (Bullock et al., 1981; Kirkpatrick and McDougal, 1976).

On the other hand, when the external K concentration is increased to 40 mM, the interaction between Na and Ca becomes less significant. It is probable that under this condition the Na-Ca exchange may be masked by the increase in Ca conductance of the membrane. The possibility of an increase in Ca conductance is indicated by the strong suppressing effect of verapamil in the depolarized preparation in 40 mM K. Thus, in the excess K solution, it is possible that removal of Na increases the Ca conductance and readmission of Na reduces it, and that the effects are somehow related to the concentration gradient of Na. The tension maintained in Na-free solution is probably not due to the inability to relax due to lack of Na influx, but due to a sustained increase in Ca conductance, because relaxation can be produced at a reasonably fast rate by Ca removal in the absence of Na. However, since clearly different effects of Na removal and readmission on the Ca-induced contraction can be demonstrated in the presence of verapamil, the Na-Ca exchange mechanism may also be involved at least during the early phase of the response.

The importance of an increase in Ca conductance has been considered for the mechanical response to Na removal in the myometrium (Osa, 1971; Masahashi and Tomita, 1983). In the myometrium, Na removal in the normal Krebs solution produces a large depolarization, although a causal relationship between the depolarization and the increase in Ca conductance is not clear. In the guinea-pig tracheal muscle, the change in the membrane potential on Na removal needs to be investigated. However, since there is no fundamental difference in the presence and absence of verapamil at 5.9 mM K, depolarization of the membrane and the increase in Ca conductance may not be large factors in the Na-Ca interaction.

The weak effect of verapamil in the solution containing a normal concentration of K is likely due to the fact that under this condition prostaglandins and leukotrienes are responsible for the contraction, since a combination of indomethacin ($10^{-6}$ M), FPL 55712 (a leukotriene antagonist, $5 \times 10^{-6}$ M), and verapamil ($10^{-5}$ M) blocks the tension development on Na removal (unpublished observations). The mechanism of the contraction produced by prostaglandins and their related substances seems different from that produced by depolarization of the membrane with excess K, which is verapamil sensitive. Although an involvement of the Na-Ca exchange process in the mechanical response observed at

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5.9 mM K is suggested at least during the early phase of Na removal and Na re-admission, the relationship between the external Na or K concentration and the contribution of prostaglandins should be carefully investigated before drawing conclusions on the mechanism of the effect of Na removal.

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