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Roles of Sodium Ions in Mechanical and Electrical Activities in Smooth Muscles

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ABSTRACT

In several smooth muscles effects of removal of the external sodium ions (Na^+) were studied by measuring tension development, intracellular pH (pH_i) and intracellular calcium ion concentration ($[Ca^{2+}]_i$) and also by recording membrane currents produced by the Na^+–Ca^{2+} exchange process. When the external Na^+ was removed by replacing with choline or other substitutes, a tonic contraction (0-Na contraction) was produced. This was accompanied by an increase in $[Ca^{2+}]_i$ and a decrease in pH_i. A part of the contraction could be due to noradrenaline released from nerve terminals and also due to Ca^{2+} influx through voltage-gated Ca^{2+} channels. The 0-Na^+ contraction was significantly potentiated by ouabain and the ouabain-potentiated contraction was not reduced by verapamil, a Ca^{2+} channel blocker. These results strongly suggest that smooth muscles have the Na^+–Ca^{2+} exchange mechanism. This idea was supported by direct observation of the membrane current induced by the electrogenic Na^+–Ca^{2+} exchange activated by alteration of Na^+ or Ca^{2+} concentration gradient.

INTRODUCTION

In most nerve cells the action potential is generated by influx of Na^+, so that their excitability depends on Na^+ concentration ($[Na^+]_o$) in the external medium. On the other hand, in smooth muscles, Ca^{2+} are known to be the main charge carrier for the action potential. Na^+ removal is, therefore, expected to have little effect on the excitability. In many smooth muscles, however, reduction of $[Na^+]_o$ produces depolarization of the plasma membrane and contraction. A possible explanation for this difference could be that in smooth muscle the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increases due to disturbance of a Na^+–Ca^{2+} exchange mechanism (Blaustein, 1977, 1984; Ozaki and Urakawa, 1981; Ashida and Blaustein, 1987). Another possibility is that, due to impairment of a Na^+–H^+ exchange, intracellular acidification may occur, resulting in a decrease in the potassium (K^+) permeability and in turn membrane depolarization. The depolarization would produce Ca^{2+} influx through voltage-gated Ca^{2+} channels. Therefore, in this experiment we have tried to analyse the mechanism underlying the contraction in Na^+ deficient solutions by measuring membrane currents mediated through the Na^+–Ca^{2+} exchange, in addition to changes in $[Ca^{2+}]_i$ and pH_i in several smooth muscle preparations.

METHODS

Guinea pigs weighing 250–350 g or rabbits weighing 950–1450 g of either sex were stunned and bled and the portal vein, vena cava, aorta, stomach and taenia coli were excised. Muscle strips were carefully dissected out after removing fat and mucosa.

pH_i was measured mainly in the guinea pig aorta, vena cava and stomach. A pH-sensitive dye, dimethylcarboxy-fluorescein (Me_2CF) was loaded intracellularly using its acetoxymethylester and changes in absorption spectra were measured by a spectrophotometer (MCPD-100, Otsuka Denshi). pH_i was calculated from a ratio of absorbance at 476 nm and 513 nm, based on a calibration curve obtained by using nigericin (a proton ionophore). Similarly, intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured with a Ca^{2+}-sensitive dye, fura-2, using a fluorescence microscope (OSP-3, Olympus). $[Ca^{2+}]_i$ was estimated from a ratio of fluorescence at 380 nm and 340 nm.

In some experiments, tension developments of muscle strips were also recorded using a strain gauge. For pH_i and tension recordings, a preparation was mounted in a small organ bath (1 ml in capacity) and superfused with solution at a constant rate of 3 ml/min. All experiments were carried out at 34°C.

For electrophysiological experiments, single cells

were dispersed from the rabbit portal vein or the guinea pig taenia coli by enzymatic treatments using collagenase. As schematically shown in Fig. 1, membrane currents were recorded, using the whole-cell clamp technique (Hamill et al., 1981), with a patch-clamp amplifier (L/M EPC-7, List). In order to record the current mediated by Na^+–Ca^{2+} exchange, other membrane transport systems were inhibited pharmacologically, i.e. K^+ current by cesium and tetraethylammonium, Na^+ current by tetrodotoxin (only in some experiments), Ca^{2+} current by verapamil, Na^+–K^+-pump current by ouabain.

Physiological solution contained (mM): NaCl 127, $KHCO_3$ 6, $CaCl_2$ 2.4, $MgCl_2$ 1.2, glucose 11.8, Tris-HCl buffer 10 (pH 7.4). NaCl was reduced by replacing it isoosmotically with LiCl (for the patch-clamp experiments), choline-Cl (with 10 μM atropine), *N*-methyl-D-glucamine-Cl (NMDG), or sucrose. Most chemicals were obtained from Sigma. Verapamil and phenoxybenzamine were obtained from Eisai and Smith Kline & French, respectively.

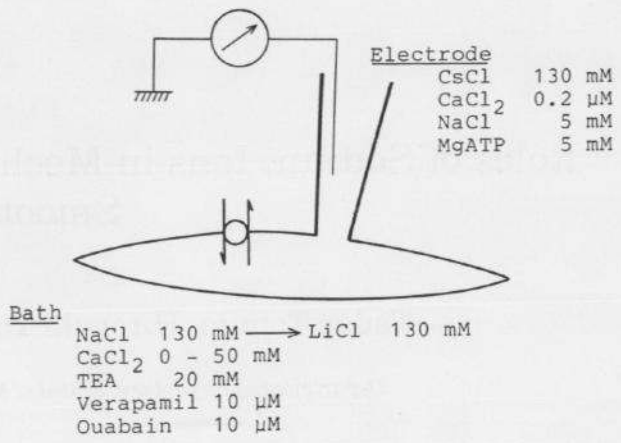

Fig. 1. Schematic diagram of whole-cell clamp experiment in a single smooth muscle cell. After obtaining giga-seal between a glass pipette and cell membrane, a membrane patch was broken by applying a negative pressure intracepillarly to make whole-cell clamp configuration and membrane currents of the whole cell were recorded through the pipette with a voltage-clamp amplifier. Pipette and bath solutions had compositions as indicated to record Na^+–Ca^{2+} exchange currents.

RESULTS

Tension development

Figure 2 shows an example of tonic contractions produced by removal of the external Na^+ (0-Na^+ contraction) in the guinea pig vena cava. When Na^+ was replaced with choline a contraction was slowly developed and this recovered quickly on reapplication of Na^+ (a,b). An irreversible α-adrenoceptor blocking agent, phenoxybenzamine (3 μM) partially inhibited the 0-Na^+ contraction (c), suggesting an involvement of noradrenaline released from nerve terminals. An inhibitor of Na^+–K^+-pump, ouabain (10 μM) potentiated the contraction and a Ca^{2+} channel blocker, verapamil (3 μM) had no significant inhibitory effect in the presence of ouabain (e). Before the ouabain treatment verapamil produced a partial inhibition. When magnesium (Mg^{2+}) was increased to 10 mM, 0-Na^+ contraction was markedly inhibited, confirming the previous report on the rat aorta (Altura et al., 1990) and also on Na^+–Ca^{2+} antiport studied with ^{45}Ca (Smith et al., 1987). The inhibition by Mg^{2+} was reversible even in the absence of Na^+.

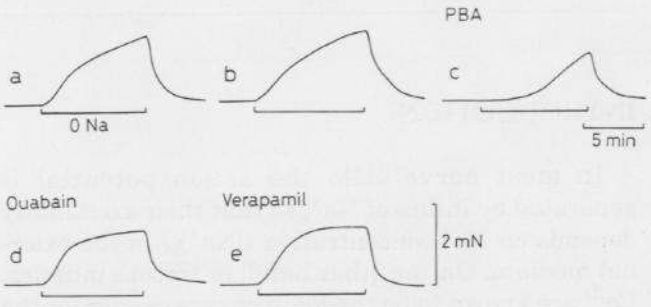

Fig. 2. Contractions produced by removal of Na+ from the external medium. Effects of phenoxybenzamine (PBA, 3 μM), ouabain (10 μM), and verapamil (3 μM) on the 0-Na^+ contractions (choline substitute) in a muscle strip of guinea pig vena cava.

Changes in pH_i

pH_i was measured after intracellular loading of Me_2CF by exposing tissues to 50 μM Me_2CF for about 2 h. Changes in pH_i were clearly observed with NH_4Cl (20 mM) which is known to cause intracellular alkalinization during application and transient acidification on removal, as shown in Fig. 3a. Removal of the external Na^+ gradually produced a slow decrease in pH_i (b). When NH_4Cl was applied in the absence of Na^+, acidification on NH_4Cl removal was potentiated and no recovery was observed until Na^+ was reintroduced (c). 0-Na^+ contraction was inhibited during NH_4Cl application, but it was markedly potentiated on removal of NH_4Cl, suggesting that intracellular alkalinization produces relaxation whereas acidification causes contraction. Similar results were obtained in the smooth muscles of guinea pig aorta and stomach.

Changes in $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was measured in the guinea pig vena cava, using fura-2 (Fig. 4). When the external Na^+ was removed by replacing with NMDG, $[Ca^{2+}]_i$ was in-

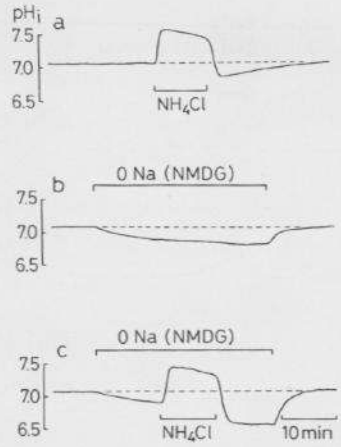

Fig. 3. Measurements of intracellular pH. Changes in intracellular pH (pH_i) produced by ammonium chloride (NH_4Cl, 20 mM) and Na^+ removal (0-Na) in guinea pig vena cava were estimated by the ratio of absorbance spectra at 513 nm and 476 nm of intracellularly loaded Me_2CF. Na^+ was completely replaced with NMDG.

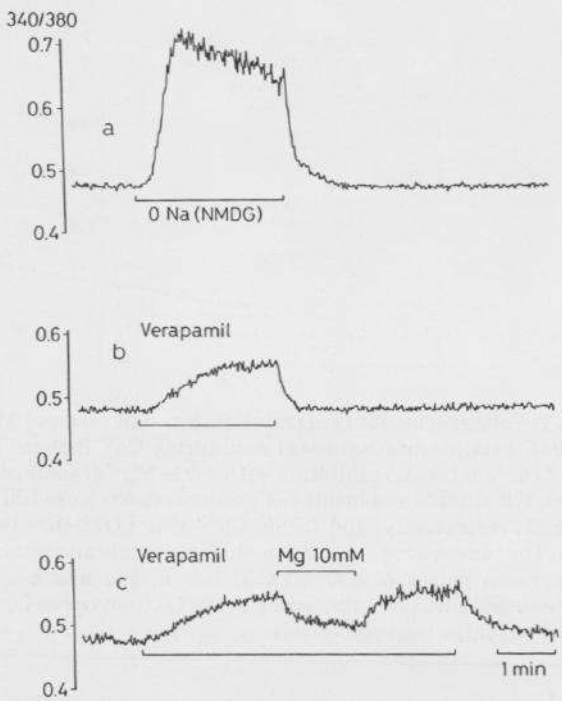

Fig. 4. Measurements of intracellular Ca^{2+}. Changes in $[Ca^{2+}]_i$ caused by Na^+ removal from the external solution were measured with fura-2 in the guinea pig vena cava by estimating from the fluorescence ratio at 340 nm and 380 nm. a: Control; b and c: in the presence of 3 μM verapamil, and in c, Mg^{2+} was increased from 1.2 to 10 mM during exposure to 0-Na^+ solution (NMDG substitute). Continuous record.

creased (a), as observed in the guinea pig taenia coli (Pritchard and Ashley, 1987). This was partially reduced by verapamil (3 μM) (b), suggesting a contribution of Ca^{2+} influx through voltage-gated Ca^{2+}

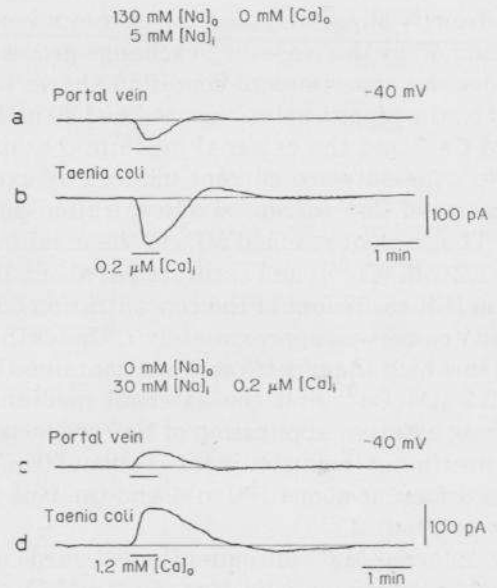

Fig. 5. Na^+–Ca^{2+} exchange currents. Membrane currents were recorded from single cells dispersed from rabbit portal vein (a,c) and guinea pig taenia coli (b,d) with the whole-cell clamp method. Membrane potential was held at –40 mV. Currents were elicited by applying Ca^{2+} to patch pipette (a,b) or bathing solution (c,d) as indicated by horizontal bars, under the Na^+ and Ca^{2+} concentrations shown in the figure. In c and d the external Na^+ was substituted by choline. Na^+, K^+ and Ca^{2+} channels were blocked as described in the methods. Upward deflection indicates outward current.

channels caused by membrane depolarization, in addition to possible Ca^{2+} influx through Na^+-dependent pathways, such as Na^+–Ca^{2+} exchange. The verapamil-insensitive component of the increase in $[Ca^{2+}]_i$ was markedly inhibited by increasing Mg^{2+} to 10 mM (c), as found in the mechanical experiment.

Membrane current generated by Na^+–Ca^{2+} exchange

In single smooth muscle cells dispersed from the rabbit portal vein and the guinea pig taenia coli, it was possible with the whole-cell clamp method to record membrane currents by either applying Ca^{2+} intracellularly or extracellularly, under the condition in which Na^+, K^+, and Ca^{2+} channels and also Na^+-K^+-pump were blocked (Fig. 5). When the external medium contained 130 mM Na^+ and 0 mM Ca^{2+} and a patch pipette contained 5 mM Na^+, an intracellular application of 0.2 μM Ca through the pipette produced inward currents (a, b). When the Ca^+ concentration gradient was reversed by having 30 mM Na^+ in the bath and 0 mM Na^+ in the pipette, an external application of 1.2 mM Ca^{2+} produced outward currents (c,d). Thus, the currents flowed in the direction of Na^+ concentration gradient. These re-

sults strongly suggested that the currents recorded were carried by the Na^+–Ca^{2+} exchange process.

Under the experimental condition shown in Fig. 5c, d: i.e., the pipette solution contained 30 mM Na^+, 0.2 μM Ca^{2+} and the external medium contained 0 mM Na^+, the outward current induced by external application of Ca^{2+} increased concentration-dependently. The current reached 50% of the maximum at about 1.2 mM $[Ca^{2+}]_o$ and saturated at about 10 mM and the Hill coefficient of the concentration-current intensity curve was approximately 1. Under the condition in which the pipette solution contained 0 mM Na^+, 0.2 μM Ca^{2+} and the external medium was Ca^{2+}-free, external application of Na^+ produced 50% of the maximum inward current at about 50 mM and the maximum at about 100 mM and the Hill coefficient was about 2.7.

The external Mg^{2+} inhibited the outward current induced by external application of 1.2 mM Ca^{2+} (Fig. 6). In this experiment, Ma^{2+}-free solution was used as control solution. The current was reversibly reduced to 50% by 3 mM Mg^{2+} without altering the time course. 10 mM Mg^{2+} had strong suppressing effects, as observed with $[Ca^{2+}]_i$ measurements.

Figure 7A shows the voltage–current relationship obtained by applying ramp pulses before (a) and during outward current induced by external Ca^{2+} application (1.2 mM) (b) and also during inhibition of the current in the presence of 3 mM Mg^{2+} (c). Even under the condition in which all possible membrane currents were blocked as described in the methods, there was some leak current before the external Ca^{2+} application. The nature of this current was not analyzed in the present experiments.

The curve (b–a) shown in Fig. 7B indicates the current component increased by external application of 1.2 mM Ca^{2+}.

The current was outward and increased with membrane depolarization. This current was inhibited by 3 mM Mg^{2+} increasingly with larger membrane depolarization (c–a). According to the theoretical consideration, assuming a coupling ratio of 3 Na^+ to 1 Ca^{2+}, the direction of the current carried by Na^+–Ca^{2+} exchange is determined by a relative magnitude of driving forces generated by Na^+ and Ca^{2+} concentration gradients across the plasma membrane (Baker, 1972, Kimura et al., 1986). Since the reversal potential (E_r) for the Na^+–Ca^{2+} exchange current is expressed by $E_r = 3E_{NA}-2E_{Ca}$ (E_{Na} and E_{Ca} are the equilibrium potential for Na^+ and Ca^{2+}, respectively), the E_r is –112 mV (E_{Na} = +38 mV, E_{Ca} = +113 mV) under the experimental condition shown in Fig. 7. Thus, although the external Na^+ concentration (130 mM) was greater than the internal concentration (30 mM), Na^+ was transported in the outward direction by the Na^+–Ca^{2+} exchange process, generating outward currents at a membrane potential larger than –112 mV.

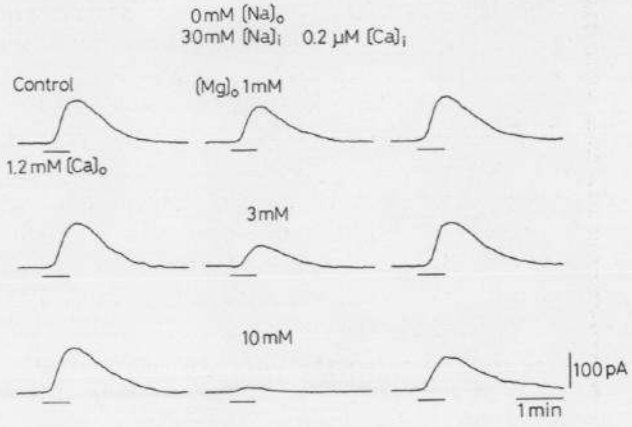

Fig. 6. Effects of magnesium on Na^+–Ca^{2+} exchange current. External Mg^{2+} (1–10 mM) inhibited outward currents produced by external application of 1.2 mM Ca^{2+} under the same condition as that of Fig. 5c,d in rabbit portal vein. Control bathing solution contained no Mg^{2+} and Mg^{2+} was applied 3 min before Ca^{2+} application. 1.2 mM Ca^{2+} was applied for 30 s at 10 min intervals.

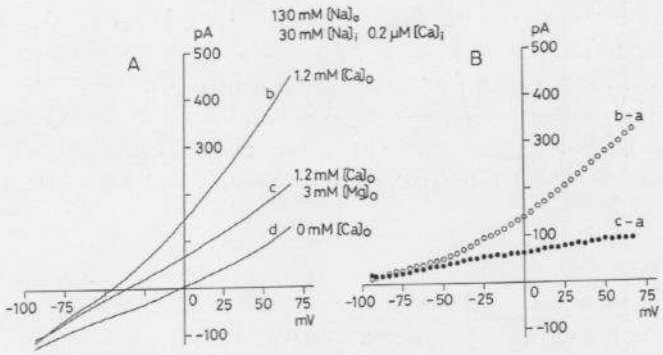

Fig. 7. Voltage–current (V–I) relationship and external Mg^{2+}. A: V–I relationship before (a) and during Ca^{2+}-induced outward current (b) and inhibition with 3 mM Mg^{2+} (rabbit portal vein). The outside and inside Na^+ concentrations were 130 and 30 mM, respectively, and inside Ca^{2+} concentration was 0.2 mM. The curves were obtained by shifting membrane potential with ramp pulses of 200 mV/250 ms. B: b–a and c–a are currents obtained by subtracting curve (a) from curve (b) and also from curve (c), respectively.

DISCUSSION

In several smooth muscles, removal of the external Na^+ is known to produce a tonic contraction (0-Na^+ contraction). Different mechanisms seem to be involved in this contraction. In some vascular smooth muscles, noradrenaline released from nerve terminals within the tissue is probably largely re-

sponsible for the 0-Na^+ contraction (Karaki and Urakawa, 1977). On the other hand, the contribution of catecholamines to the 0-Na^+ contraction is considered to be minor in the guinea pig aorta (Ozaki et al., 1978) and canine coronary artery (Maseki et al., 1990). In the guinea pig vena cava, phenoxybenzamine partially reduced the 0-Na^+ contraction, suggesting some participation of a nervous component. It seems that the contribution of catecholamine varies depending on smooth muscles, species and experimental conditions.

In the canine coronary artery, verapamil significantly inhibited 0-Na^+ contraction before ouabain treatment, but only very weakly in the presence of ouabain (Maseki et al., 1990). Similar results were obtained in the present experiment on the guinea pig portal vein. It is likely that removal of the external Na^+ produces membrane depolarization, probably due to intracellular acidification as found in the guinea pig vena cava. A decrease in pH_i can be explained by an inhibition of Na^+–H^+ exchange. Since Ca^{2+}-activated K^+ is known to be inhibited by a decrease in pH_i (Kume et al., 1990), intracellular acidification caused by Na^+ removal may lead to membrane depolarization, resulting in an increased Ca^{2+} influx through verapamil-sensitive voltage-gated Ca^{2+} channels. Therefore, at least some part of the 0-Na^+ contraction is due to membrane depolarization.

In the guinea pig vena cava, the 0-Na^+ contraction was potentiated by and became less sensitive to, verapamil. This may be due to the fact that ouabain increases the $[Na^+]_i$ by blocking a Na^+–K^+ pump and this increases a contribution of increased $[Ca^{2+}]_i$ through the Na^+–Ca^{2+} exchange which is verapamil-insensitive. When the external Na^+ is removed, the driving force would move Na^+ outward and this results in an increase of Ca^{2+} influx through the Na^+–Ca^{2+} exchange process, producing the 0-Na^+ contraction. Ouabain-induced $[Na^+]_i$ increases a driving force for outward Na^+ movement on Na^+ removal and this potentiates Ca^{2+} influx coupled with the Na^+ efflux, as observed in other vascular muscles (Blaustein, 1977; Ozaki and Urakawa, 1981; Ashida and Blaustein, 1987; Maseki et al., 1990).

Membrane currents recorded in the present experiment are considered to be carried by the Na^+–Ca^{2+} exchange, because all possible current pathways except for Na^+–Ca^{2+} exchange have been blocked and also because the property of the current is similar to the N^+–Ca^{2+} exchange current recorded in cardiac muscle cells (Kimura et al., 1986; Li and Kimura, 1990). The direction of the current produced by changes in Na^+ or Ca^{2+} concentrations is in accord with the theoretical prediction. Inhibition of the current by excess Mg^{2+} also agrees with the observations on tension development and measurement of $[Ca^{2+}]_i$.

The results obtained in the present experiments all strongly suggest the presence of Na^+–Ca^{2+} exchange mechanism in the smooth muscles studied. It seems, however, the some Na^+–independent process is also involved in the regulation in $[Ca^{2+}]_i$. Excess Mg^{2+} inhibits not only Na^+–Ca^{2+} exchange current but also 0-Na^+ contraction and 0-Na^+-induced increase in $[Ca^{2+}]_i$. This means that $[Ca^{2+}]_i$ can be reduced in the absence of Na^+, i.e., by a process other than Na^+–Ca^{2+} exchange, such as an ATP-driven Ca^{2+}-pump, as considered for the guinea pig ureter (Aaronson and Benham, 1989). After the inhibition with excess Mg^{2+}, $[Ca^{2+}]_i$ can be again increased by reducing Mg^{2+} concentration in the absence of Na^{2+}. Since $[Na^+]_i$ is considered to be very low after a prolonged exposure to Na^+-free medium, Ca^{2+} influx responsible for the increase in $[Ca^{2+}]_i$ can not also be mediated by the Na^+–Ca^{2+} exchange process. A leak pathway for Ca^{2+} influx may be activated by removing Na^+. Whatever the pathway is, the result suggests Na^+ has an inhibitory role in Ca^{2+} influx, in addition to the regulation of $[Ca^{2+}]_i$ and pH_i by Na^+–Ca^{2+} and Na^+–H^+ exchange process, respectively. Physiological significance of these Na^+-dependent regulations of $[Ca^{2+}]_i$ should be further investigated.

CONCLUSIONS

At least three different mechanisms were considered to be involved in the tonic contraction produced by removal of the external Na^+. The main mechanism is probably an increase in Ca^{2+} influx mediated through Na^+–Ca^{2+} exchange, because the contraction, accompanied by an increase in $[Ca^{2+}]_i$, was only partly inhibited by verapamil, and greatly potentiated by a ouabain treatment. Furthermore, the presence of Na^+–Ca^{2+} exchange was directly demonstrated by recording the membrane current generated by this process. Intracellular pH decreased following the Na^+ removal, probably due to inhibition of Na^+–H^+ exchange. This may produce membrane depolarization and in turn increase Ca^{2+} influx through voltage-dependent, verapamil-sensitive, Ca^{2+} channels. In addition, noradrenaline released from nerve terminals is partially responsible for the 0-Na^+ contraction.

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