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CONTRACTILITY AND ^{45}Ca FLUXES IN HEART MUSCLE OF FLOUNDER AT A LOWERED EXTRACELLULAR NaCl CONCENTRATION

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SUMMARY

The twitch force of isolated electrically paced ventricular strips of flounder, *Platichthys flesus* L., increased after lowering the extracellular sodium chloride concentration by 50 mmol l^{-1}. This response was markedly reduced by replacing the sodium chloride with either Tris-HCl or sucrose, so that osmolarity was unchanged.

The ^{45}Ca efflux decreased and the ^{45}Ca influx increased when the extracellular sodium concentration Na_o^+ was lowered. In contrast, changing only the osmolarity had no observable effect on these fluxes.

An increased resting tension appeared in strips exposed to a Na^+-, Ca^{2+}-free solution. This was transient at an unchanged osmolarity but became permanent at an osmolarity lowered by 100 mosmol l^{-1}.

These results suggest that both a lowered Na_o and a lowered osmolarity have a positive inotropic effect, due respectively to an increased cellular uptake of Ca^{2+} and a redistribution of cellular Ca^{2+}.

INTRODUCTION

Changes in extracellular osmolarity occur naturally in many vertebrates, mainly due to changes in the concentration of sodium chloride (Vislie & Fugelli, 1975; Holeton, Neumann & Heisler, 1983). Such changes affect many cellular functions. A lowering of extracellular sodium concentration, Na_o, has a positive effect on twitch tension development in cardiac muscle. This effect is probably due to an increased net uptake of Ca^{2+} *via* the sarcolemmal Na^+- for Ca^{2+}-exchange believed to exist in myocardial cells (Lüttgau & Niedergerke, 1958; Chapman, 1979). In addition, however, a lowering of the osmolarity itself has been shown to stimulate the twitch force development of frog myocardial tissue (Chapman, 1978). This was tentatively explained as an effect of lowered osmolarity on the intracellular Ca^{2+}-sequestering mechanisms (Chapman, 1978). However, information about possible additional effects of osmolarity on sarcolemmal Ca^{2+} fluxes appears to be scarce.

The purpose of the present study has been to examine the influence of lowered Na_o, with or without a parallel lowering of the osmolarity, on force development and

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sarcolemmal Ca^{2+} fluxes in myocardial tissue. The study has been carried out on the ventricular myocardium of the euryhaline flounder. When this fish moves from sea water to fresh water its plasma osmolarity decreases from about 350 to about 250 mosmol l^{-1} mainly as a result of a lowered sodium chloride concentration (Vislie & Fugelli, 1975).

MATERIALS AND METHODS

Flounders (*Platichthys flesus* L.) were kept in tanks with recirculating sea water at 10–15 °C. Composition of salines is given in Table 1. Their temperature was $10 \pm 0{\cdot}5$ °C. When containing bicarbonate they were gassed with 99 % O_2 and 1 % CO_2; otherwise 100 % O_2 was used. The resulting pH was 7·6. Osmolarities of the standard solution and low osmolarity solutions as measured by freezing point depression (Knauer) were 352 and 249 mosmol l^{-1}, respectively. After decapitation of the experimental fish, the heart was isolated and rinsed in standard solution. A ventricular strip of less than 1 mm diameter was cut. It was quickly blotted with a filter paper and weighed to the nearest 0·1 mg. The weight ranged from 16·2 to 26·1 mg. The strip was fixed at one end while the other end was connected to a transducer (Statham) with surgical silk and its isometric force development was recorded (Beckman R511A). The strips were electrically paced at a frequency of 12/min *via* two Ag-AgCl electrodes on opposite sides of the strips with square pulses of 5 ms duration and twice threshold voltage delivered by a stimulator (Grass SD9). The distance between the transducer and the fixed end of the strip could be varied with a micrometer screw. This allowed determination of the peak of the length-twitch force relationship, where the experiments were carried out after a stabilization period of not less than 20 min.

Directly after the determination of this peak, the length of the strip was estimated to the nearest mm with a ruler. Cross-sectional area was then calculated assuming that the strip was cylindrical, with a specific weight of 1·0 g cm^{-3}. This allowed a rough estimation of the force in mN mm^{-2}. The reference level of 100 % corresponds to $4{\cdot}2 \pm 0{\cdot}7$ mN mm^{-2} ($N = 76$). The effects of the different treatments on the force development was, however, evaluated in relative terms since the cellular density and orientation probably vary largely in the flounder ventricle, which has a markedly spongy structure (Santer & Greer Walker, 1980).

For the ^{45}Ca-efflux experiments the strip was incubated in 1 ml standard solution (Table 1) with 4 μCi ^{45}Ca ml^{-1} for 2 h. Thereafter, while being mounted for the force recording and stabilized, it was washed three times for 20 min in about 5 ml unlabelled

Table 1. *Incubation solutions, composition in mmol l^{-1}*

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	$H_2PO_4^{2-}$	SO_4^{2-}	HCO_3^-	Tris (total)	Sucrose
Standard	162	5	1·8	1	157	1	1·8	11	0	0
Low Na_o^+ (Tris-HCl)	112	5	1·8	1	154–157	1	1·8	11	50	0
Low Na_o^+ (sucrose)	112	5	1·8	1	107	1	1·8	11	0	100
Low Na_o^+ (low osmo)	112	5	1·8	1	107	1	1·8	11	0	0
$-Na^+$, $-Ca^{2+}$	0	5	1·8	0	100–110	0	1·8	0	112	122
$-Na^+$, $-Ca^{2+}$ (low osmo)	0	5	1·8	0	100–110	0	1·8	0	112	22

standard solution. It was then, while paced to contraction, transferred through a series of baths of 4 ml unlabelled solution. The incubation in each bath lasted 10 min. Finally, the strip was digested overnight in 2 ml 30 % H_2O_2. The residue was dissolved in 2 ml H_2O. 0·1 ml 50 % TCA was added to each ml of this solution and the incubation solutions. 2 ml of each of the resultant solutions was transferred to a plastic vial containing 10 ml of a Triton-X-100/toluene scintillation solution. Counts min^{-1} were measured for 20 min in the ^{14}C-channel of a scintillation counter (Beckman LS 250). These values allowed the efflux rate of the tissue to be calculated as fractional loss of $^{45}Ca\ min^{-1}$.

In the ^{45}Ca uptake experiments the strip was mounted for force recording. In some experiments, after being appropriately stretched, the strip was left unstimulated for the rest of the experiment. After stabilization for at least 30 min in standard solution, the strip was exposed to a bath with 0·2 $\mu Ci\ ml^{-1}$ of ^{45}Ca in either a low osmolarity, a low Na_o^+ with sucrose, or a control solution (Table 1). After 10 min in this bath the strip was incubated for 15 min in each of three baths with 10 ml of an ice-cold Na^+-, Ca^{2+}-free solution (Table 1) in which 40 mmol l^{-1} of the sucrose had been replaced by 10 mmol l^{-1} $LaCl_3$. Thereafter the strip was digested as above. Its ^{45}Ca content was then measured together with that of the incubation solution as described for the efflux experiments. The values obtained allowed the Ca-uptake to be calculated and expressed as mmol $min^{-1}\ kg^{-1}$ tissue. The results are given as mean ± S.E. The level of significance was estimated by Student's *t*-test for either paired or unpaired samples, and considered significant at $P < 0{\cdot}05$.

RESULTS

Isometric force development and ^{45}Ca efflux were measured simultaneously in flounder ventricular strips exposed to different solutions. Lowering the NaCl concentration by 50 mmol l^{-1} without any compensation for the lowered osmolarity (low osmo, Table 1) caused an elevation of the twitch force, which lasted throughout the 30-min exposure to this test solution (Fig. 1). A return to control conditions was followed by a steep decrease in force to values well below those recorded for the controls.

Upon replacing 50 ml NaCl with either Tris-HCl or sucrose (low Na_o^+, Table 1), the twitch force increased, although only transiently and to a level well below that observed in the low osmolarity solution (Fig. 1). Until the return to control conditions the strips exposed to sucrose behaved like those exposed to Tris-HCl. Thereafter, however, the force of the strips that had been exposed to low NaCl with sucrose was the same as for the controls, while the force of the strips treated with Tris-HCl fell well below the control level. This may indicate an adverse effect of Tris-HCl on contractility. Toxic effects of Tris-HCl have previously been reported for different tissues (Gillespie & McKnight, 1976). Therefore, sucrose was used in the rest of the experiments to increase the osmolarity.

Although the different treatments had large effects on the twitch force, the resting tension remained unchanged.

It was difficult to obtain a perfect stabilization of twitch force before the different salines were applied, i.e. a small transitory decrease occurred for the strips which were

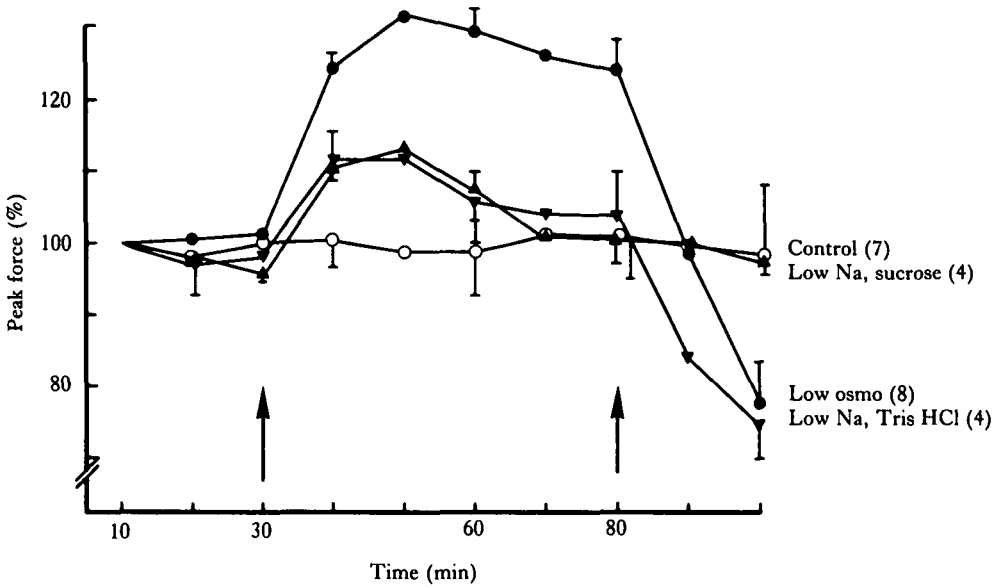

Fig. 1. Effects of decreases in osmolarity and/or Na_o^+ on the twitch force development of electrically paced flounder ventricular tissue. The force is given as a percentage of that developed after the initial period of stabilization. Control (○); low osmolarity (●); low Na_o with sucrose (▲); low Na_o with Tris-HCl (▼). Number of hearts is given in brackets. The arrows signify the application of experimental solutions and return to standard solution.

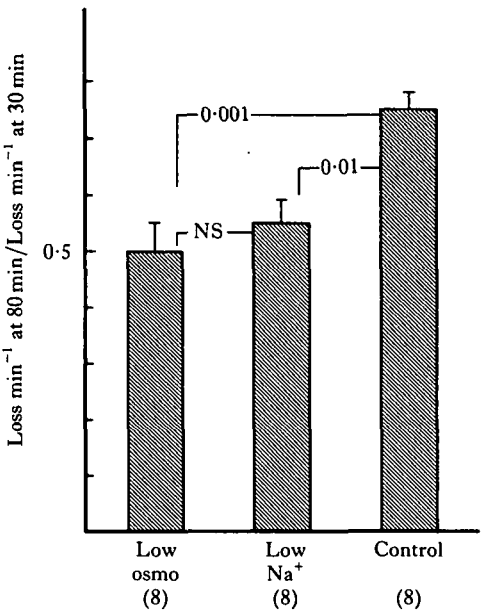

Fig. 2. Effects of decreases in osmolarity and/or Na_o^+ on the ratio between the $^{45}Ca^{2+}$-efflux rate at the 30th and the 80th minute (see arrows in Fig. 1) of electrically paced flounder ventricular tissue. This ratio is thus the fraction of the initial efflux rate left after the different exposures. The $^{45}Ca^{2+}$-efflux and the force depicted in Fig. 1 were measured simultaneously in the same strips. Results at low Na_o consist both of those obtained with sucrose to maintain osmolarity and those obtained with Tris-HCl. Number of hearts as in Fig. 1, figures in brackets.

to be exposed to a low Na_o with sucrose. The force of the controls also varied somewhat throughout the experiment (Fig. 1). These variations were possibly due to mechanical disturbances caused by the change of bath every 10 min.

The ^{45}Ca efflux was measured in parallel with these force recordings and according to the experimental protocol given in Fig. 1. The effect of the different salines on this parameter was estimated by calculating the ratio between the rate obtained at the 80th minute, i.e. when the test solution was removed, and that obtained at the 30th minute, i.e. when the test solution was applied. The efflux was reduced to a similar extent when Na_o was lowered with a reduction in osmolarity as at constant osmolarity (Fig. 2). (There was no significant difference between values obtained with Tris-HCl or sucrose, so they were treated as belonging to the same experimental group.)

Specific ^{45}Ca uptake during stimulation to contraction at a frequency of 12 min^{-1} was the same as during rest. Reduction in Na_o caused an increased Ca uptake, and, as for the efflux values, the lowered osmolarity appeared to have no effect of its own (Fig. 3).

To further investigate the specific effect of osmolarity on contractility, the approach of Chapman (1978) was used. In four preparations, stimulation was halted after stabilization of the twitch force. A solution free of Na^+ and Ca^{2+} was then applied in order to inhibit the sarcolemmal Ca^{2+}- for Na^+-exchange. The resting tension

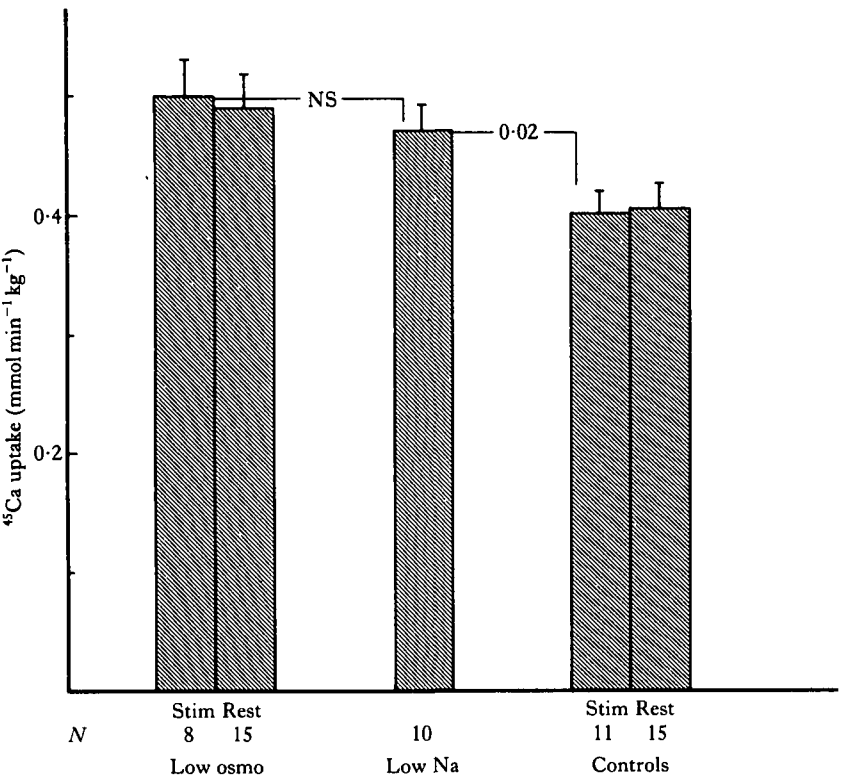

Fig. 3. Effects of decreases in osmolarity and/or Na_o^+ on the $^{45}Ca^{2+}$-uptake rate in flounder ventricular tissue, either when stimulated (Stim) to contraction, or when indicated left at rest (Rest). *N* indicates number of hearts.

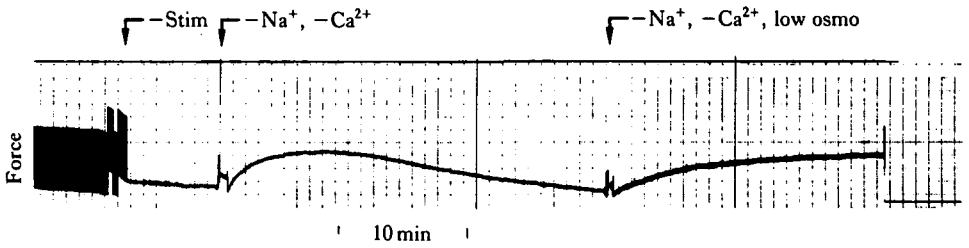

Fig. 4. Direct recording of the resting tension of unstimulated flounder ventricular tissue exposed to a nominally Na^+-, Ca^{2+}-free solution. At first the osmolarity was about 350 mosmol l^{-1}, but after 30 min it was lowered to about 250 mosmol l^{-1} by removing 100 mmol l^{-1} sucrose.

increased for about 10 min and then started to decrease. This transient reaction of cardiac tissue to a removal of extracellular Na^+ and Ca^{2+} is commonly seen (e.g. Chapman, 1974). However, when the solution was replaced by one which not only was free of Na^+ and Ca^{2+} but also had a low osmolarity, the resting tension again increased (Fig. 4). Response was slower than in the experiments of Chapman (1974), probably because the cardiac preparations were considerably larger in the present study.

DISCUSSION

A bulk of evidence suggests that regulation of the cardiac force development depends heavily on a Ca^{2+}- for Na^+-exchange across the sarcolemma (Lüttgau & Niedergerke, 1958; Chapman, 1979). The effects of a decreased Na_o observed in the present study, to increase twitch force and Ca uptake and lower Ca efflux, are consistent with such an exchange.

The stimulation of cardiac force development produced by a reduction in osmolarity does not appear to depend on a changed net uptake of Ca^{2+}, since the efflux and uptake rate of ^{45}Ca measured after a decreased Na_o were the same whether the osmolarity was changed or not. Similar to the finding of Chapman (1978), however, the osmolarity affected the tension development of flounder cardiac tissue exposed to a nominally Na^+- and Ca^{2+}-free solution. This suggests that lowered osmolarity causes an intracellular redistribution of Ca^{2+}, which in turn may stimulate the force development. Chapman (1978) proposed that a changed osmolarity may affect the Ca^{2+}-accumulating capacity of the sarcoplasmatic reticulum.

The results presented are of interest as concerns euryhaline animals, for example the flounder. This fish often inhabits estuarine waters, where it is likely to be exposed to rapid salinity changes. There exists substantial evidence that the heart cells of the flounder can acclimate to a changed osmolarity by a regulation of the intracellular levels of taurine and potassium. These processes, however, seem to require several hours (Vislie, 1980). Therefore the heart cells may still be exposed to sudden osmotic shocks. Furthermore, the described mechanism of acclimation does not appear to compensate for the specific effects of changes in Na_o, commonly accompanying a changed osmolarity (Vislie & Fugelli, 1975). Consequently, the flounder heart may also be presented to such changes when slowly moving between different salinities.

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