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Comparison of the Action of La^{3+} and Ca^{2+} on Contraction Threshold and Other Membrane Parameters of Frog Skeletal Muscle

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Summary. The influence of La^{3+} on contraction threshold, on membrane input resistance, and on action potential parameters was investigated in fibers of the sartorius muscle of the frog, and it was compared to that of Ca^{2+}. The dependence of the contraction threshold on $[La^{3+}]_0$ in the presence of 0.5 mM Ca^{2+} gave a sigmoid relationship between 0.1 and 5 mM La^{3+} with a shift of 23 to 34 mV to less negative potentials following a 10-fold increase of $[La^{3+}]_0$. The membrane input resistance was increased to various degrees in La-containing solutions, the increase being *irreversible*. The threshold of action potential generation was shifted to less negative potentials by 28 mV, and the duration at half-maximal amplitude was tripled by 0.5 mM La^{3+}. In comparison a 10-fold increase of $[Ca^{2+}]_0$ in the range of 0.5 to 50 mM shifted the contraction threshold by 15 mV to less negative potentials. 17 mM Ca^{2+}, a concentration having the same effect on contraction threshold as 0.5 mM La^{3+}, increased membrane input resistance *reversibly*, shifted the action potential threshold by 16 mV to less negative potentials, and had only minor effects on action potential duration. Conduction was never blocked by Ca^{2+} as it was with 1 mM La^{3+}. In a theoretical treatment, it is shown that the influence of Ca^{2+} on contraction threshold, but *not* that of La^{3+}, may be accounted for by its screening and binding to negative surface charges according to the Gouy-Chapman theory of the diffuse double layer. To describe the action of La^{3+} on the contraction threshold an additional interaction of La^{3+} with neutral but amphoteric sites was considered.

Key words: excitation-contraction coupling, action potential, membrane input resistance, lanthanum effects, surface charge model, frog skeletal muscle

The trivalent rare earth alkaline metal ion lanthanum has been reported to influence contractile activation of isolated twitch muscle fibers in much the same way as the divalent cation calcium. Low concentrations of lanthanum. $[La^{3+}]_0$ (<0.3 mM), resulted in a shift of the curve relating membrane depolarization to tension to less negative potentials (Weiss, 1970; Andersson & Edman, 1974*a*; Dörrscheidt-Käfer & Lüttgau, 1974). The maximal amplitude of the potassium-induced contracture was not altered, whereas twitch tension was slightly potentiated (Andersson & Edman, 1974*b*).

The aim of the present paper was to compare the effect of La^{3+}, which had been termed "supercalcium" by Takata, Pickard, Lettvin and Moore (1966), on mechanical and electrical properties in frog sartorius to that of Ca^{2+}. It will be shown that La^{3+} exerts a strong effect on the contraction threshold being 10 to 50 times more effective than Ca^{2+} in shifting the contraction threshold to less negative potentials.

The action of Ca^{2+} and La^{3+} appeared to be different only in quantitative aspects. The formal description used earlier for the effects of Ca^{2+}, Mg^{2+} and H^+ on contraction threshold, which followed the Gouy-Chapman theory of the diffuse double layer involving electrostatic screening of and binding to negative charges fixed to the surface of the T-tubular wall (Dörrscheidt-Käfer, 1976, 1979*a*, *b*), proved to be too small an effect to describe the strong influence of La^{3+}. A satisfying approach was found taking also into account adsorption of La^{3+} to neutral but amphoteric lipids, e.g. phosphatidyl ethanolamine (McLaughlin, Szabo & Eisenman, 1971).

The effect of La^{3+} on the amplitude and time course of the action potential, its blocking of conduction and the effect on membrane input resistance were found to be in accordance with data reported

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by Hartz and Ulbricht (1973) for crayfish giant axon, and with those given for frog semitendinosus (Andersson & Edman, 1974*b*; Parry, Kover & Frank, 1974). The threshold of the action potential was shifted to less negative values. This finding agrees with the results on crayfish axon given by D'Arrigo (1973).

Parts of the results presented here have been published in preliminary form (Dörrscheidt-Käfer & Lüttgau, 1974; Dörrscheidt-Käfer, 1978).

Materials and Methods

All experiments were performed with the sartorius muscle of *Rana temporaria* in the winter season 1973/74 and during autumn 1978.

For measurements of the contraction threshold muscles were fixed in a lucid Plexiglas chamber and stretched to *ca.* 120% of their slack length. Individual fibers were impaled by two microelectrodes, one for potential recording, and the other for intracellular stimulation. The potential recording electrode had a resistance of 8 to 20 MΩ with tip potentials less than 5 mV; the stimulus electrodes had resistances between 8 and 15 MΩ. The latter were electrically shielded against the potential electrode to eliminate stimulus artifacts. The contraction threshold was determined as follows: Once a fiber was impaled by the two electrodes *ca.* 100 μm apart, stimuli of 150 msec and increasing current strength were applied until a well-observable local contraction could be detected. Then stimulus strength was gradually decreased until the contraction was on the verge of disappearing. The measured membrane potential was then defined as the *contraction threshold*. It was photographed together with the stimulus and further evaluated. The stimulus applied through a 22 MΩ resistor in series with the electrode proved to be fairly rectangular.

Membrane input resistance was determined measuring the stimulating current as the voltage drop across a 20 kΩ resistor placed in the stimulus circuit between the bath electrode (an Ag-AgCl electrode) and ground. Together with the corresponding voltage change the resistance was calculated by linear regression. It was determined only between resting voltage and the contraction threshold because of the nonlinearity encountered at voltages more positive than contraction threshold (Costantin, 1968).

For action potential measurements the muscle was stretched to *ca.* 140% of its slack length to minimize artifacts due to movement of the fiber, and the stimulating electrode was placed at a distance of *ca.* 1000 μm from the potential recording electrode. Stimulus duration ranged from 0.3 to 1 msec. The threshold of the action potential was determined with a stimulus of 5-msec duration to obtain a slowly rising electrotonic component with microelectrodes placed *ca.* 100 μm apart. The threshold potential was taken as the intersection of tangents to the electrotonic potential and to the rising phase of the action potential.

The membranes became rather tough during treatment with La^{3+}; therefore penetration was facilitated by bevelling the electrodes. The accurateness of the optical determination of the threshold of contraction was improved by the use of polarized light.

Test solutions contained in general (mM): 123 choline Cl, 2.5 KCl, 3 HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid), and no calcium ("zero-calcium" solution), or 0.5, 1.5 or 5 mM $CaCl_2$. With 15, 17 and 50 mM $CaCl_2$ choline Cl was reduced to 90, 85 or zero mM, respectively, and the osmolarity was kept constant by addition of sucrose. For action potential measurements choline Cl was replaced by NaCl. The pH was adjusted to 6.5 to avoid precipitation of La^{3+} which was added as $LaCl_3$ from a stock solution. All precautions were taken to prevent precipitation of La^{3+}, i.e. tubings and glassware which came into contact with La-containing solutions were carefully cleaned, and as a last step washed with 0.1 N HCl and distilled water immediately before use. As the solid substance $LaCl_3$ is highly hygroscopic, and thus the effective molecular weight is subject to uncertainties, the $LaCl_3$ concentration of the stock solution was determined by analyzing the chloride content. Chemicals were reagent grade, from Merck, Darmstadt. Experiments were performed at room temperature. Significance was checked by the two-sided Student *t*-test, and the theoretical curves were calculated on a PDP 12 computer (Digital Equipment Corp.).

Results

Contraction Threshold

In excitable tissues, calcium is known to "stabilize" the electrical membrane functions. Omission of Ca^{2+} from the bathing medium usually results in a depolarization of the membrane which often leads to repetitive firing, and which may be irreversible (*see also* Dalton, 1958; Jenden & Reger, 1963; Lüttgau, 1963; Armstrong, Bezanilla & Horowicz, 1972; Frank & Inoue, 1973; Barrett & Barrett, 1978). Fig. 1*a* shows an example where after a change in the Ca concentration ($[Ca^{2+}]_o$) from 5 mM to "zero", which merely means omission of Ca^{2+} from the bath, the resting potential of a sartorius fiber (fiber No. 10) became less negative by *ca.* 20 mV, and its former negative value was not restored by going back to 5 mM Ca^{2+}, although the resting potential of other fibers from the same muscle exhibited normal values around −82 mV.

The contraction threshold showed the reverse behavior. It became more negative after omission of Ca^{2+}, and it was fully reversed after readdition of 5 mM Ca^{2+}. When La^{3+} was added instead of Ca^{2+} (Fig. 1*b*, 0.65 mM La^{3+}) the resting potential remained unaffected, whereas the contraction threshold was shifted to a less negative potential by *ca.* 30 mV. The effect of La^{3+} on the contraction threshold was completely reversible. The subsequent change in Ca^{2+} concentration from 0.5 to 50 mM had almost the same effect on contraction threshold as that of adding 0.65 mM La^{3+}, and further addition of 0.65 mM La^{3+} to the 50 mM Ca^{2+} shifted the threshold to a still less negative potential value. In a more detailed study, however, the difference between these latter threshold values was not significant. Again the threshold shift was completely reversed after returning to 0.5 mM Ca^{2+}.

It should be noted that in contrast to the action of Ca^{2+}, which occurred in the first 30 sec of super-

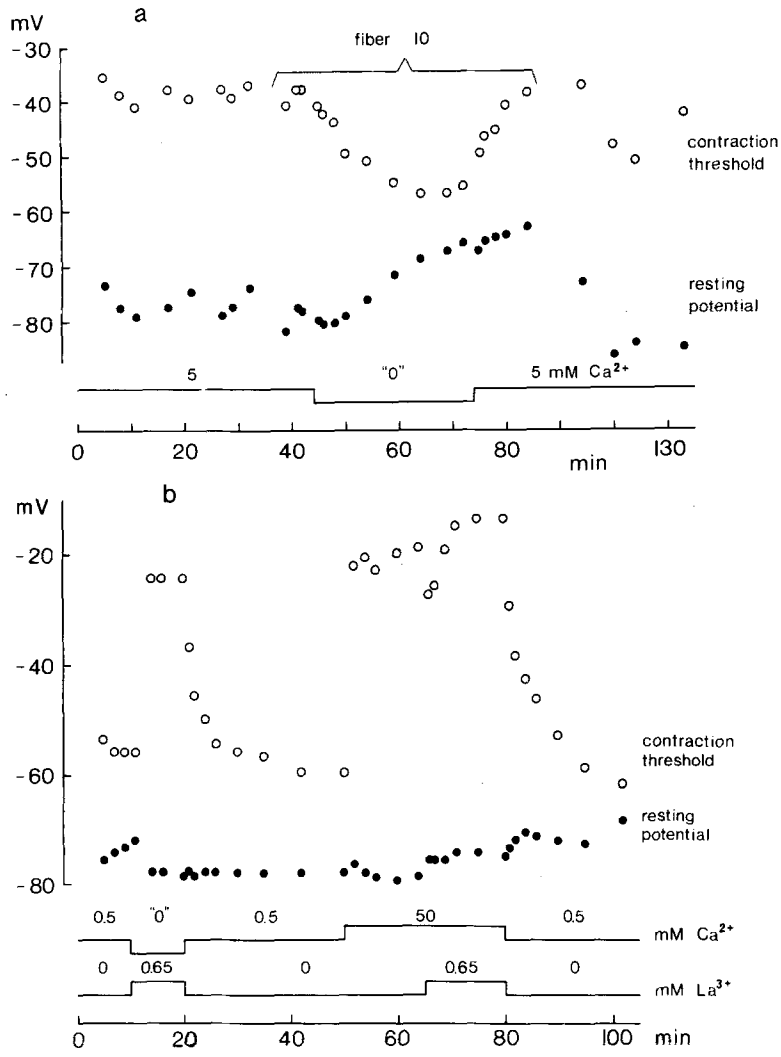

Fig. 1. Resting potential (●) and contraction threshold (○) in different bathing solutions. (*a*) In 5 mM Ca^{2+} and in "zero-calcium" solution. Values are from different fibers of one muscle except for fiber 10 showing the effect of changing from a Ca-containing solution to one without added calcium. (*b*) Values of one fiber of a second muscle. The solution was changed, as indicated at the bottom, from one containing 0.5 mM Ca^{2+} to a "zero-calcium" solution with 0.65 mM La^{3+} and back again. Further change was to 50 mM Ca^{2+} without and with 0.65 mM La^{3+} and back to 0.5 mM Ca^{2+}

fusion, the effect of La^{3+} on the contraction threshold developed slowly, taking up to 20 min at low $[La^{3+}]_0$, and *ca.* 5 min at higher concentrations (1–5 mM) to reach a constant new value. In all instances contraction threshold values were read only after a constant value was reached. The La effect being very variable, this precaution was necessary, and it turned out that in one-third of the experiments La^{3+} exerted no effect even after 1 hr of superfusion (0.1–0.65 mM). At higher $[La^{3+}]_0$ the contraction threshold was stable at the new level for more than 30 min.

Results of experiments like those in Fig. 1*b* at different $[Ca^{2+}]_0$ without and with 0.65 mM La^{3+} are shown in Fig. 2. The mean values of contraction threshold were shifted by 30 mV to less negative potentials when going from 0.5 to 50 mM Ca^{2+}. Addition of 0.65 mM La^{3+} resulted in a shift of 25 mV at 0.5 mM Ca^{2+}. At 5 mM and 50 mM Ca^{2+} the shift produced by the addition of 0.65 mM La^{3+} was 19 and 6 mV, respectively, the mean absolute potentials being not significantly different from each other and from the value at 50 mM Ca^{2+} without La^{3+}.

The dependence of the contraction threshold on $[La^{3+}]_0$ is shown in Fig. 3. Here and for the rest of the experiments all La solutions contained 0.5 mM Ca^{2+} as well. Between 0.2 and 2 mM La^{3+} the dependence of the threshold potential on $[La^{3+}]_0$ was very steep with a shift to less negative potentials of 34 mV. At lower $[La^{3+}]_0$ the effect of La^{3+} was weak, and at $[La^{3+}]_0$ greater than 2 mM no further shift in contraction threshold was seen ("ceiling effect"). Comparing the influence of $[Ca^{2+}]_0$ on contraction threshold (Fig. 2) with that of $[La^{3+}]_0$ (Fig. 3) shows that the ratio of effectiveness was not constant at 20:1 as suggested by Takata et al. (1966), or at 50 to 60:1 (D'Arrigo, 1973; Hartz & Ulbricht, 1973; Vogel, 1974), but that it varied between 10:1 for low $[La^{3+}]_0$ and 30 to 50:1 at higher $[La^{3+}]_0$ (0.5 to 2 mM), which may indicate an interaction of

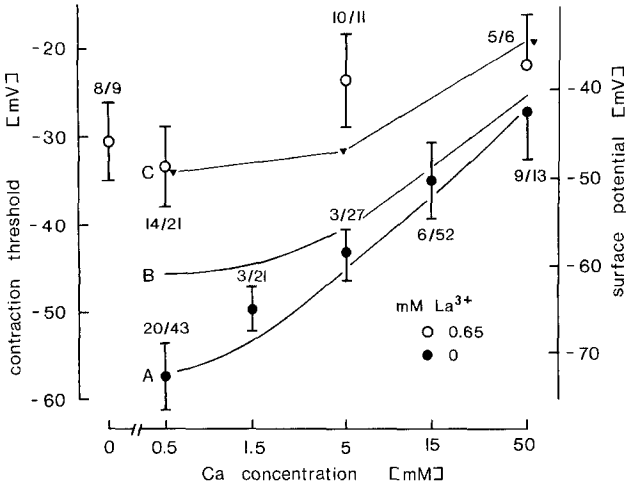

Fig. 2. The dependence of the contraction threshold (mean ± one SD) on $[Ca^{2+}]_0$ without (●) and with 0.65 mM La^{3+} (○). Numerals beneath symbols denote number of muscles and fibers investigated. Curves *A* and *B* are calculated surface potentials according to the surface charge model [Eq. (1) and Eq. (2), *see text*]. Curve *A*: surface potential in dependence on $[Ca^{2+}]_0$; curve *B*: surface potential for 0.65 mM La^{3+}-containing solutions. Surface potential values on "curve" *C* were obtained by taking adsorption of La^{3+} to neutral sites also into account (*see text*). Parameters are given in the legend of Fig. 6

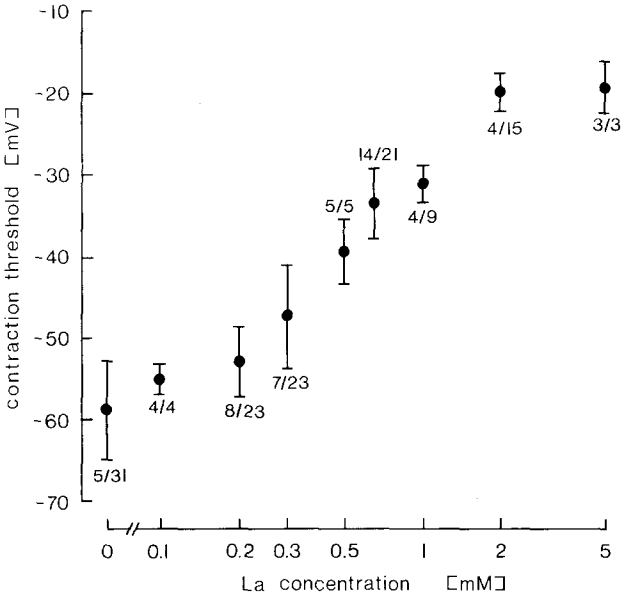

Fig. 3. The dependence of the contraction threshold (mean ± one SD) on $[La^{3+}]_0$ at constant $[Ca^{2+}]_0$ (0.5 mM). Numerals beneath symbols denote number of muscles and fibers investigated

La^{3+} with the membrane surface different to that proposed for Ca^{2+}.

Membrane Input Resistance

Hartz and Ulbricht (1973) reported that La^{3+} (1 mM) caused a 20% increase in membrane input resistance

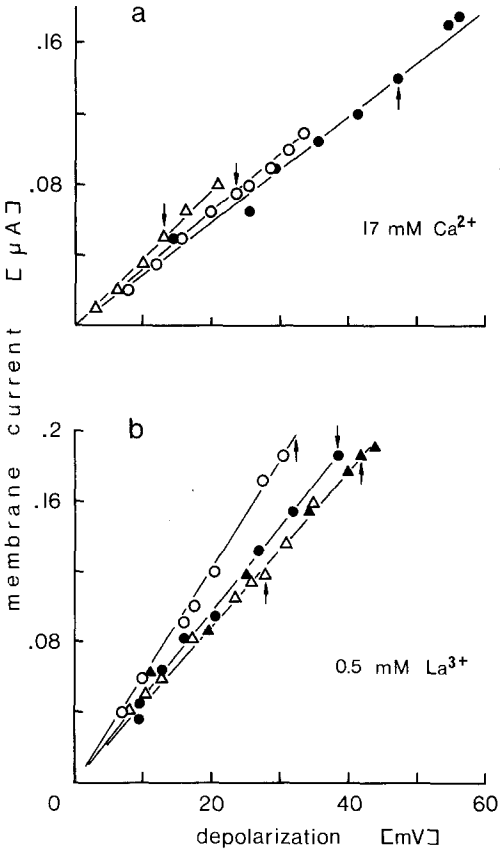

Fig. 4. Current-voltage relation of two fibers of two different muscles. *Abscissa:* Depolarization from resting potential; *Ordinates:* Corresponding membrane current. (*a*) (○) control value in 0.5 mM Ca^{2+}; (●) after 5 min in 17 mM Ca^{2+}; and (△) after 15 min of wash-out in 0.5 mM Ca^{2+}. (*b*) (○) control in 0.5 mM Ca^{2+}; (●) after 25 min in 0.5 mM La^{3+}; (▲) after 40 min in 0.5 mM La^{3+}; and (△) after 30 min of wash-out in 0.5 mM Ca^{2+}. La solutions contained 0.5 mM Ca^{2+} as well. Straight lines are calculated regression lines, the slope indicating membrane input conductance. For numerical values of the membrane input resistance, i.e. the reciprocal of membrane input conductance, *see text*. Arrows denote depolarizations at contraction threshold

for the crayfish giant axon. In some of the experiments presented here the input resistance was measured together with the contraction threshold. In most cases (six out of nine), an increase in input resistance was seen; in two fibers it decreased, and in one fiber it stayed constant.

Fig. 4 shows two typical experiments. Input resistance was taken as the regression line of the linear part of the current-voltage curve relating the depolarization from the resting potential to the membrane current (for determination of the membrane current *see* Materials and Methods). In Fig. 4, the straight lines represent the calculated membrane input conductance, i.e. the reciprocal of the membrane input resistance.

In Fig. 4*b*, the input resistance was $1.6 \times 10^5\ \Omega$ in 0.5 mM Ca^{2+} (open circles). After superfusing the

muscle with 0.5 mM La^{3+} the input resistance steadily increased from $1.9 \times 10^5\,\Omega$ after 25 min (closed circles) to $2.3 \times 10^5\,\Omega$ after 40 min (closed triangles). The corresponding contraction threshold (arrow) became constant at -45 mV only after 40 min in La^{3+}. A wash-out period of 30 min was sufficient to restore the contraction threshold to the control value of -59 mV, but the input resistance was not reversed after this period (open triangles). Subsequent superfusion with 1 mM La^{3+} had no further effect on input resistance (not shown in Fig. 4*b*); it remained at $2.4 \times 10^5\,\Omega$ even after another wash-out period of 30 min, whereas the contraction threshold was shifted to a less negative potential in 1 mM La^{3+} and returned to control value within 30 min.

For comparison, membrane input resistance was measured in 17 mM Ca^{2+} (Fig. 4*a*), a concentration having the same effect on contraction threshold as 0.5 mM La^{3+}. Results are from a different muscle than those in Fig. 4*b*. The input resistance increased slightly from $3.1 \times 10^5\,\Omega$ (open circles) to $3.4 \times 10^5\,\Omega$ after 5 min of superfusion (closed circles). At this time the contraction threshold had reached a constant value of -37 mV. After returning to 0.5 mM Ca^{2+} the input resistance was decreased by *ca.* 20% below the initial control value (open triangles). A similar relative change in input resistance has been obtained in a second fiber.

Action Potentials

La^{3+} has been reported to exert a strong effect on the activation and inactivation of the sodium current in nerve fibers (Hartz & Ulbricht, 1973; Vogel, 1974).

To see whether La^{3+} possesses similar effects in muscle fibers, the action potential was investigated in 0.3 and 0.5 mM La^{3+}, and the results compared to those of 17 mM Ca^{2+}. Higher concentrations of La^{3+} led to a conduction block within the first minutes of superfusion. Fig. 5 shows action potentials of different fibers of the same muscle with 1.5 mM Ca^{2+} (control, *A*, *C*, *E*) and with 0.5 mM (*B*) and 0.3 mM La^{3+} (*D*). In another muscle the effect of 17 mM Ca^{2+} (Fig. 5, *G*) was compared to that of 1.5 mM Ca^{2+} (*F*, *H*). It should be noted that the time scale in Fig. 5, parts *B*, *D* and *G*, is 1 msec as compared to 0.5 msec in the others. 1.5 mM Ca^{2+} had to be used in the control solution since with lower $[Ca^{2+}]_0$ fibers often began to contract spontaneously.

The rate of rise and fall of the action potential of La^{3+}-treated fibers was markedly slowed down, and the overshoot was decreased. These effects were not so evident with 17 mM Ca^{2+}. Quantitative results from the two muscles of Fig. 5 are given in Table 1.

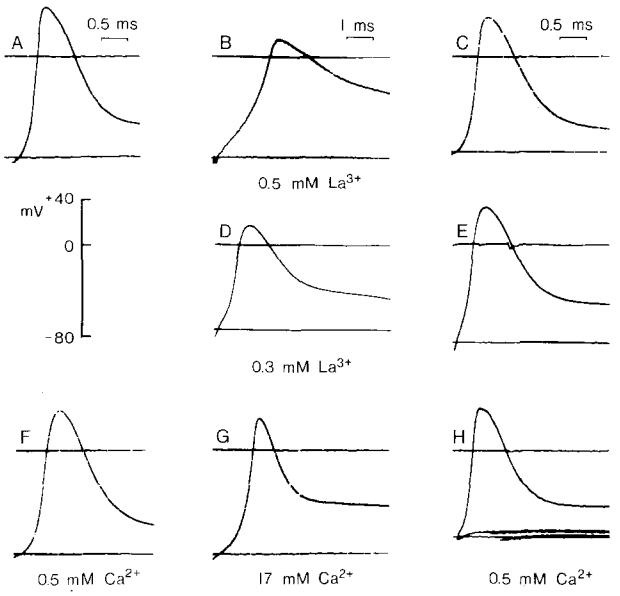

Fig. 5. Action potentials of different fibers of two muscles (*A–E* and *F–H*). (*A*) control in 1.5 mM Ca^{2+}, (*B*) after 10 min in 0.5 mM La^{3+}, (*C*) after 15 min of wash-out in 1.5 mM Ca^{2+}, (*D*) after 10 min in 0.3 mM La^{3+}, (*E*) after 15 min of wash-out in 1.5 mM Ca^{2+}, (*F*) and (*H*) control before and after treatment with 17 mM Ca^{2+}. (*G*) after 15 min in 17 mM Ca^{2+}. Note that time scale in control solution is half that in the test solution

Table 1. Overshoot, duration of conducted action potential at 0 mV (t_0) and half maximal amplitude (t_{50}) of one muscle in lanthanum and of a second in high calcium[a]

	Overshoot (mV)	t_0 (msec)	t_{50} (msec)
a) In lanthanum, experiment 28/78			
1.5 mM Ca^{2+}	$+38.8 \pm 4.4$ $n=7$	0.73 ± 0.06 $n=7$	1.08 ± 0.11 $n=7$
0.5 mM La^{3+}	$+17.4 \pm 2.3$ $n=7$	1.07 ± 0.24 $n=6$	3.13 ± 1.05 $n=6$
1.5 mM Ca^{2+}	$+36.5 \pm 3.1$* $n=3$	0.71 ± 0.11* $n=3$	1.11 ± 0.19* $n=3$
0.3 mM La^{3+}	$+21.9 \pm 4.0$ $n=7$	1.08 ± 0.23 $n=8$	2.86 ± 0.62 $n=5$
1.5 mM Ca^{2+}	$+29.3 \pm 4.4$ $n=4$	0.82 ± 0.08* $n=4$	1.45 ± 0.62 $n=4$
b) In high calcium, experiment 30/78			
1.5 mM Ca^{2+}	$+33.7 \pm 1.3$ $n=3$	0.77 ± 0.04 $n=3$	1.2 ± 0.06 $n=3$
17 mM Ca^{2+}	$+19.5 \pm 3.8$ $n=11$	0.77 ± 0.05* $n=11$	1.84 ± 0.25 $n=11$
1.5 mM Ca^{2+}	$+32.4 \pm 1.6$* $n=6$	0.73 ± 0.04* $n=6$	1.14 ± 0.09* $n=6$

[a] Mean $\pm$ SD; *n* denotes number of fibers; stimulus and recording electrode were *ca.* 1,000 µm apart; stimulus duration ranged from 0.3 to 0.6 msec; data follow experimental sequence.

* Denotes value not significantly different from value in the first run with 1.5 mM Ca^{2+}. Significance is taken as $p < 2\%$.

Table 2. Threshold of action potential generation, overshoot, and duration of action potential measured at 0 mV, t_0, and half maximal amplitude, t_{50}, of one muscle[a]

	Threshold (mV)	Overshoot (mV)	t_0 (msec)	t_{50} (msec)
1.5 mM Ca^{2+}	−46.1±3.3	+45.2±5.6	0.78±0.06	0.97±0.10
	$n=8$	$n=8$	$n=8$	$n=8$
17 mM Ca^{2+}	−30.2±5.9	+37.0±2.8	0.87±0.09*	1.36±0.13
	$n=11$	$n=11$	$n=10$	$n=8$
0.5 mM La^{3+}	−18.5±3.7	+40.9±6.7*	1.79±0.33	–
	$n=8$	$n=9$	$n=9$	
1.5 mM Ca^{2+}	−43.4±3.9*	+42.8±3.3*	0.92±0.08	1.17±0.12
	$n=8$	$n=8$	$n=7$	$n=8$

[a] Mean ± SD; n denotes number of fibers; data follow experimental sequence (for further details *see text*); stimulus duration was 5 msec; electrodes were placed *ca.* 100 µm apart.

* Denotes value not significantly different from value in the first run with 1.5 mM Ca^{2+}. Significance is taken as $p \leqq 2\%$.

The action potential threshold was measured in another preparation (Table 2). The threshold was shifted to less negative potential values by *ca.* 16 mV with 17 mM Ca^{2+}, and by *ca.* 28 mV with 0.5 mM La^{3+}. Table 2 shows the averaged data together with the mean overshoot, the mean action potential duration measured at 0 mV (t_0) and at half-maximal amplitude (t_{50}). Contrary to the values of Table 1, the mean overshoot in 0.5 mM La^{3+} was not significantly smaller than that obtained in 1.5 mM Ca^{2+}. The difference may be due to the fact that Table 1 gives results of action potentials which were conducted over *ca.* 1000 µ, whereas action potentials of Table 2 were recorded in the near vicinity of the stimulus electrode. Superfusion with the control solution for 45 min did not lead to a complete recovery of the action potential duration, whereas the action potential threshold returned to its initial value within the limits of error.

Discussion

The trivalent rare earth alkaline cation lanthanum has been shown to affect ion permeabilities in nerve and the activation of contraction in skeletal muscle in a similar way as calcium (Takata et al., 1966; Blaustein & Goldman, 1968; D'Arrigo, 1973; Hartz & Ulbricht, 1973; Andersson & Edman, 1974*b*; Dörrscheidt-Käfer & Lüttgau, 1974; Vogel, 1974). These authors reported a shift in sodium and potassium permeability curves to less negative potentials in a variety of nerve preparations at relatively low $[La^{3+}]_0$ (<1 mM), and an eventual block of action potential conductance after longer application or at concentrations of *ca.* 1 mM (Takata et al., 1966; Hafemann, 1969). The curve relating membrane depolarization to tension in skeletal muscle was shifted by 10–15 mV to less negative potentials by 0.1 mM La^{3+} (Andersson & Edman, 1974*b*). The same range of shift was experienced by Dörrscheidt-Käfer and Lüttgau (1974) with 0.15 mM La^{3+} while maximum potassium contracture seemed to be little affected.

The general idea could be that La^{3+}, as does Ca^{2+}, affects membrane permeabilities by a specific interaction with membrane-bound negative surface charges. The interaction can be binding or adsorption to surface charges, as was first suggested by A.E. Huxley in Frankenhaeuser and Hodgkin (1957). Another possible interaction is given by electrostatic screening of charges without a change in net charge density (Grahame, 1947). McLaughlin et al. (1971) adapted this approach with respect to conductance changes in lipid bilayer membranes, and Hille, Woodhull and Shapiro (1975) for the myelinated nerve fiber conductances. In an earlier paper the dependence of the contraction threshold on the concentration of monovalent and divalent cations has been described in a similar way (Dörrscheidt-Käfer, 1976).

$$\sigma_f = \frac{1}{G}\left\{\sum_{i=1}^{n} C_{io}\exp - z_i\psi_0 F/RT - 1\right\}^{\frac{1}{2}}. \quad (1)$$

Screening is formally expressed by the Grahame-equation (Eq. 1; Grahame, 1947). It relates the surface potential, ψ_0, to the ion concentrations in the bath, C_{io}, at constant free charge density, σ_f. G is a constant, z_i the valency of the i-th ion species, and R, T, and F have their usual meaning. Eq. (1) yields a maximal change in ψ_0 of 58 and 29 mV at 22 °C following a 10-fold change in monovalent and divalent cation concentration, respectively.

$$\sigma_f = \frac{\sigma_t}{1 + \frac{[H^+]_s}{K_H} + \frac{[Ca^{2+}]_s}{K_{Ca}} + \frac{[La^{3+}]_s}{K_{La}}}. \quad (2)$$

From Eq. (1) it follows that a 10-fold concentration change of the trivalent cation La cannot result in a change of ψ_0 larger than 19.3 mV. The shift becomes even smaller when binding of the cations to negative fixed charges is also taken into account [Eq. (2); σ_t = total negative charge density, K_i = apparent dissociation constants of ion species i; $[H^+]_s$, $[Ca^{2+}]_s$ and $[La^{3+}]_s$ are the concentrations at the surface with $C_{is} = C_{io}\exp - z_i\psi_0 F/RT$].

This is shown in Fig. 6 where curve A was calculated assuming screening of La^{3+} to be its only interaction with surface charges. Curve B shows the

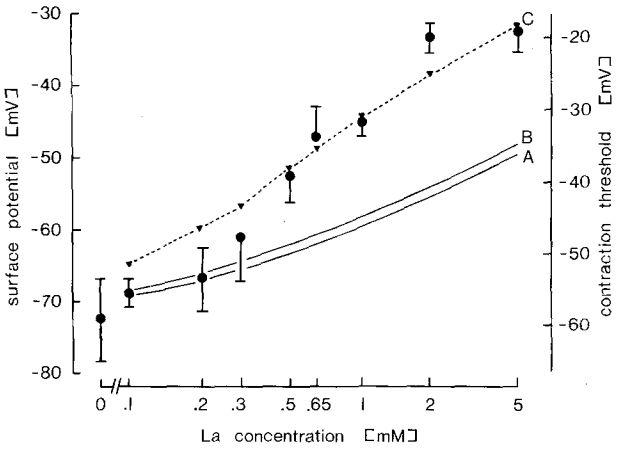

Fig. 6. The dependence of the contraction threshold (closed circles, same values as in Fig. 3) and the surface potential (curve *A*, *B*, and values on "curve" *C*) on $[La^{3+}]_0$. Curves were calculated assuming screening to be the only La effect [curve *A*, Eq. (1)], and considering binding as well [curve *B*, Eqs. (1) and (2)]. Values of "curve" *C* were obtained by assuming an additional adsorption of La^{3+} to neutral sites. Parameters are: $\sigma_t = -5.9 \times 10^{-3}/Å^2$, $K_H = 1.78 \times 10^{-3}$ M, $K_{Ca} = 4.5$ M, $K_{La} = 30$ M. Adsorption was expressed by $\Gamma_\infty = 3.2 \times 10^{-3}/Å^2$, and $K_e = 4.5 \times 10^{-4}$ M (*see* Fig. 7 and text)

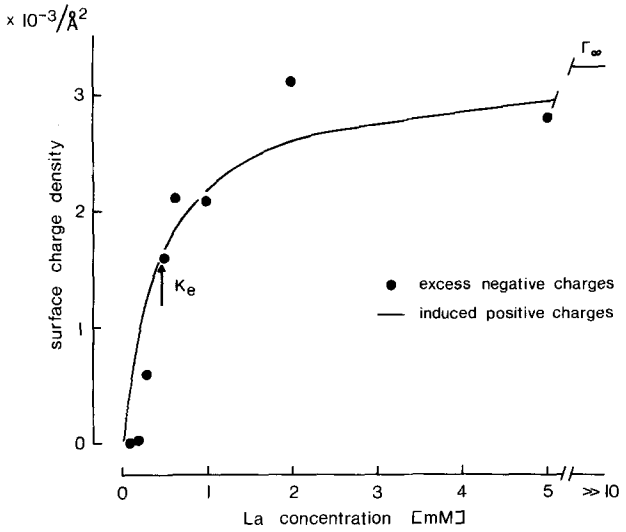

Fig. 7. The dependence of the density of "excess negative charges" on $[La^{3+}]_0$, which was obtained by subtracting the values of σ_f calculated for the surface potentials at the measured contraction threshold values (Fig. 6) from the negative net charge density calculated for curve *B* (Fig. 6) at each $[La^{3+}]_0$ investigated (filled circles). The smooth curve was calculated according to Eq. (3) (Langmuir adsorption isotherm) with $\Gamma_\infty = 3.2 \times 10^{-3}/Å^2$ and $K_e = 4.5 \times 10^{-4}$ M. It represents the positive charge density Γ induced by adsorption of La^{3+} to neutral sites

case when La binding was also considered. (For parameters see legend to Fig. 6.) σ_t was chosen to give $\psi_0 = -72.5$ mV at pH 6.5 and 0.5 mM Ca^{2+}. This value proved to be relevant in earlier investigations (Dörrscheidt-Käfer, 1979*a*, *b*). Ca^{2+} and H^+ were taken to bind to σ_t under all circumstances.

The two curves are parallel to each other as $[La^{3+}]_s$ is nearly constant independent of the bulk concentration. The parallel shift of curve *B* would increase upon decreasing K_{La} without a significant increase in steepness. It is clear from Fig. 6, that neither screening of La^{3+}, nor screening and binding accounts for the measured effect of La^{3+} on the contraction threshold, the dependence on $[La^{3+}]_0$ being considerably steeper then that of the calculated surface potential.

Curves *A* and *B* in Fig. 2 were calculated in the same manner: Curve *A* relates the surface potential to $[Ca^{2+}]_0$ (0 La^{3+}) following screening and binding (same parameters as for Fig. 6), curve *B* was obtained with the additional screening and binding of 0.65 mM La^{3+}. Again, curve *B* poorly reproduces the measured shift in contraction threshold.

A way out of this difficulty could be to consider La adsorption to neutral but amphoteric lipids. McLaughlin et al. (1971) have shown that polyvalent cations like UO_2^{2+} or Th^{4+} strongly bind to a bilayer membrane formed by the neutral phospholipid phosphatidyl ethanolamine inducing a positive surface potential.

To assess this problem the net negative charge density was calculated for curve *B* (Fig. 6), and for the contraction threshold values with Eq. (1), assuming a constant relation to the surface potential. The difference of these charge density values are those charges ("excess negative charges") which have to be compensated to obtain the full description of the contraction threshold behavior by surface charge effects. The "excess negative charges" are given in Fig. 7 in dependence on $[La^{3+}]_0$. The smooth curve in Fig. 7 was calculated by the Langmuir adsorption isotherm

$$\Gamma = \frac{\Gamma_\infty}{1 + \frac{K_e}{La_0}} \tag{3}$$

where Γ_∞ is thought to represent the maximal density of positive fixed charges induced by La binding to neutral sites, Γ the induced positive charge density at a certain $[La^{3+}]_0$, and K_e the equilibrium constant of this reaction. The bulk concentration $[La^{3+}]_0$ is to be taken since no surface charge, and hence no surface potential exists near neutral sites (McLaughlin et al., 1971). The calculated adsorption curve is in good agreement with the "excess negative

charges" so that their compensation by the induced positive charges may well be considered to be plausible.

The values of Γ were added to the free charge density, σ_f, calculated for curve *B* yielding the new net charge density, σ_f'. The surface potential for any σ_f' value was calculated by Eq. (1) and values given as filled triangles on "curve" *C*. The same procedure was undertaken in Fig. 2, where the Γ value at 0.65 mM La^{3+} was added to the calculated screening-binding curve (curve *B*). In both instances the agreement of the calculated surface potential with the measured contraction threshold values is satisfactory, although the range of low $[La^{3+}]_0$ and the "ceiling effect" at 2 and 5 mM La^{3+} were not completely reproduced. No reasonable fit could be obtained when considering the La concentration at the surface, $[La^{3+}]_s$, being relevant for adsorption as it is nearly constant independent of the bulk concentration.

The La effects on the action potential in the skeletal muscle fiber are in accordance with those reported by Andersson and Edman (1974*b*), and similar to those obtained by Hartz and Ulbricht (1973) in crayfish giant axon. The increased action potential threshold was already described by D'Arrigo (1973) for the crayfish giant axon. The drastic shift of the action potential threshold, as reported in the present paper, and the decreased overshoot may equally be due to an altered surface potential near the sodium channel, whereas the prolonged action potential duration may be seen in context with La affecting the potassium channel in this way. The charge density near the sodium channel should be somewhat different to that on the T-tubular wall near the triad, as La^{3+} had a stronger influence on the action potential than Ca^{2+} in concentrations that had equal effects on contraction threshold.

The irreversible increase of the membrane input resistance can best be explained by the well-known action of La^{3+} on Ca binding sites. In various tissues La^{3+} readily displaces Ca^{2+} from its binding sites (Langer, 1973; Martin & Richardson, 1979). Ca is necessary for membrane stabilization in linking the external lamina to the surface coat of the membrane by fucose-bridges (Cook & Bugg, 1974; Frank, Langer, Nudd & Seraydarian, 1977). La^{3+}, which normally has a higher affinity for Ca binding sites than Ca^{2+} itself, could replace Ca^{2+} from these sites hereby inducing a structural change which might be irreversible. This also could explain the irreversible increase of the membrane stiffness always experienced after La treatment.

An intracellular action of La^{3+} can be discarded as La precipitates only were found at extracellular sites such as the outer surface of the T-tubular wall (Fink, Grocki & Lüttgau, 1980).

To summarize La^{3+} affected the contraction threshold, the action potential and the membrane input resistance in a similar but more efficient way than Ca^{2+}. With respect to membrane input resistance, action potential duration and conduction La treatment even led to irreversible alterations. In a quantitative analysis it was shown that the Ca effect on contraction threshold may well be described by a surface charge model following the Gouy-Chapman theory of the diffuse double layer. This includes screening of and binding to negative charges supposed to be part of the surface of the T-tubular wall. This surface charge model did not account for the effect exerted by La^{3+} on the contraction threshold, its dependence on $[La^{3+}]_0$ being much steeper than that of the calculated surface potential.

A further interaction of La^{3+} with the surface of the triad had to be assumed consisting in an adsorption of La^{3+} to neutral but amphoteric sites. The interaction of La^{3+} with neutral sites induces additional positive charges leading to an overall less negative surface potential. Its dependence on $[La^{3+}]_0$ now yields a good description of the measured contraction threshold.

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