Effect of stretch on contractility and ionic contents of the sartorius muscle of *Rana pipiens*

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Rapoport, Stanley I., and Jeannette M. Bidinger. Effect of stretch on contractility and ionic contents of the sartorius muscle of *Rana pipiens*. Am. J. Physiol. 226(2): 452-457, 1974.—When sartorius muscles of frogs are soaked in Ringer at room temperature, they gain Na, lose K, and lose contractility with time. These changes are retarded by stretching the muscles to 20-30% of their rest length. Conditions which inhibit the Na pump, such as soaking muscles in ouabain, K-free, or cold Ringer, abolish the differences in ionic contents and contractility between stretched and unstretched muscles. However, ouabain and K-free Ringer accelerate and cold retards the changes that occur in unstretched muscles in Ringer at room temperature. The results suggest that stretch stimulates the Na pump, either directly or indirectly through its effect on general metabolism. Contractility lasts longer in those muscles whose K contents are increased either by stretch or by different conditions of soaking.

STRETCH OF SARTORIUS MUSCLE in vitro stimulates metabolism in several species of frogs and retards the loss of twitch tension (or muscle contractility) which takes place with prolonged soaking in Ringer (9, 17, 49). We wanted to assess the possible relation between these effects. Harris (23) suggested that stretch stimulates the Na pump, and since a high muscle K is correlated with retention of contractility (18), stretch might retard loss of contractility by promoting retention of muscle K through its effect on the Na-K exchange pump.

Harris’ (23) conclusion about the Na pump, however, is open to question. He showed that stretch increased Na tracer efflux equally at 18 and 0°C, although the Na pump is inhibited at 0°C (15, 33). Furthermore, changes in one unidirectional flux cannot be interpreted necessarily to indicate changes in net ionic content, since tracer flux in the other direction may change also. It thus seemed important to reassess the effect of stretch on net Na and K in the presence of the Na pump and when the pump was inhibited and to relate ionic changes to contractility, if possible. A report of this work has been published (43).

METHODS

A pair of sartorius muscles of *Rana pipiens* was dissected at room temperature (about 20°C) and mounted together with the attached pelvic bone in a solution stirred with bubbling 96.3% O₂-3.7% CO₂ (the CO₂ level in amphibian blood) (2). The solution (Table 1) was kept at room temperature (about 19–21°C) or at 2 ± 0.5°C by an external cooling system and contained 1.5 × 10⁻⁵ M tubocurarine to eliminate spontaneous miniature end-plate potentials (16, 29).

The pelvic bone was grasped with a hemostat, and the thial ends of the muscle pair were tied to FT-03 tension transducers with matched springs, whose self-resonant frequency was 330 Hz (Grass Instrument Co., Quincy, Mass.). Muscle length and tension were adjusted by raising or lowering the transducers with micromanipulators. The transducers fed into Type 900 strain-gauge couplers, which led to Dynograph amplifiers (type 482; Beckman Instruments, Schiller Park, Ill.) and a pen recorder.

One muscle was adjusted to rest length, or “the distance between tendons when legs are pulled into a straight line” (9). The second was stretched to 1.2–1.3 times rest length, the extension needed to stimulate metabolism (9). The muscles were stimulated through flat platinum electrodes along their sides every 1–3 h with a single 30- to 50-ms pulse or a single 50-ms pulse followed 10 s later by a 40-Hz train of 2-ms pulses lasting 0.5 s. Pulse voltages were adjusted to give maximum contractile responses.

Bathing solutions were replaced every 8 h or more often to reduce effects of evaporation or bacterial growth. Experiments were rejected if bacterial clouding occurred before contractility was lost.

Gastrocnemius muscles were kept in separate baths and were stretched from rest to about 1.09 rest length and initial tensions of about 186 g (Table 2, below). Tendons broke on contraction at further extensions. Contractile tensions were measured as for sartorius muscles. Since gastrocnemii have anoxic central fibers in vitro (26), we did not determine their ionic contents.

Ionic determinations. Na, K, and Mg were determined on sartorius muscles washed in Na- and K-free Tris Ringer for 10 min to elute extracellular Na and K (Table 1) (46), and the muscles were blotted on Whatman no. 541 filter paper. The muscles were cut from their tendons and weighed wet in silica crucibles of known weight. Muscles were less than 0.8 mm thick (wet weight between 40 and 80 mg) to secure more than 98% elution, assuming an extracellular Na diffusion coefficient as low as 2.6 × 10⁻⁶ cm²/s (12, 24).

The muscles were dried at 110°C for 24 h, weighed, and then ashed at 470°C (13). The ash was dissolved in 5 ml of 0.1 N HNO₃ containing 1% I₂ as I₂ (NO₃)₂ (solution A,
TABLE 1. Composition of solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>Bathing Solutions*</th>
<th>Rel O.P.</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na, mM</td>
<td>120</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>K, mM</td>
<td>12.5</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>Cl, mM</td>
<td>2.15</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>Ca, mM</td>
<td>0.85</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>HPO₄₂⁻, mM</td>
<td>2.15</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>H₂PO₄, mM</td>
<td>0.85</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>Trizma, mM</td>
<td>120.1</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>Ringer, mM</td>
<td>0.12</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>K⁺ free, mM</td>
<td>1.05</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>Trizma, mM</td>
<td>120.1</td>
<td>1</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Stock solutions for ionic determination: Solution A: take-up solution—0.1 N HNO₃; 1% La as La(NO₃)₃. Solution B: μg/ml—Na-50, K-200, Mg-25, Ca-5, as chlorides. Solution C: μg/ml—Na-50, K-200, Mg-25, Ca-5, as chlorides; 1% La as La(NO₃)₃; 0.1 N HNO₃. Rel O.P. = relative osmotic pressure. * All solutions contained 1.5 X 10⁻⁵ M d-tubocurarine chloride. † Determined by freezing-point depression. $ pH of aerated solution.

Table 1. La stops phosphate from interfering with atomic absorption measurements of Ca and probably of Mg as well (6, 41).

Diluted take-up solution was used to determine Na and K with flame emission and Mg with atomic absorption spectrophotometry (model 153 spectrophotometer, Instrumentation Laboratories, Lexington, Mass.). Reference solutions, made by diluting stock standard B (Table 1) with distilled water, contained the correct amounts of La(NO₃)₃ and HNO₃.

Ca was determined by dissolving the ash of muscles not washed in Tris Ringer in 5 ml of solution A and by using atomic absorption spectrophotometry. Readings from take-up solutions were compared with those of dilutions of stock standard C with equivalent amounts of La(NO₃)₃ and HNO₃.

RESULTS

Contrastility. The time course of changes in twitch and tetanic contractions in Ringer soak is illustrated in Fig. 1 for a stretched and unstretched pair of sartorius muscles. Stretch retards by several hours the decline of contractility that appears after 24 h of soaking. Resting tension of the stretched muscle falls by half after 30 min in soak, then decreases more slowly to 25-30% of its initial level within 24 h (Table 2). The initial adjustment in resting tension, or stress relaxation, is accelerated discontinuously by a twitch or tetanus, as shown in Fig. 2 (1). Contraction height increases when resting tension falls.

Table 3 shows that when contractile tensions have fallen below 30-40% of their initial values in Ringer, stretch has retarded loss of twitch and tetanic contraction by about 4 h (P < 0.05). Retardation is found also in gastrocnemius muscles (cf. 49). In ouabain Ringer, the potentiation of contractile tension following stress relaxation of stretched sartorius muscles continues until contraction falls below 20% of its initial value. For the later part of the soak, however, stretch in ouabain Ringer does not retard the loss of contractility, as it does in Ringer.

Cold and K-free Ringer, as well as ouabain Ringer, inhibit the Na pump (7, 33). Table 3 illustrates that stretch does not retard the loss of twitch contraction of sartorius muscles bathed in these solutions. The solutions themselves modify the rate of loss of contractility. K-free and ouabain Ringer accelerate it and cold retards it.
The presence of stretch on Na uptake and K loss in individual muscles at zero time was only about 364 mmol/kg dry wt of Na for an equivalent quantity of K, but K content showed that muscles in Ringer exchanged about 22 mmol/kg dry wt. The differences in net water content.

Table 5 illustrates the effects on Na, K, and Mg of soaking muscles in different solutions and of stretch. Changes in Table 5 represent changes in intracellular ion concentrations because Na and K were eluted from the extracellular space before analysis and Mg was absent from the bath. The Na and K contents of freshly dissected sartorius muscles agree with published values, but the Mg content is somewhat higher (5, 14, 20, 40, 41).

After 18-24 h in Ringer at room temperature, muscles have gained about 30 mmol Na/kg dry wt and have lost about 90 mmol K/kg dry wt. Although these changes are not calculated for paired muscles, it is apparent that K gain exceeds Na loss. This imbalance is due to the exchange of H for K at the Ringer pH of 6.3 (18). Bianchi (5) found that muscles soaked in Ringer for 6 h accumulate about 2.5 mmol/kg dry wt. Since stretch decreases muscle Na uptake by 19 mmol/kg dry wt and reduces K loss by 28 mmol/kg dry wt. These mean reductions are not significantly different from each other (P > 0.05), and the influence of stretch on Na uptake and K loss in individual muscles can be represented as an effect on a 1:1 exchange (Fig. 3). Muscles also lose Mg at a ratio of 1 Mg for every 10 Na gained or 10 K lost (Fig. 3, Table 5). The 1:1 correlation with K loss, but not with Na gain, holds also in the cold. The stretch-induced change in muscle Mg may be related to direct effects on Na or H (20, 39).

Muscules in the cold were sampled after soaking for 18-24 h, and those in ouabain and K-free Ringer were sampled after 6-8 h in order to compare them all at about 50% loss of initial contractility (see Fig. 1, Table 3). Ouabain accelerates the Na uptake and K loss found in Ringer (Table 5). A retardation of K loss by stretch is not seen, but Na uptake is accelerated in stretched muscles soaking in ouabain rather than retarded. K-free Ringer accelerates and cold retards the Na and K changes of control muscles. Cold, 2°C (tw) and Ringer (te t) 5 41.7 31 2.7* 47.8 3~ 3.5 50.1 zt 5.0 53.1 & 6.5 2.8 zt 7.4 9.0 zt 4.6 13.9 rt 2.8* 13.7 Zt 2.2* Values are means ± SE. Tetanic (tet) and twitch (tw) tensions are calculated as percentages of initial (zero time) tensions. Tetanic measurements were always done with twitch measurements on the same muscle pair, but twitch measurements were sometimes done without tetanic stimuli. The twitch measurements done together with tetanus and done without tetanus are lumped together under the tw heading in the table. * Mean significantly different from zero (P < 0.05).
TABLE 5. Ionic and water contents of sartorius muscles under different experimental conditions and with and without stretch

<table>
<thead>
<tr>
<th>Exp. Condition</th>
<th>No. of Pairs</th>
<th>Percent H2O</th>
<th>Dry Wt, mmol/kg, Unstretched</th>
<th>Dry Wt, mmol/kg, Stretched-Unstretched</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unstretched</td>
<td>Stretched, unstretched</td>
<td>Na</td>
</tr>
<tr>
<td>Ringer, 0-4 h</td>
<td>11</td>
<td>79.6±0.4</td>
<td>±0.34±0.1</td>
<td>27.6±2.3</td>
</tr>
<tr>
<td>Ringer, 18-24 h</td>
<td>19</td>
<td>79.9±0.6</td>
<td>-0.93±0.4*</td>
<td>66.5±9.4</td>
</tr>
<tr>
<td>Cold, 2°C, 18-24 h</td>
<td>10</td>
<td>78.7±0.6</td>
<td>-0.83±0.3*</td>
<td>16.0±2.7</td>
</tr>
<tr>
<td>10⁻⁶ M ouabain, 6-8 h</td>
<td>11</td>
<td>78.7±0.3</td>
<td>-1.4±0.5*</td>
<td>55.0±1.9</td>
</tr>
<tr>
<td>K⁺ free, 6-8 h</td>
<td>11</td>
<td>78.0±0.4</td>
<td>-1.04±0.3*</td>
<td>80.3±7.8</td>
</tr>
</tbody>
</table>

Values are means ± SE for unstretched muscles and as differences between stretched and unstretched muscles. Differences for Ringer at 0-4 h are given as ± and represent experimental error and biological variation. Ionic contents represent only intracellular contents, since K and Na were washed out from the extracellular space and Mg was not included in the bathing solution. * Mean significantly different from zero, P < 0.05.

ATPase activity, Ca binding by the sarcoplasmic reticulum, or excitation contraction coupling (5, 8, 34). After 24 h in Ringer, both contracture sensitivity to caffeine and contractility to electrical excitation have fallen when resting and action potentials have not (35). The resting potentials calculated for the muscles whose ionic contents are given in Table 5, using the constant field expression for potential when PNa/PK = 0.01 (20), are about -80 mV, well above contraction threshold (32).

Although a pH of 6.3 led to an excess of K lost as compared to Na gained by both stretched and unstretched muscles, stretch effects on both Na and K could be distinguished from these base-line changes because the paired muscles were studied together in the same soak. Stretch produced a retention of K and a reduced uptake of Na at a ratio of 1:1, superimposed on the base-line changes. These effects would be expected if the Na pump were stimulated (42). A low pH does not stop the Na pump (25), and the experimental conditions that inhibit it—ouabain, cold, and K-free Ringer—also abolished the specific effects of stretch on ionic contents and muscle contractility.

Mg did not differ significantly (P > 0.05) between unsoaked muscles and those soaked for 18-24 h in Ringer, and equivalent changes in Ca are expected from soaking in Ringer at pH 7.0 (5). Retardation of loss of contractility by stretch in Ringer at pH 6.3 is comparable to retardation found by Weiss at pH 7.0 (49) and is observed in gastrocnemii, in which central fibers would be expected to be both anoxic and acidic (26).

Accelerated uptake of Na and loss of K, as found in K-free and ouabain Ringer, might obscure a stretch effect, whereas reduced changes in cold might obscure further reduction by stretch; taken together, however, these observations suggest that Na pump inhibition reduces or eliminates a stretch effect on ions. Weiss (49) pointed out that stretch need be applied only in the later stages of soak to reduce loss of contractility, whereas we show that ionic effects occur at least at the midpoint. They may precede any retardation effect on contractility.

The results lend support to Harris’ (23) conclusion that stretch stimulates the Na pump. However, the effect of stretch on 1:1 exchange of Na for K differs from his finding that 10% stretch did not affect K content (22), possibly because a 20-30% stretch is required to stimulate metab-

studied. Ouabain and K-free Ringer accelerate loss of contractility, uptake of Na, and loss of K, and cold retards these changes. In Ringer, stretch retards loss of contractility and of K but does not have a significant effect on either in cold, K-free, or ouabain Ringer. Muscles in different bathing solutions have approximately equal contractilities (Tables 3, 5). In cold Ringer, however, contractility remains above 40% for 24 h but Na content is lower and K content higher than for muscles in Ringer with an equivalent loss of relative contractility.

The intracellular ionic environment may affect myosin
within close to normal limits longer than at room temperature. Conditions are even more favorable in cold, when high-

3) and a reduced rate of change of muscle Na and K (Table 5) are in accord with other observations (15, 22). The Na pump would appear to operate well enough in cold, although at a lower rate (7, 15), to maintain ionic contents within close to normal limits longer than at room temperature. Decreased pumping in cold would have to be compensated by lowered Na and K permeability so as to reduce passive ionic leakage (15, 21, 22, 47; see below).

The difference in Na content between muscles soaked in cold for 18–24 h and those kept at room temperature for 0–4 h is probably not biologically significant. Paired muscles are not compared under these conditions, and soaking at room temperature for even a short time (cf. 5) can produce the small difference shown in Table 5.

The sartorius uses about 1.6 μmol ~ l/g per h at 0°C (36, p. 202). Thirty percent of this could support an active Na efflux of 2 pmol/cm² per s, assuming an extracellular space of 130 ml/kg and a fiber radius of 10 μ (42). At 21°C, Na efflux and influx are each about 4 pmol/cm² per s (27), and a Q10 of 2–3 (15) would reduce this flux to below 2 pmol/cm² per s at 0°C. As noted, decreased pumping in the cold would require lowered ionic permeabilities to maintain ionic concentrations unchanged (15, 21, 47).

Although unstretched fibers probably deteriorate in Ringer at room temperature (19, 45), in principle they have an adequate energy supply to maintain ionic contents. Winter frogs use about 175 cal/kg per h (10). Oxidation of lactic acid produces about 100 cal/g per kg, as calculated from the O₂ consumption of 0.5 mm³/g per min (26, 31). Together with the energy stores of 100 μmol/g glucose (as glycogen), 20–30 μmol/g phosphocreatine, and 5–7 μmol/g ATP (37, 38), normal ionic contents could be maintained for about 55 h, about the length of retention of contractility. Conditions are even more favorable in cold, when high-energy phosphate is consumed at a rate of 1.6 μmol/g per h (37). From the O₂ utilization rate of 8 × 10⁻² mm³/g per min (26), about 1.3 μmol/g per h of ATP would be expected to be produced by lactic acid oxidation (51). Together with the energy stores, muscles would be expected to maintain ionic contents far beyond the time in which contractility is lost (40).

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REFERENCES


