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Mechanism of Inotropic Action by Hypotonic Solution in the Frog Atrial Muscle

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Abstract The effects of hypotonic solution on the mechanical activities and action potential of the bullfrog atrium were investigated. Exposure of muscle to hypotonic solutions (70% of normal solution) produced initially a transient increase in twitch after which twitch declined below the control level. The response is independent of the kinds of salts withdrawn to make the medium hypotonic and of the presence of beta-blocker (5×10^{-7} M propranolol). The resting potential and the plateau level of action potential were little changed initially. When the twitch declined, a small amount of depolarization and a shortening of action potential duration were observed; however, the plateau level of action potential was not reduced. The initial increase in twitch was not observed, and only the gradual decline of twitch remained in the caffeine containing hypotonic solution. The weight of muscle increased 10% in the hypotonic solution. The resting tension was also increased trasiently and then declined to reach a maintained plateau with exposure to hypotonic solution. In the 0-Ca^{2+} or caffeine containing medium, the transient component of contracture was suppressed but the plateau tension remained. It is suggested that the initial transient increase of twitch by the perfusion of the hypotonic solution was induced by the Ca^{2+} released from the sarcoplasmic reticulum (SR), and the resultant decline of twitch resulted from the depletion of Ca^{2+} from the SR and/or from the shortening of action potential duration.

Key Words: cardiac muscle, hypotonic solution, sarcoplasmic reticulum, contraction.

In the previous study on the twitch potentiation by hypertonic solution in the frog atrial muscle, it was suggested that an elevation in the myoplasmic calcium content plays an important role in the positive inotropic action (KAWATA *et al.*, 1983). The reversed mechanism is expected when the muscle is exposed to hypotonic fluids. There is some evidence that hypotonicity does affect the contraction

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of heart muscle, although the effects are complicated, an initial phasic increase of twitch which then decreases below the original level was observed (KAWATA *et al.*, 1974; CHAPMAN, 1978). The application of strongly hypotonic solutions evoked a contracture (KAWATA and KAWAGOE, 1975) and in Na-free fluid the contracture induced by reducing the tonicity increased in size (CHAPMAN, 1978). These contractures are unaffected by removal of ionic Ca from the bathing medium.

The rather complicated effects of hypotonic Ringer solution on the heart are caused partly by reduction of the $[Na^+]_o$ which will modify the myoplasmic $[Ca^{2+}]$ through the Na^+-Ca^{2+} exchange mechanism and affect contractility (see CHAPMAN, 1983; for review). In the present work, when the tonicity was lowered, muscle fibers had been first equilibrated on the isotonic low-Na solution and then bathed in the hypotonic low-Na solution without altering the $[Na^+]_o$. The study was undertaken to further elucidate the effects of hypotonic solutions on contraction and action potential of the frog atrium.

MATERIAL AND METHODS

Small strips consisting of a few trabeculae, 50–200 μm in diameter and 2–3 mm long, were excised from the atrial wall of frogs (*Rana catesbeiana*). They were mounted in a rapid perfusion chamber, where the replacement of fluid occurred within about 3 sec. An initial 1 hr period of equilibration in the normal Ringer solution was allowed before exposure to the low Na solution for another 1 hr. The muscle fibers were stimulated with 5 msec square pulses (50% above threshold) at 12/min through platinum electrodes placed parallel to the long axis of the fibers.

The constituents of the solutions used in these experiments are listed in Table 1. The osmolarity of all the solutions listed in Table 1 was examined by freezing-point depression osmometer (Advance, 3W). The osmolarity of the normal Ringer solution was around 200 mOsm; this value is very close to that obtained on the blood plasma of frogs (177–210 mOsm) determined by the osmometer. The value of the osmolarity of hypotonic solutions was chosen and fixed at 70% of the normal osmolarity since the muscle became less excitable at lower osmolarity due to the inevitable reduction of $[Na^+]_o$. The solutions were equilibrated with 100% O_2, and pH of all these solutions was adjusted to 7.2 with NaOH. Reagents except propranolol (ICI) and l-isoproterenol (Nikken Chemicals, Co., Tokyo) were all guaranteed grade and purchased from Nakarai Chemicals, Ltd., Kyoto.

Twitch tension and intracellular action potentials, or in many experiments twitch tension only, were displayed on a storage oscilloscope (Tektronix, 7313) with camera (Tektronix, C50) and simultaneously recorded on a pen-recorder (San-ei Sokki Co., 8K). For force measurement, a strain gauge transducer (Minebea, Co., Ltd., UL-2GR) was used. A conventional glass microelectrode, filled with 3 M KCl and having a resistance of 10 to 20 MΩ, was used for recording action potentials.

Table 1. Composition of the various Ringer solutions used in the present experiments.

	Ringer solution	NaCl	KCl	$CaCl_2$	$MgCl_2$	HEPES	Glucose	Sucrose	LiCl	Caffeine
I	Normal	85	5	2	2	5	10	—	—	—
II	Sucrose, 40-Na	40	5	2	2	5	10	81	—	—
III	Li, 40-Na	40	5	2	2	5	10	27	29	—
IV	Hypotonic, 40-Na	40	5	2	2	5	10	27	—	—
V	Caffeine, 40-Na	40	5	2	2	5	10	54	—	30
VI	Caffeine, hypotonic, 40-Na	40	5	2	2	5	10	—	—	30
VII	0-Ca, 40-Na	40	5	0	2	5	10	86	—	—
VIII	0-Ca, hypotonic, 40-Na	40	5	0	2	5	10	32	—	—

Numbers are expressed in mM and conversion tables ("Handbook of Chemistry and Physics," 63rd ed., CRC Press) was used to calculate the osmolarity of sucrose and LiCl.

In order to estimate the volume change due to reduction of tonicity we measured the muscle volume according to the method previously stated (KAWATA *et al.*, 1983). The measurement was performed on small sections of about 20 mg separately from that of tension.

All experiments were done at room temperature (18–24°C).

RESULTS

Effect of the hypotonicity on twitch tension

NaCl must be deducted from the isotonic Ringer fluid to lower the tonicity. In such a condition, decreasing the tonicity (from solution I to IV) results in an initial increase in the twitch tension followed by a gradual decrease in twitch (Fig. 1A), and when the perfusion time is long enough, the amplitude of the twitches decreases below the control level (not shown). On returning to the solution of normal tonicity, a rapid reduction in the strength of the twitches was caused and followed by slow recovery to the control level (Fig. 1A). After the muscle fibers have been incubated for 1 hr in low-Na Ringer solutions (solution II or III), exposure to the hypotonic solution results in a rapid but somewhat smaller increase in the twitches, and the following decline in twitch occurs more rapidly and profoundly. Returning the fibers into the isotonic solution, however, does not induce the rapid reduction (Fig. 1B and C).

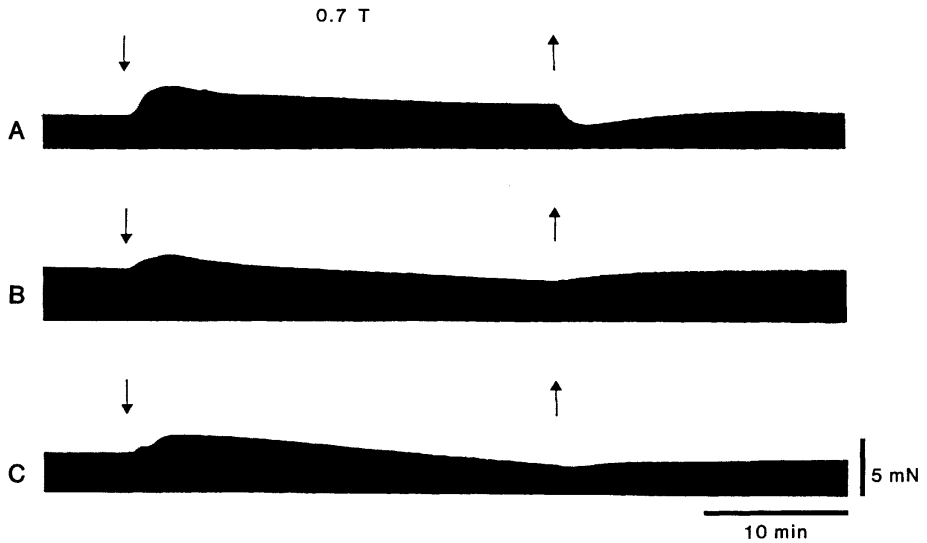

Fig. 1. Effect of hypotonicity (70% of normal) on twitch contraction. Fibers were pre-incubated in (A) normal Ringer solution, (B) low-Na (sucrose) solution, and (C) low-Na (LiCl) solution. Hypotonic solutions were perfused at downward arrow and withdrawn at upward arrow. Note the disappearance of the transient decrease in twitch on returning to isotonic solutions in B and C.

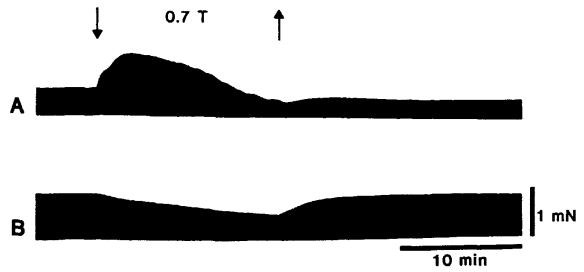

Fig. 2. Effect of caffeine on the initial increase in twitch. Solution II in Table 1 was used as a control solution. A: Control (solution II to IV in Table 1). B: Hypotonicity in a caffeine (30 mM) containing solution (solution V to VI in Table 1).

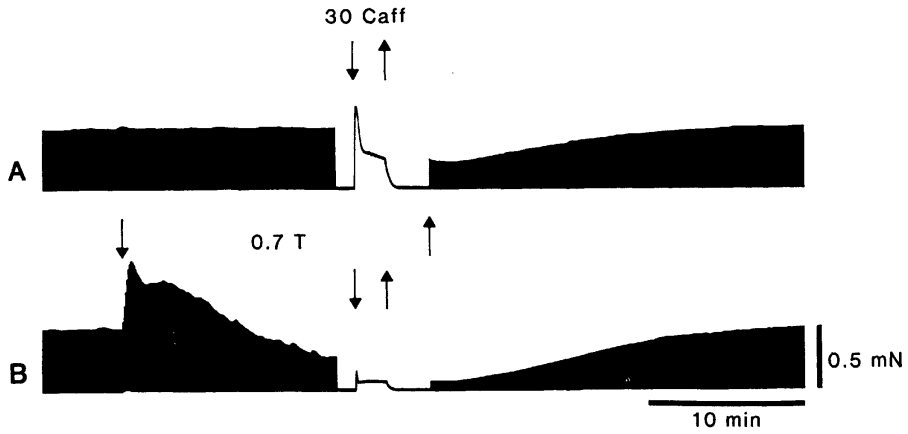

Fig. 3. Caffeine contracture in the hypotonic solution. Contractures with 30 mM caffeine were induced in (A) isotonic (solution II to V in Table 1) and hypotonic (solution IV to VI in Table 1) solution. In B tonicity was lowered at the first downward arrow and at the second one 30 mM caffeine was applied.

In some cases, although the cause is unclear, the initial rapid increase in twitch by hypotonic solutions occurred in two steps (Fig. 1C; see also Figs. 2A and 3B).

Caffeine on the inotropic action of hypotonicity

When the muscle was exposed to the 40 mM Na Ringer solution containing 30 mM caffeine (solution V) the resting tension subsided to the original level within 20 min. Although there are some discrepancies among preparations with regard to the caffeine effect on surface membrane, in frog atrial muscle cells the enhancement of calcium inward current was suggested (KIMOTO *et al.*, 1974; NIEDERGERKE and PAGE, 1981). The amplitude of twitch remained at about 70% augmented level but did not exceed the maximum level which might be attained by hypotonicity. At this stage an exposure to the hypotonic solution (solution VI) did not

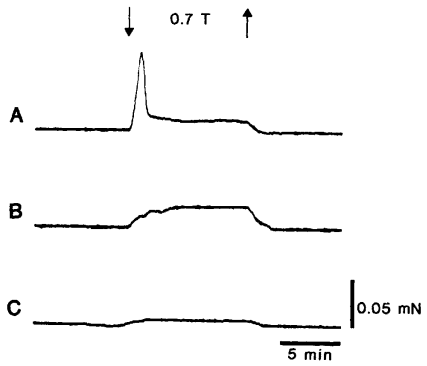

Fig. 4. Effect of hypotonicity on resting tension. Single thin trabecula was used in this experiment to reduce the diffusion delay. Solutions were made hypotonic in (A) control solution, (B) Ca free solution, and (C) 30 mM caffeine containing solution (from solutions II, VII, and V to IV, VIII, and VI, respectively, in Table 1).

induce the initial increase in twitch, and only the monotonic reduction in the twitch tension remained (Fig. 2).

Caffeine contractures were greatly suppresed in the hypotonic solution when caffeine was applied after the twitch tension had been decreased below the control level by the hypotonic solution (Fig. 3). In Figs. 2A and 3B, the resting tension increased slightly and trasiently at the beginning of the perfusion with the hypotonic solution. To investigate this effect in detail, a single trabecula, the diffusion delay accompanying solution changes being minimized, was used without twitch. Exposure of such a thin fiber to the hypotonic medium (solution IV) produced a rapid rise in the resting tension, after which the tension declined to reach a sustained level (Fig. 4A). When the fibers were exposed to the hypotonic medium (solution VIII) after preincubating in the Ca^{2+} free medium (solution VII) until the twitch had disappeared (not shown), the initial transient rise in the resting tension was abolished; however, the maintained tension remained at a higher plateau level (Fig. 4B). The abolition of the rapid rise in the resting tension also occurred when the fibers had been equilibrated in a caffeine (30 mM) containing medium (solution V), and the amplitude of plateau tension was smaller than that seen in A and B (Fig. 4C).

Change in the muscle weight by hypotonicity

Rapid increase followed by slow decrease in tension are observed by exposure to the hypotonic solution. The increase occurs within 3 min and the decrease lasts for 30 min or more. When muscles were soaked in the hypotonic medium (solution IV), they could be expected to gain water and thus increase in volume and weight. This may consequently lower the intracellular concentration of electrolytes. Figure 5 shows the time course of the change of muscle weight ob-

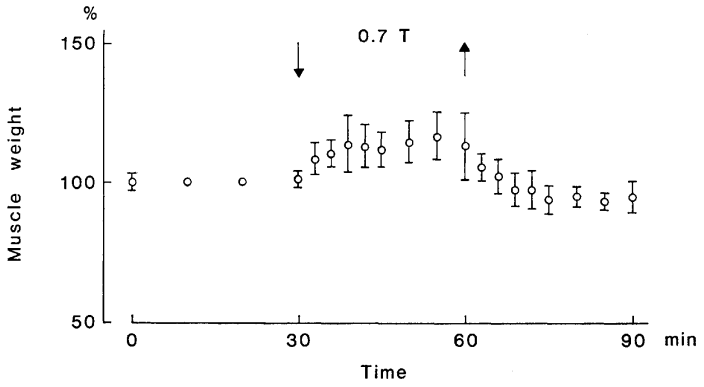

Fig. 5. Time course of changes in muscle weight exposed to hypotonic solution (solution IV). Mean of five quiescent strips (mean weight 20 mg) was expressed as percent of mean weight during the equilibration for 30 min in control solution (solution II in Table 1). Bars on each point represent standard error.

tained from five experiments. The increase in muscle weight occurred with the time course corresponding to the slow decrease in tension, and muscle weight increased 10% during the 30 min perfusion of hypotonic solutions. In the bullfrog ventricle, a 20% increase was obtained when exposed to the hypotonic solution (down to 50% of normal tonicity) (KAWATA *et al.*, 1974). The muscle preparation used for the measurement of weight was composed of multifibers, thus it might take more time for diffusion than in the muscle fibers used for tension measurements. Since an accurate measurement of weight was not possible in a short period, it is not clear whether any transient change in weight occurred in the 3 min at the beginning of the soaking.

Effects of hypotonic solutions on the membrane potential

When the fiber had been incubated in the low-Na Ringer (solution II in Table 1) for 1 hr, the action potential plateau was somewhat depressed by the low $[Na^+]_o$ and the twitch accompanied was larger (Fig. 6A, marked C) compared to those usually seen in normal Ringer solutions (not shown). Switching a solution to the hypotonic one (solution IV) increases the action potential duration and the twitch tension within a minute (Fig. 6A, no mark). Then the action potential duration shortens markedly, corresponding to the reduced twitch by exposure to the hypotonic medium for 10 min (Fig. 6B, no mark). The change in resting potential was less obvious at the beginning of perfusion (Fig. 6A), but the small amount of depolarization (about 4 mV) was measured in 10 min (Fig. 6B).

DISCUSSION

The initial increase and subsequent suppression in atrial muscle twitch due to

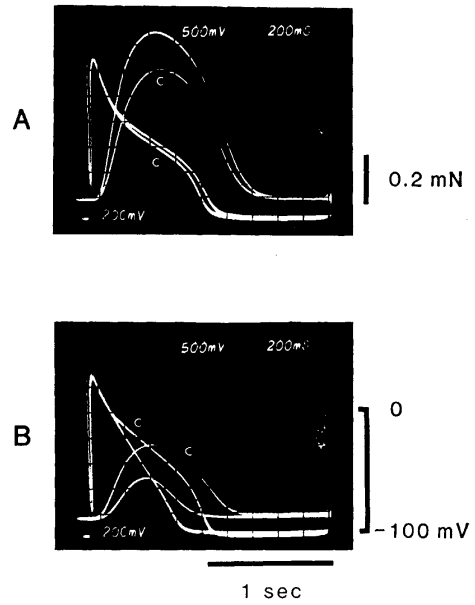

Fig. 6. Effects of hypotonicity on the action potentials and twitches. A: Action potentials and twitches are photographed after equilibration for 1 hr in low-Na solution (solution II) (marked C) and after 1 min perfusion with the hypotonic solution (solution IV). B: Taken at 10 min in the hypotonic solution (solution IV) and 11 min after switching back to the isotonic solution (solution II) (marked C). The data in A and B were obtained from the same fiber.

hypotonic treatment reported in the present study have not been obtained previously. The initial increase in the twitch tension is hardly explained by the mechanism opposite to the one proposed for the twitch potentiation by a hypertonic solution (KAWATA *et al.*, 1983). However, the phenomenon is similar to the one called "off-response" produced by a switch back to the control Ringer solution from a hypertonic solution (KAWATA and KAWAGOE, 1975; CHAPMAN, 1978). Since the initial twitch potentiation was not abolished with the β-blocker, it is not attributed to an endogenous catecholamine release (not shown).

Lowering the tonicity of the Ringer fluid must be accompanied with the reduction of the $[Na^+]_o$. It is possible to activate tension through the Na^+-Ca^{2+} exchange mechanism when $[Na^+]_o$ is lowered (see CHAPMAN, 1983; for review). In the present experiments, the heart has been equilibrated in the low-Na solution for a sufficient time to reach the steady but increased twitch tension before exposure to hypotonic solutions. In the low $[Na^+]_o$ condition, caffeine can cause the release of Ca^{2+} from the SR of cardiac muscle (SUZUKI, 1962; CHAPMAN and MILLER, 1974). It has been shown that 30 mM caffeine is enough to release most of the Ca stored in the SR of mammalian cardiac skinned fibers (ENDO and

KITAZAWA, 1978). The initial increase in twitch was absent when the fibers were exposed to the hypotonic solution after incubation in 30 mM caffeine containing isotonic solution. It was suggested that a reduction of 30 mM potassium methanesulfonate to make a solution hypotonic was effective to induce a release of calcium from heavily loaded SR in a skinned fiber from the skeletal muscles of toads (ENDO and THORENS, 1975). Thus the twitch potentiation induced by the hypotonic solution here can be attributed to the release of Ca^{2+} from the SR.

The cell hydration estimated from the measurement of muscle weight when the strips were soaked in the hypotonic solution (70% of normal solution) for 30 min may lower the intracellular concentration of electrolytes. The time course of water gain was comparable to that of the decline in twitch tension. Not all electrolytes are likely to play a role in the observed decrease in the twitch tension. A reduced $[K^+]_i$ may account for the depolarization of the cell membrane at a late stage of perfusion. Depolarization by about 25 mV with the hypotonic solution (12.5% of normal tonicity) in the frog ventricle (KAWATA *et al.*, 1974) and by 5 mV (67% of normal tonicity) in the rabbit papillary muscle (AKIYAMA and FOZZARD, 1975) were shown. In the present experiment, since the depolarization was small it is not plausible to cause inactivation of the Na channel and to reduce the excitability of muscle cells. A decreased $[Na^+]_i$ may reduce the contractility through the Na^+-Ca^{2+} exchange. Among them, the lowered $[Ca^{2+}]_i$ and Ca stored in the SR would affect more directly and profoundly the muscle contractility. The size of caffeine contracture in the hypotonic solutions was far less than that in the isotonic solution. The level of plateau of action potentials, the genesis of which is sensitive to the slow inward calcium current, was less changed in the present work. Thus the negative inotropic effects of lowering the tonicity may mainly be due to the depletion of Ca^{2+} from the SR in the heart muscle cells.

The transient increase in the resting tension was absent in both the caffeine-containing and calcium-omitted solutions. Hence the sharp increase in the resting tension would also be induced by the release of Ca^{2+} from the SR by the hypotonic solution; this indicates that the hypotonicity (30% of normal) does release Ca^{2+} from the SR and develops the tension without an additional Ca-influx through a cell membrane since action potentials were not elicited. Such a hypotonic release of Ca^{2+} has been shown in skinned skeletal muscle fibers (ENDO and THORENS, 1975; MOBLEY, 1979). The remaining plateau tension was not affected by those treatments; rather, the amplitude of sustained tension was larger in the 0-Ca^{2+} solution. This indicates that the increased resting tension in the hypotonic solutions is not dependent on Ca^{2+}. The contractures induced by a strongly hypotonic and/or by a hypotonic solution of Na-free Ringer are unaffected by removal of Ca^{2+} from the bathing medium in intact muscles (KAWATA and KAWAGOE, 1975; CHAPMAN, 1978). An increase in tension, with decreasing ionic strength in the absence of Ca^{2+}, has been shown in skinned frog muscle fibers (GORDON *et al.*, 1973) and in glycerol-treated rabbit psoas muscle fibers (YANAGIDA *et al.*,

1982). Although it is difficult to know what effects hypotonicity may have on the ionic strength of the intracellular fluid of living fibers studies here, there would be some direct effects of the ionic strength on the interaction of actin and myosin and on the SR of frog atrial muscles (Ohba, unpublished observation).

The effects of hypotonicity on the electrical properties of frog atrial cells were the slight depolarization and shortening of action potential duration without accompanying changes in both plateau level and overshoot. The shortening of action potential duration may contribute to the decline in twitch by the exposure to the hypotonic solution. Although the mechanism of this shortening was not clarified, the same amount of shortening is observed on the frog ventricle (KAWATA *et al.*, 1974). In frog ventricle muscle fibers, neither hypertonic nor hypotonic solutions reduce the level of plateau component of the action potential (HERMSMEYER *et al.*, 1972; KAWATA *et al.*, 1974). EHARA and HASEGAWA (1983) reported that $\dot{V}_{max}$ of the slow action potentials changed little or not at all within a few min after the onset of the hypertonicity in the guinea-pig ventricle muscle. Hence neither the positive nor negative inotropic effects of hypotonicity would be mediated through the change in calcium inward current.

In conclusion, the hypotonicity may exert a rapid positive inotropic action on the frog atrial muscle through the osmotic release of Ca^{2+} and a subsequent negative inotropic action due to Ca^{2+} depletion from the SR and/or to a shortening of action potential duration. Additional observations regarding the influence of changing osmolarity on contraction properties of skinned frog atrial fibers will be reported in a subsequent article.

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