Mechanism of Positive Inotropic Actions of Hydrazinium Ions on Bullfrog Heart Muscles

Tsunetaka MATOBA, M.D.

SUMMARY

An investigation was carried out on isolated bullfrog's ventricular muscles so as to study the mechanism of an increasing positive inotropic effect of hydrazine, using a conventional microelectrode technique and a force-displacement transducer; membrane potentials and isometric twitch tensions were observed. A low concentration, 1.5 to 15 mM, of hydrazinium ions produced an increase of isometric twitch tension, having a shorter contractile duration with an increasing velocity of contraction and relaxation. The developments of twitch contractions illustrated a typical sigmoid curve. However, the shortened contractile duration with the deeper negative deflection of dT/dt remained after the increased contraction was returned to the initial value. These increasing twitches were markedly enhanced in the Ca^{++} -rich solution as compared with those observed in normal Ringer's solution. The action potentials generated in the bullfrog's ventricular muscles revealed the shorter duration and the decreased maximum rate of rise of the action potential. Phase II, the late portion of the upstroke of the action potential, was augmented by hydrazine in the Na^+ -deficient and the Ca^{++} -rich solutions. The resting potentials were slightly reduced.

In conclusion, it is postulated that the positive inotropic actions of hydrazinium ions on this tissue are mainly due to the depolarization of cell membrane and the increasing permeability of cell membrane to calcium ions. In one respect, the mode of action of hydrazinium ions on these cells would be essentially similar to that of adrenaline.

Additional Indexing Words:

Depolarization of membrane Membrane permeability to calcium ions Action potential Contraction Adrenaline Ouabain

HYDRAZINE, which is structurally one of nitrogen compounds, was synthesized in 1887 and has been used as high energy fuel in rockets.¹⁾ In 1959 Lorente de Nó et al introduced first this substance as one of the substitutes for sodium ions in the generation of action potential in nerve fibers.²⁾ Since then several investigators have had experiments to clarify the nature of

From the Department of Physiology, Kurume University School of Medicine, Kurume 830.
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hydrazine from a viewpoint of physiology and pharmacology.³⁾⁻⁷⁾

As has been observed in our previous experiments, hydrazinium ions under favorable conditions can completely substitute for sodium ions in the generation of action potentials in bullfrog's heart muscles, and induce the developments of the twitch contractions.⁸⁾ This twitch developments are characterized by an increasing velocity of contraction and a markedly prolonged relaxation time. Even a low concentration has induced an increase of the twitch with a shorter contraction time in our observations. These findings are of great interest, and hydrazine may make it possible to use in a way in clinical field.

Among the positive inotropic agents, there are adrenaline and ouabain. These 2 substances act on heart muscles in different modes. Ouabain might inhibit the sodium pump, leading on an increase of intracellular sodium stores and secondary on an increased calcium stores by Na-Ca exchange mechanism.⁹⁾ Adrenaline increases the calcium inward current and also enhances calcium influx during excitation.¹⁰⁾⁻¹²⁾

In the present experiment, the effects of hydrazine on the isometric twitch contractions and action potentials of bullfrog's ventricular muscles were measured at various extracellular concentrations of Na⁺ and Ca⁺⁺ ions in comparison with adrenaline and ouabain, in order to clarify the mechanism of the positive inotropic action.

METHODS

The isolated ventricular strips, 3 × 6 mm in size, of bullfrogs (*Rana catesbiana*) were used throughout. The isometric twitch contraction and its differential (dT/dt) were measured by a force-displacement transducer in a tissue bath which had a ventricular strip soaked in Ringer's solution, continuously bubbled by air, was stretched with an initial load of 1 Gm. The submaximal electrical stimulation (5/min) was given to the strip by means of bipolar silver wire electrodes placed on the preparation at one end of ventricular muscle. The twitch changes were recorded by an ink-writing oscillograph.

Intracellular membrane potentials and their differentials (dV/dt) were observed by a conventional microelectrode technique. The resistance of microelectrode was approximately 15 megohm (12-20 megohm). The preparation was driven by submaximal electrical stimulation through a 2 M NaCl glass-capillary microelectrode in a constant rate of 5/min throughout experiments.

The composition of Ringer's solution in millimoles per liter was as follows: NaCl, 112; KCl, 2.0; CaCl₂, 1.8; NaCO₃, 2.4 and glucose, 2.5. The pH of Ringer's solution was 6.8 to 7.0. The hydrazine hydrate, adjusted to pH 6.8 to 7.0 by adding hydrochloric acid, was prepared in a high concentration, of which a desired amount was flushed into the perfusate in one injection. The chemical structure of hydrazine is as follows:

The drugs used were adrenaline bitartrate (Sigma) and ouabain (Merck). All experiments were carried out at room temperature (22–24°C).

RESULTS

1. Mechanical responses of the strips in low concentrations of hydrazinium ions

A steady isometric twitch of bullfrog's ventricular strip in Ringer's solution was obtained at about 30 min after applying electrical stimulations. These

Fig. 1. Mechanical responses of ventricular strips in low concentrations of hydrazinium ions. It illustrates a characteristic sigmoid curve. The open circle shows the means of 4 experiments. Vertical bars: standard deviation. The ordinate shows the scale (per cent) of isometric twitch development: the control value 100%. The abscissa the concentration of hydrazinium ions (mM in logarithmic scale).

steady state continued during approximately 3 hours. After such a procedure, all experiments were started.

The effects of low concentrations of hydrazinium ions on the isometric twitch tensions were tested in 4 preparations (Fig. 1). The range of hydrazine concentration was 1.5 to 15 mM (mM indicates millimoles per liter). The isometric twitches were increasing immediately after an addition of hydrazine into the perfusate, and attained to the maximal values at between 10 and 25 min in proportion to the hydrazine concentration. When hydrazine concentration was increased in steps from 1.5 to 15 mM, the maximal twitch tensions in each concentration inscribed a dose-response curve as shown in Fig. 1. This curve had a characteristic sigmoid shape. The amount of less than 1 mM hydrazine did not appreciably increase the twitch tensions.

A typical time course and the intensity of the twitch development and its differential (dT/dt) in the concentration of 7 mM hydrazine were illus-

Fig. 2. A typical time course and the intensity of twitch contraction in the concentration of 7 mM hydrazine. Upper traces show twitch contraction every 5 min after the injection of hydrazine. Lower traces their first differentials (dT/dt). A, B and C show the changes of tension development, dT/dt and contractile duration at 50% level, respectively. The solid and dotted curves in B represent the positive and negative deflections of dT/dt , respectively. When hydrazine is flushed into Ringer's solution (arrow mark), the twitch contraction increases progressively to the maximum at 20 min and thereafter reduces to the control level (A). However, the shortened contractile duration remains with the deeper negative deflection of dT/dt , which demonstrates that the process of relaxation is accelerated (B and C). The strips are initially loaded with 1 Gm.

Fig. 3. The changes of the action potential of bullfrog's ventricular strips in 7 mM hydrazine solution. 2 and 3 show the action potentials immersed in 7 mM hydrazine solution after 3 and 5 min, respectively. They show the reduced maximum rate of rise of the upstrokes and the shortened duration of action potentials. Ten min after changing of the perfusate by Ringer's solution, the action potential of the strip is gradually recovering (4).

trated in Fig. 2. When hydrazine was added to Ringer's solution, the progressively enhancing twitches attained to the maximum within 20 to 25 min. The increasing tension was characterized by an increasing positive amplitude of dT/dt . The duration of the twitch contraction became shorter than that of the control value, especially shorter relaxation phase with the deeper negative deflection of dT/dt . The time-to-peak tension was also slightly shortened. After the increment of the twitch to the maximal value, the twitch tension was gradually decreasing to the initial value within about 70 min. The shortened contractile duration, however, remained with the deeper negative deflection of dT/dt .

2. Changes in action potentials of bullfrog's ventricular muscles

Addition of 7 mM hydrazine to Ringer's solution produced the changes of the action potential of the ventricular strip as shown in Fig. 3; the maximum rate of rise of action potential was markedly reduced with a decreasing amplitude of dV/dt . The resting potential was slightly decreased. The peak height of action potential was also slightly decreased. The duration of action potential was shortened. Typical action potentials 3 and 7 min after addition of hydrazine illustrated in the record 2 and 3 of Fig. 3, respectively. When the strip was transferred into Ringer's solution, the shape of the action potential was almost recovered to that of the control after 10 min (record 4).

3. Influence of extracellular sodium ions

In Ringer's solution, the twitch contraction patterns affected by 7 mM hydrazine resembled those by adrenaline. There appeared the enhanced

Fig. 4. The influence of Na⁺ ions on the actions of hydrazine (A, 7 mM), adrenaline (B, 0.8 μ M) and ouabain (C, 0.3 μ M). In Ringer's solution, the twitch curve pattern affected by hydrazine is similar to that by adrenaline (A and B). Note the deeper negative deflection of dT/dt. While, ouabain increases progressively the twitch contraction with a broader duration and the unchanged negative deflection of dT/dt (C). Even in the Na⁺-deficient (56 mM) solution, the effects of hydrazine on the twitch contraction was similar to that of adrenaline.

amplitude of twitch tensions, the shorter contractile durations and the accelerated relaxation phase with deeper negative deflection of dT/dt as shown in Fig. 4. In ouabain, the twitch contraction was progressively increased with the broader contractile duration in shorter time course. When the strip was transferred into a modified Ringer's solution with 56 mM of Na⁺ ions (namely, a half of Ringer's solution), the twitch contractile force was usually increased. The addition of hydrazine led to a detectable increase in twitch contraction, while adrenaline showed a marked increase. Whereas, ouabain decreased remarkably the twitch contraction.

Fig. 5 showed the changes of the action potential in the Na⁺-deficient solution. Reduction of the sodium level was found to decrease the maximum rate of rise and the amplitude of the action potential, and to delay conduction velocity (record 1 and 2). The application of 10 mM hydrazine produced the more decreasing maximum rate of the upstroke, the appreciably higher amplitude of action potential, especially phase II,¹²⁾ and the more prolonged conduction velocity.

Fig. 5. The effect of 10 mM hydrazine on the action potential under the influence of Na^+ -deficient (56 mM) solution. Record 1 shows the action potential in Ringer's solution. The maximum rate of rise and the amplitude of the action potential in Ringer's solution (1) are decreased in the Na^+ -deficient solution (2). Record 3 shows the action potential induced by the addition of hydrazine: the more decreased maximum rate of rise and the higher amplitude of action potential, and the prolonged conduction velocity.

Fig. 6. The influence of Ca^{++} ions on the twitch contractions under the effects of hydrazine (A, 7 mM), adrenaline (B, $0.8 \mu\text{M}$) and ouabain (C, $0.3 \mu\text{M}$). Ca^{++} -rich solution (double asterisks, 9 mM) induces the augmentation of the twitch contraction. In this solution, hydrazine and adrenaline increase the amplitude and the duration of the twitch contractions (A and B). However, ouabain decreases the twitch contractile forces (C).

4. Influence of extracellular calcium ions

The isometric contraction of bullfrog's ventricular strip in a modified Ringer's solution with 9 mM of Ca^{++} ions developed with a shorter contraction time. The administration of hydrazine led to increase the amplitude and the duration of the contractile twitch. There was no appreciable change of the rate of fall in the relaxation phase. In case of adrenaline, the same tendency as observed by the addition of hydrazine was obtained in the Ca^{++} -rich solution. However, ouabain decreased progressively the amplitude of the twitch contraction. In a lower concentration of Ca^{++} (0.36 mM), the contractile force was rapidly decreased. In this solution, hydrazine, adrenaline or ouabain increased the contractile forces as compared with those before the addition of each drug.

The changes of the action potential in the Ca^{++} -rich solution were also

Fig. 7. The influence of Ca^{++} ions on the upstroke of the action potentials under the effects of hydrazine (A, 7 mM) and adrenaline (B, 0.8 μM). In a modified Ringer's solution with Ca^{++} -rich (9 mM), the maximum rate of rise and the peak height of the action potentials are augmented as compared with those in normal Ringer's solution (record 1). Hydrazine in the Ca^{++} -rich (9 mM) solution leads to the changes of the action potentials associated with the reduced maximum rate of rise and the comparatively enhanced the late portion of the upstroke (phase II) after 10 min (A-2). While, adrenaline leads to increase the maximum rate of rise and the phase II of the action potentials after 5 min (B-2).

observed; the maximum rate of rise and the amplitude, especially the peak level, of action potential were increased, and the duration of plateau was shortened. A rapid depolarization phase in frog's myocardium has been reported to be composed of 2 phases: phase I is mainly due to sodium currents, and phase II calcium currents.¹²⁾ This phase II was augmented by hydrazine or adrenaline. Fig. 7 illustrated the typical changes of the action potential in the Ca^{++} -rich solution with hydrazine or adrenaline. Namely, hydrazine in the Ca^{++} -rich solution decreased the maximum rate of rise of the upstroke and, however, enhanced the peak height, especially phase II of the upstroke. While, adrenaline in the same solution produced appreciably an increase in the maximum rate of rise and the phase II of the upstroke of the action potential.

DISCUSSION

Hydrazine has revealed to have a positive inotropic action on bullfrog's heart muscles. This effect of hydrazine was also observed on isolated papillary muscles of rabbit's right ventricles (our unpublished data).

Kavaler,¹⁴⁾ Wood et al¹⁵⁾ and Bravený et al¹⁶⁾ have reported that a cause of tension development in mammalian ventricular muscle was due to membrane depolarization. Goto et al¹⁷⁾ have observed as well in frog ventricular muscles that membrane depolarization elicited an increase of contractile force. As has been indicated, hydrazine depolarizes the membrane of cardiac cells. This evidence can be notably seen in the high concentration of hydrazine.⁸⁾ The depolarization due to this substance is about 25 mV per tenfold increase in hydrazine concentration by the calculation on the basis of the previous and present results. Therefore, the cause of the positive inotropic action of hydrazine could be partly explained by this evidence.

The upstroke of the action potential in frog's myocardium can be regarded as being composed of 2 phases: an initial phase of rapid depolarization preceding the step (phase I) and the following slower phase, reaching the peak of the overshoot (phase II).¹²⁾ Phase I seems to be mainly related to Na^+ currents, whereas phase II is related to Ca^{++} currents. Phase II, preceding plateau phase, to plateau phase has been proposed to call "slow channel".¹³⁾ Since this proposal by Rougier, the nature of this pathway has been clarified by several investigators.¹⁸⁾⁻²¹⁾ Namely, this pathway is composed of 2 components: slow outward sodium currents and inward calcium currents. This "slow channel" is activated by sympathomimetic amines such as adrenaline.^{10), 12)} Adrenaline was reported to augment the phase II in Na^+ -depleted, Ca^{++} -rich as well as K^+ -rich solutions by increasing calcium

currents.^{12), 21), 22)} This augmentation would be due to an increase of calcium influx.²³⁾ In our experiment, hydrazine augmented the phase II in the Na⁺-deficient and the Ca⁺⁺-rich solutions. And the twitch contractions due to hydrazine as well as adrenaline were developed in these various solutions. Thus the mode of action of hydrazine seems to be essentially the same manner as adrenaline; in other word, hydrazine can increase the permeability of cell membrane to calcium ions. The increase of calcium currents could in some part explain the positive inotropic action by hydrazine. This possible role of hydrazine was suggested in the sympathetic ganglion cells of bullfrogs.^{3), 4)}

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