Influence of Ouabain on the Force-frequency Relationship in Cardiac Muscle


Summary

The effects of different concentrations (10^{-8} to 10^{-5} M) of ouabain on frequency-dependent positive inotropy were studied in rabbit papillary muscles. In the absence of ouabain an increase in the frequency of stimulation (from 15/min 30, 60, 90, and 120/min) produced a frequency-dependent increase in the contractility in papillary muscles. Ouabain (10^{-8} M), which did not produce any change in the contractility, also did not produce any change in the frequency-dependent increase in the contractility. However, in the presence of 10^{-7} M of ouabain the frequency-dependent increases in the contractility were greater than those in the absence of ouabain. At a concentration of 10^{-6} M of ouabain the frequency-dependent inotropic effect was positive only at low frequencies (30, 60/min) whereas at higher frequencies (90, 120/min) the positive effect was transient. In the presence of still higher concentrations (2, 5 \times 10^{-6} M and 10^{-5} M) of ouabain the frequency-dependent positive inotropy was changed to negative inotropy and this effect was dependent upon the concentrations of ouabain. This ouabain-dependent change to negative inotropic effect of frequency increases was not due to the lack of oxygen or energy supply. Also the negative inotropy for the same concentration of ouabain was directly related to the frequency of stimulation. There results indicate that the frequency-dependent increase in the cardiac contractility is mediated through an inhibition of the sarcolemmal Mg^{++}-dependent, Na^{+}-K^{+}-ATPase. Pre-inhibition of this enzyme system abolishes the frequency-dependent positive effect and the latter shows an inverse relationship with the degree of pre-inhibition.

Additional Indexing Words:
Cardiac contraction Force-frequency relationship Sarcolemmal Na^{+}-K^{+}-ATPase Ouabain Positive inotropy Negative inotropy

Frequency-dependent positive inotropy in cardiac muscle has been reported by various investigators.1-4 An increase in the frequency of stimulation has been reported to produce an increase in the rate of tension
development and a reduction in time to peak tension similar to cardiac glycosides.\(^1,2\) It has been suggested that the positive inotropic effect of cardiac glycosides is mediated through an inhibition of Mg\(^{++}\)-dependent, Na\(^+-\)K\(^+-\)activated sarcolemmal ATPase.\(^5\)-\(^9\) The frequency-dependent increase in the cardiac contractility is also suggested to be mediated through an inhibition of the sarcolemmal Na\(^+-\)K\(^+-\)ATPase.\(^4\),\(^11\),\(^12\) If this is the case then the rate dependent increase in the cardiac contractility should be abolished by the inhibition of sarcolemmal Na\(^+-\)K\(^+-\)ATPase. Also there would be an inverse relationship between the degree of pre-inhibition of sarcolemmal Na\(^+-\)K\(^+-\)ATPase by ouabain on the frequency-dependent positive inotrophy in the cardiac muscle of rabbit.

**Methods**

Papillary muscles (thickness never more than 0.75 mm) were obtained from the right ventricle of the rabbit after it was stunned by a blow on the head. The muscles were tied horizontally in a jacketed 100 ml constant-temperature bath at 37°C containing Krebs-Ringer solution of the following composition in mEq/L: Na, 146.8; K, 5.0; Ca, 5.31; HCO\(_3\), 20.14; Po\(_4\), 41.36; Cl, 137.14; and glucose, 45.0 mM. The Krebs-Ringer solution in the bath was constantly bubbled with 95% oxygen and 5% carbon dioxide. One end of the muscle was tied to a fixed holder while the other end was tied to a Grass FT-03 force displacement transducer through a movable holder in order to permit isometric contraction. The muscle was stimulated supramaximally with rectangular wave stimuli of 5 msec duration at desired rates by a Grass stimulator through platinum electrodes placed at one end of the muscle. The resting tension was adjusted to give maximum contraction. The contraction was recorded on Beckman recorder (RB-4) and monitored on a Tektronix 564A storage oscilloscope and photographed on a polaroid film. The muscles were equilibrated in Krebs-Ringer solution for at least 1 hour before control contractions were recorded.

Force of contraction of rabbit papillary muscle was recorded at 5 different stimulation rates (15, 30, 60, 90, and 120/min) in an increasing order. Prior to changing the stimulation rate to any next higher frequency the muscle was always stimulated at the rate of 15/min which served as control.

**Results**

*Force-frequency relation:*

Twenty-five experiments were conducted in which the effects of increasing frequency (from 15/min to 30, 60, 90, and 120/min) on the contractility of papillary muscles were observed. A representative recording from 1 experiment is shown in Fig. 1 and the results are summarized in Fig. 4. A positive inotropic effect was observed when the stimulation rate was increased from
Fig. 1. Force-frequency relationship in the rabbit papillary muscle. Arrows mark the increase in frequency of stimulation from 15/min to higher values indicated by numbers on top.

Fig. 2. Effects of ouabain (10⁻⁷ M, Panel I and 2 × 10⁻⁵ M, Panel II) on the force-frequency relationship.

A in I and II: Control in normal Krebs-Ringer solution. Tracings from bottom to top represent contractions at the stimulation rates of 15, 30, 60, 90, and 120/min respectively.

B in I and II: Contractility in normal solution (lower tracing) and 20 min after ouabain (upper tracing) at the stimulation rate of 15/min. Peak tension is marginally higher at low concentration of ouabain (Panel I) and significantly higher at higher concentration of ouabain (Panel II).

C in I: Sequence of tracings of contraction is the same as in A. Note that the frequency-dependent response is positive and the percentage increase is slightly more than in the absence of ouabain (control).

C in II: In this record the calibration line at the right for this figure (c) represents 1 Gm. Tracings from bottom to top represent contractions at the stimulation rates of 120, 90, 60, 30, and 15/min respectively. A negative force-frequency relationship is apparent at this dose of ouabain. Effects of increasing frequency on the duration of contraction in the presence of ouabain are qualitatively similar to that of control.
lower to higher frequencies. Frequency-dependent increase in the peak tension was associated with a decrease in the time of peak tension and duration of contraction (Figs. 2. I and II). The resting tension was not affected at any frequency of stimulation employed in the present investigation.

**Ouabain and contractility:**

Effects of various concentrations (10^{-8}, 10^{-7}, 10^{-6}, 2 \times 10^{-6}, 5 \times 10^{-6}, 10^{-5} M) of ouabain on the contractility of papillary muscle were investigated. A minimum of 4 experiments were conducted for each concentration of ouabain. Ouabain in the concentration of 10^{-8} M did not produce any positive inotropic effect in the muscles up to 2 hours period of study. A slight but not significant positive inotropic effect was observed with a concentration of 10^{-7} M of ouabain at 20 min (Fig. 2. I). At still higher concentrations (10^{-6}, 2 \times 10^{-6}, 5 \times 10^{-6}, 10^{-5} M) the increase in contractility was concentration-dependent till 2 \times 10^{-6} M after which the maximum response was unchanged. Also the time for maximum positive inotropic effect was inversely related to the concentration of ouabain used. Resting tension was unaffected up to 10^{-6} M of ouabain. A concentration higher than 10^{-6} M produced an increase in the resting tension at control rate of stimulation (15/M). There was a decrease in time to peak tension associated with an increase in peak tension in the presence of ouabain (Fig. 2. II).

**Ouabain and force-frequency relation:**

The effects of increasing frequency of stimulation on the contractility of the papillary muscles were studied in the presence of various concentrations (10^{-8}, 10^{-7}, 10^{-6}, 2 \times 10^{-6}, 5 \times 10^{-6}, 10^{-5} M) of ouabain. A change in the rate of stimulation was made after a steady state of contraction was reached in the presence of ouabain. A minimum of 4 experiments were carried out with each concentration of ouabain. The typical results are shown in Fig. 2 and 3 and the results are summarized in Fig. 4. The maximum increase in the force of contraction with the increase in the frequency of stimulation was inversely related to the concentration of ouabain used (Figs. 2, 3, and 4).

In the presence of low concentrations of ouabain (10^{-8} and 10^{-7} M), the response of the muscle to increasing frequency of stimulation was qualitatively similar to that in the absence of ouabain. In some of the experiments the force-frequency relationship was potentiated in the presence of 10^{-8} M of ouabain but the effect was not consistent. The percentage increases in the contractility in the presence of 10^{-7} M of ouabain in response to an increasing frequency of stimulation were greater than the increases in the contractility in the absence of ouabain. Also the percentage increases in the contractility
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Fig. 3. Effects of different concentrations of ouabain on the frequency-force relationship. The records are from 6 different muscles taken from 6 different rabbits. Arrows at the top mark the time of change in the stimulation rate from 15/min to higher values shown beside each arrow. Numbers at right indicate the bath concentration of ouabain. Note that frequency-force relationship changes from positive to negative as the amount of ouabain is increased.

in response to increasing rate of stimulation in the presence of ouabain (10^{-7}) were greater, at higher frequencies than those in the absence of ouabain.

In the presence of 10^{-6} M of ouabain, although the positive force-frequency relationship was present, it was transient at the frequencies of 90 and 120/min and it changed to negative response within 45 sec (Figs. 3 and 4). Resting tension was not affected at any frequency in the presence of 10^{-8}, 10^{-7}, 10^{-6} M of ouabain. Ouabain in the concentration of 2\times10^{-6} M changed positive force-frequency relationship to a negative one at all frequencies of stimulation. The change to a negative effect was immediate at all frequencies except at 120/min where first 3 to 5 contractions were positive (Figs. 3 and 4). At a concentration of 5\times10^{-6} M of ouabain, the frequency-dependent response was negative at stimulation rates of 30 and 60/min. However at stimulation rates of 90 and 120/min intermittent contractions were
Fig. 4. Effects of different concentrations of ouabain on force-frequency relationship. Numbers at the bottom represent rate of stimulation per minute. Figures below bars indicate number of experiments. A—normal in the absence of ouabain. B, C, and D—in the presence of $10^{-7}$ M, $10^{-6}$ M and $2 \times 10^{-6}$ M of ouabain respectively.

observed indicating the increase in the threshold voltage of stimulation and hence cardiac toxicity of ouabain. Increasing the concentration of ouabain to $10^{-5}$ M, the inexcitability of the preparation developed quickly and hence only the effect of one increase in the rate of stimulation was observed. At these 2 concentrations of ouabain ($5 \times 10^{-6}$ and $10^{-5}$ M) also the force-frequency relationship changed from positive to negative. Resting tension increased at all concentrations of ouabain higher than $10^{-6}$ M and this increase was more at higher frequencies of stimulation (Fig. 3).

Individual contraction cycles were studied at 2 concentrations of ouabain vis a vis $10^{-7}$ M, where the drug effect was marginal but force-frequency relationship was positive (Fig. 2. I) and at $2 \times 10^{-6}$ M, where the force-frequency relationship was negative at all the stimulation rates employed in the study (Fig. 2. II). At a low dose of ouabain ($10^{-7}$ M) the frequency-dependent effects on contraction curves were essentially similar to those of
Fig. 5. Effects of epinephrine on force-frequency relationship in the presence of ouabain. Muscle was exposed to ouabain for 25 min. At arrow 1 the frequency of stimulation was increased from 15 to 120/min. Epinephrine was added to the bath (arrow 2). Note that epinephrine produced an increase in the force of contraction when the latter was reduced to below control at a higher frequency of stimulation (120/min) in the presence of ouabain. Chart speed is indicated on top.

normal preparations. At this dose, increase in frequency of stimulation decreased time to peak, reduced total duration of the active state and increased peak tension (Fig. 2. I). At a concentration of $2 \times 10^{-6}$ M, ouabain changed the nature of response with regard to the peak tension which was reduced when the stimulation rate was increased. However, time to peak as well as total duration of the active state were still reduced when the rate was increased (Fig. 2. II).

Experiments were conducted to test the possibility that the ouabain-dependent conversion of frequency response from positive to negative might be due to a shortage of oxygen or energy supply, especially at higher frequencies of stimulation and at a bath temperature of 37°C. The record shown in Fig. 5 is representative of the results obtained. In this experiment, after a steady state contraction in normal Krebs-Ringer solution, the muscle preparation was exposed to $5 \times 10^{-7}$ M of ouabain. As expected the drug caused a significant increase in the force of contraction and the new steady state was attained within 25 min. At this point the frequency of stimulation was increased from 15 to 120/min, which resulted in a transient increase in the force of contraction, and within 3 min the contractility was less than the control value. Epinephrine ($10^{-7}$ M) was added to the bath at this point. The drug caused an immediate increase in the force of contraction, while the frequency of stimulation was kept unchanged at 120/min. In separate experiments less pronounced but similar effects were observed when calcium (2 mEq)
was added to the bath in place of epinephrine.

**Discussion**

The present investigation demonstrated that the increase in the rate of stimulation produced a frequency-dependent increase in the contractility of papillary muscles. Ouabain produced a concentration dependent increase in the contractility. Frequency-dependent increase in the contractility in the presence of ouabain was inversely related to the concentration of ouabain. In the presence of high concentrations (2×10⁻⁶, 5×10⁻⁶, and 10⁻⁵ M) of ouabain, the increase in the frequency of stimulation not only failed to produce any increase in the contractility but also produced a decrease in the contractility. In low concentration (10⁻⁵ M) of ouabain the frequency-dependent increases in contractility were greater than those with absence of ouabain.

In our previous studies⁴,¹¹,¹² in dogs and monkeys it has been shown that frequency-dependent positive inotropy in cardiac muscle was somehow related to an inhibition of the sarcolemmal Na⁺-K⁺-ATPase. An indirect role of sarcolemmal ATPase has been suggested by Langer.¹³,¹⁴ According to his hypothesis, an increase in the frequency of stimulation produces an accumulation of intracellular sodium secondary to sodium-pump lag. The increased intracellular sodium secondarily increases sodium-calcium exchange and also causes increased potassium efflux. This electrically neutral Na⁺ (efflux)-Ca⁺⁺ (influx) exchange thus has been suggested to be responsible for frequency-dependent positive inotropy. However, McCans et al¹⁵ believe that it is the slow inward calcium current during systole¹⁶ which increases intracellular calcium.

While the previous reports from this laboratory⁴,¹¹,¹² have indicated that possible involvement of sarcolemmal ATPase in the force-frequency relationship in cardiac muscle, the present study shows a definitive relationship between the degree of ATPase inhibition and frequency-dependent positive inotropy. Thus at low concentration of ouabain 10⁻⁸ M the positive inotropy was not affected. At other concentrations of ouabain the frequency-dependent positive inotropy was inversely related to concentration of ouabain used. At very high concentrations (2, 5×10⁻⁶ and 10⁻⁵) there was a concentration dependent negative inotropy in response to increase in the frequency of stimulation. Our earlier studies⁴,¹² have shown that other positive or negative inotropic agents which do not inhibit the sarcolemmal Na⁺-K⁺-ATPase, did not affect the force-frequency relationship in cardiac muscle. Also the agents, which inhibited membrane ATPase, reduced the response of the muscle to an increase in frequency of stimulation.
It can be argued that the higher frequencies of stimulation at 37°C, in the presence of ouabain, may result in a higher contractile state in which muscle is liable to become oxygen or energy limited. In such an event the negative inotropic effect of increased frequency of stimulation in the presence of ouabain reported here would be artifactual. This contention of energy or oxygen limitation might further be corroborated by the fact that increased frequency of stimulation in the presence of ouabain (5×10⁻⁷, 1×10⁻⁶ M) produced a transient increase followed by a gradual decline in the myocardial contractility, which is characteristic of what happens in oxygen limited situations. However, the positive inotropic effect of epinephrine or calcium, in the muscles where the force of contraction was decreased below the control value (Fig. 5) at higher frequency of stimulation due to the effect of ouabain, demonstrate conclusively that this negative effect was not due to the limitation of oxygen or energy supply. If either of the latter was a limiting factor then no increase in muscle contraction could have been observed in the presence of epinephrine or calcium. Further, it has been reported that even the central core of the muscle is well oxygenated if the diameter of the muscle is less than 0.75 mm.¹⁰,¹⁷,¹⁸ In our studies the muscle thickness never exceeded 0.75 mm.

The present investigation shows that in the presence of very high concentrations (5×10⁻⁶ and 10⁻⁵ M) of ouabain, the positive inotropy was converted to negative inotropy in response to an increase in frequency. This could be due to an accumulation of large amount of calcium intracellularly as a result of both ouabain and an increased rate of stimulation. This increased accumulation of Ca⁺⁺ would decrease the relaxation of the muscle and hence decreased contractility. This would also produce an increase in the tone of the muscle which has been observed in the present investigation at higher concentrations of ouabain and at higher frequencies of stimulation in lower concentration of ouabain. The other possibility could be the lack of a link between calcium movements and the sodium pump as suggested by Blesa et al.¹⁹ This aspect of negative inotropy in response to increasing frequency of stimulation in the presence of ATPase inhibitors is a subject of further investigation.

In conclusion it appears that the frequency-dependent positive inotropy is mediated through an inhibition of the sarcolemmal Mg⁺⁺-dependent, Na⁺-K⁺-ATPase and pre-inhibition of this enzyme system changes the normal frequency-dependent response in accordance with the degree of enzyme inhibition.
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REFERENCES