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# The effects of tubulin-binding agents on stretch-induced ventricular arrhythmias

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**Abstract**

Stretch-activated ion channels have been identified as transducers of mechanoelectric coupling in the heart, where they may play a role in arrhythmogenesis. The role of the cytoskeleton in ion channel control has been a topic of recent study and the transmission of mechanical stresses to stretch-activated channels by cytoskeletal attachment has been hypothesized. We studied the arrhythmogenic effects of stretch in 16 Langendorff-perfused rabbit hearts in which we pharmacologically manipulated the microtubular network of the cardiac myocytes. Group 1 ($n = 5$) was treated with colchicine, which depolymerizes microtubules, and Group 2 ($n = 6$) was treated with taxol, which polymerizes microtubules. Stretch-induced arrhythmias were produced by transiently increasing the volume of a fluid-filled left ventricular balloon with a volume pump driven by a computer-controlled stepper motor. Electrical events were recorded by a contact electrode which provided high-fidelity recordings of monophasic action potentials and stretch-induced depolarizations. The probability of eliciting a stretch-induced arrhythmia increased ($0.22 \pm 0.11$ to $0.62 \pm 0.19$, $p = 0.001$) in hearts treated with taxol (5 μM), whereas hearts treated with colchicine (100 μM) showed no statistically significant change. We conclude that proliferation of microtubules increased the arrhythmogenic effect of transient left ventricle diastolic stretch. This result indicates a possible mode of arrhythmogenesis in chemotherapeutic patients and patients exhibiting uncompensated ventricular hypertrophy. The data would indicate that the cytoskeleton represents a possible target for antiarrhythmic therapies. 

*Keywords:* Arrhythmia; Mechanics; Cytoskeleton; Microtubule

## 1. Introduction

There is a high incidence of cardiac arrhythmias amongst patients with left ventricular hypertrophy (Francis, 1986; Levy et al., 1987) and for those with heart failure, arrhythmia is a significant cause of sudden cardiac death (Ghali et al., 1991). That these arrhythmias increase in conjunction with developing myocardial mechanical dysfunction (Schulze et al., 1975; Califf et al., 1987) supports the hypothesis that mechanoelectric feedback may facilitate the development of these arrhythmias. The arrhythmogenic effects of mechanical stresses have been reported (Dean and Lab, 1989; Franz et al., 1989) as have stretch-induced arrhythmias facilitated by stretch-activated channels (Stacy et al., 1992; Hansen, 1993). The literature on stretch-induced arrhythmias often uses the term "contraction–excitation" feedback (Lab, 1982; Halperin et al., 1993; Yamashita et al., 1994) or mechanoelectric feedback, but has focused almost exclusively on the role of stretch-activated channels as the transducers of arrhythmogenic effects of mechanical stretch (Hansen et al., 1991; Hu and Sachs, 1997) without attention to the role of the cellular ultrastructure. The growing attention to the role of the cytoskeleton in affecting ion channel behavior has been reviewed recently (Terzic and Kurachi, 1998), but the field of study is still relatively underdeveloped. In recognition

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of the potential electrogenic effects of cytoskeletal stresses on ion channels in both the absence and presence of diastolic stretch, we prefer the term *mechanoarrhythmogenic* to describe the effect of transient and sustained mechanical forces on arrhythmia development.

In a very elegant series of studies, Cooper and colleagues (Tsutsui et al., 1993, 1994; Tagawa et al., 1996, 1997) have identified the hyperpolymerization of microtubules as the facilitator of contractile dysfunction in hearts with pressure-overload hypertrophy. These studies demonstrated that microtubules generate a viscous load on the sarcomere, affecting the kinetics of the sarcomere. We developed a 'top–down, bottom–up' hypothesis, outlined in Fig. 1, which accounts for the role of microtubule hyperpolymerizaton in mechanoarrhythmogenesis. In the 'top–down' development of the hypothesis, we begin with patients who exhibit an increased incidence of arrhythmias in association with hypertrophy of the left ventricle (Franz, 1996). Ventricular hypertrophy is generally accompanied by an increase in the production of microtubules (Tsutsui et al., 1993). That microtubules are polymerized by the antimitotic drug taxol has been demonstrated (Schiff et al., 1979). Reports of cardiotoxic effects of taxol administration have been documented and reviewed (Rowinsky and Donehower, 1995), leading to the hypothesis that the hyperpolymerization of microtubules within the heart may have arrhythmogenic consequences. In the 'bottom–up' development of the same hypothesis, we begin at the level of the ion channel, specifically the stretch-activated channel as a transducer of stretch-induced arrhythmias (Hansen et al., 1991). That some stretch-activated channels are specific for $Ca^{2+}$ has been reported (Sigurdson et al., 1992), as has the mechanosensitivity of the L-type $Ca^{2+}$ channels (Matsuda et al., 1996). Galli and DeFelice (1994) demonstrated that taxol affects the kinetics of the L-type $Ca^{2+}$ channel, specifically, lengthening mean open time, increasing the probability of the open state, and abolishing the inactivation period. The role of the $Ca^{2+}$ channel in calcium-induced, calcium-release is well known, as is the requirement of calcium-induced, calcium release for sarcomere contraction. The studies by Tsutsui et al. (1993) confirmed that in their model of cat right ventricular hypertrophy, the hyperpolymerization of microtubules was solely responsible for the contractile dysfunction. This was confirmed through other experiments where taxol was used to polymerize microtubules, resulting in the same dysfunction. The authors hypothesized that microtubules impose a viscous intracellular load on the contracting sarcomere. Both of these paths lead us to the same hypothesis, namely, that the hyperpolymerization of microtubules is potentially arrhythmogenic, specifically, increasing the probability of a stretch-induced arrhythmia ($P$). We hypothesized that

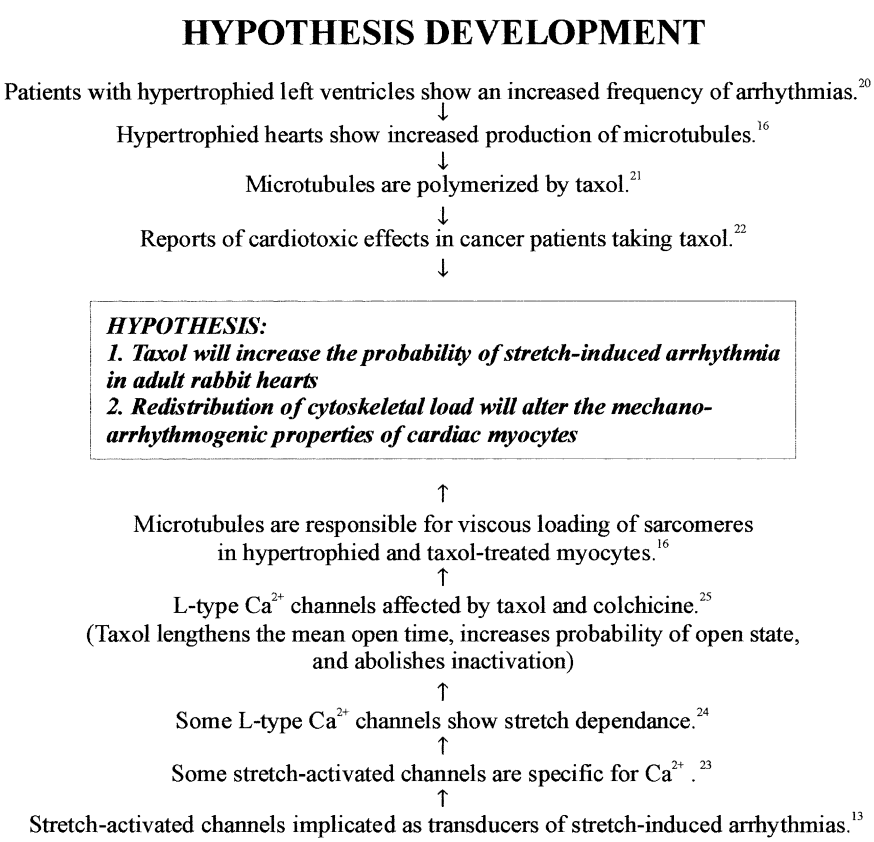


Fig. 1. Hypothesis development (see text for details).

reorganization of cytoskeletal architecture and load bearing capabilities is potentially arrhythmogenic, owing to the sensitivity of stretch-activated channels to mechanical load. We are also open to the hypothesis that there are processes other than membrane stretch which open stretch-activated channels and might facilitate arrhythmogenesis during abnormal diastolic loading of the myocardium. We base that assumption on other results previously reported by this laboratory (Parker et al., 1997a), which suggested that cellular membrane stretch is not necessarily arrhythmogenic. Preliminary results from the current study have been presented in abstract form (Parker et al., 1997b).

## 2. Materials and methods

### 2.1. Isolated heart preparation

Hearts from 16 New Zealand white rabbits (approximately 5.0 kg) were harvested while the animal was anesthetized with sodium pentobarbital (60 mg/kg) mixed with 1000 units of heparin via intravenous line. The heart was attached and perfused by a Langendorff apparatus via the aorta at approximately 36°C, as designated by digital control of the heater circulator (50-1932 Harvard Apparatus, Natick, MA) and verified with a digital temperature probe placed in the right ventricle throughout the experiment (Tandy, Dallas, TX). The heart was then submerged in perfusate while the left atria was opened and the mitral leaflets detached. A compliant balloon attached to a ventricular volume pump was inserted into the left ventricle via the prepared tract. A ring was sutured into the mitral annulus to secure the ventricle to the plastic pipe upon which the balloon is mounted and through which water flowed to inflate the balloon.

The isotonic perfusate consisted of 120 mM NaCl, 5.0 mM KCl, 20 mM sodium acetate, 1.2 mM $MgCl_2$, 5.0 mM HEPES, 1.5 mM $CaCl_2$, 10 mM glucose, 0.3 mM probenecid, an 0.05 mM NaOH. After filtration (0.45 μ), 1% neonatal calf serum was added. The solution was bubbled with 100% $O_2$ and the pH maintained at approximately 7.4. Coronary perfusion pressure was maintained between 70 and 80 mm Hg throughout the experiment and checked periodically. The AV node was ablated by suture ligation. The heart was paced electrically at 2 Hz by wire electrodes at the left ventricle apex.

The isolated rabbit heart was supported and monitored with the system schematically illustrated in Fig. 2. The monophasic action potential, left ventricle pressure, rate of change of pressure ($dP/dt$), left ventricle volume, and coronary perfusion pressure were amplified and continuously digitized during each experimental stage at 1 kHz by the LabMaster TM-100 A-to-D board and stored at 500 Hz on the computer's hard disk for subsequent data analysis. The signals were monitored in real time with software developed by our laboratory. A micromanometer-tipped catheter (SPC-330A, Millar, Houston, TX) was positioned within the balloon for instantaneous measurements of maximum pressure, maximum rate of change of pressure ($dP/dt_{max}$), and end diastolic pressure and displayed on a beat-to-beat basis throughout the experiment, allowing the stability and contractile performance of the heart to be monitored. A spring-loaded epicardial electrode on the anterior wall of the left ventricle measured monophasic action potentials in a technique described and validated by

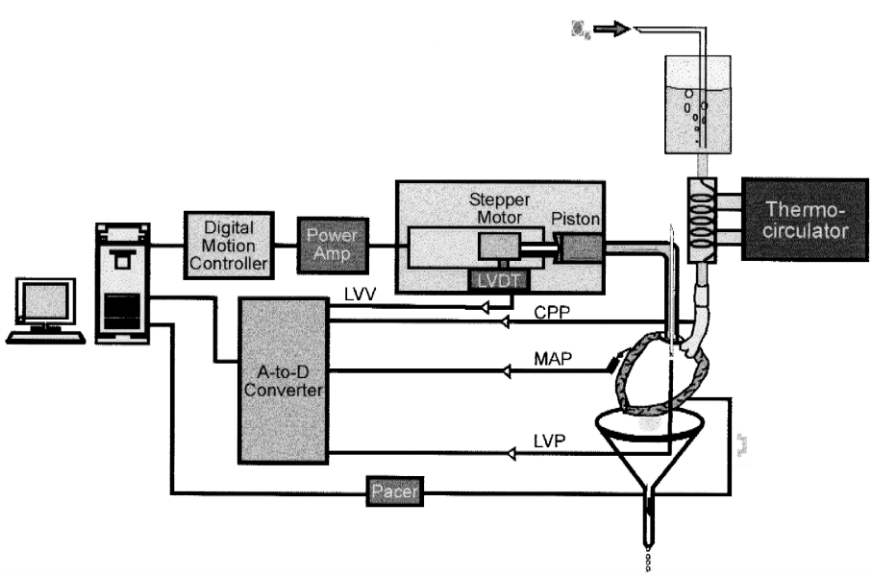


Fig. 2. Experimental setup. CPP, coronary perfusion pressure; MAP, monophasic action potential; LVP, left ventricular pressure; LVV, left ventricular volume; LVDT, left ventricular displacement transducer; $O_2$, oxygen (see text for details).

Franz et al. (1986) and was monitored throughout the experiment.

The volume pump consists of a hydraulic piston driven by a stepper motor apparatus (Compumotor Indexer model AT 6400, microstepping drive model S6, and Linear Motor Platen model PO-L20-P18, Parker Compumotor, Rohnert Park, CA). A resistive linear variable displacement transducer (DRC model LX1A-0004-BE-L10, Parker Compumotor) determined the position of the piston. The output of the linear variable displacement transducer was calibrated to measure absolute balloon volume (linearity within 1%) and was digitized at 1 kHz by a digital computer (486-DX4-66, Gateway 2000, North Sioux City, SD) using a 12-bit analog-to-digital (A-to-D) converter (LabMaster TM-100, Scientific Solutions, Solon, OH). The computer digitally calculated the desired left ventricular volume and output a signal via the AT6400 indexer to the microstepping drive, which drove the linear motor to the desired volume.

The electromechanical stimulation protocol is depicted in Fig. 3. Each sequence was initiated by a train of eight paced beats at a frequency of 2 Hz. Pacing was performed at twice the diastolic threshold with an electrically isolated 2-ms square wave of constant voltage initiated by the computer. During this period, left ventricular volume was held constant at a value determined during baseline assessment of the heart's contractility, usually less than 1 ml. Precisely 400 ms after the final pacing stimulus, the ventricle was subjected to either a transient stretch (Stretch) or quiescent period (No Stretch). In all cases, the stretch stimulus was applied during electrical diastole and when ventricular relaxation was nearly complete. The computer

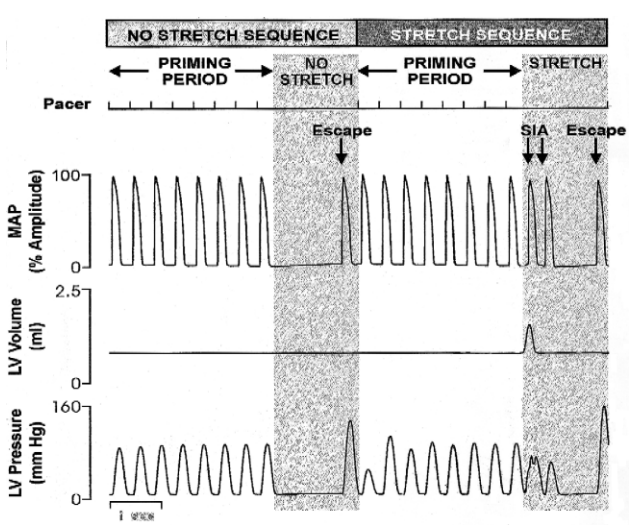


Fig. 3. Protocol for stretch-induced arrhythmia classification. In the No Stretch period, escape beats immediately following the eight beat pacing train invalidated responses during Stretch. Monophasic action potentials during the Stretch sequence were considered stretch-induced arrhythmias, provided that they were initiated during the left ventricular stretch and earlier than $t_{95}$.

was programmed to alternate between control and stretch sequences. The stretch consisted of an increase in left ventricle volume of specified amount ($\Delta V$), which was delivered over 100 ms and maintained for 250 ms. The left ventricular volume was then returned to the initial volume over 100 ms. The response to Stretch (or No Stretch) was monitored for 2 s during which pacing was held. Pacing was then resumed and the next sequence was initiated.

### 2.2. Data analysis

The digitized signals were analyzed by computer using the software developed in our laboratory. From 10 consecutive steady state beats at a paced frequency of 2 Hz, the left-ventricle peak isovolumetric pressure was determined as an index of left ventricular contractility. Left-ventricular end-diastolic pressure was also measured prior to the next paced beat.

Stretch–response data were analyzed as follows. From the control sequences, the timing of spontaneous ventricular escape complexes was determined relative to the final pacing stimulus. The longest interval which excluded 95% of the ventricular escape complexes ($t_{95}$) was then computed using all of the control sequences obtained during each experimental stage (range: 120–200 sequences). Stretch-induced arrhythmias were then defined as monophasic action potentials, which followed a stretch and arose at a time when there was at least 95% confidence that they were not ventricular escape complexes (i.e., earlier than $t_{95}$). Given this information, we could determine with at least 95% confidence that monophasic action potentials arising earlier than $t_{95}$ during stretch sequences were stretch-induced arrhythmias. The probability of a stretch-induced arrhythmia was then computed for each experimental condition as the number of stretch-induced arrhythmias divided by the total number of stretch sequences.

### 2.3. Experimental protocol

Each experiment was initiated with measurement of the pressure–volume response of the left ventricle by increasing the volume of the left ventricular balloon at 0.1 ml increments. Upon completion of this curve, the stretch–response curve, relating the probability of initiating a stretch-induced arrhythmia ($P$) to volume change ($\Delta V$) was determined. Generally, we would pick points on the curve with high probability (approximately 90%), mid-range probability (approximately 50%), and low probability (approximately 20%). Fifteen minutes after the collection of Baseline data, we would verify the response at a single point on the pressure–volume curve and selected $P$ values to determine the stability of the experimental preparation. Pharmacological agents were then administered continuously by addition to the perfusate. Hyperpolymer-

ization of microtubules was accomplished with taxol (5 μM), which was dissolved in dimethyl sulfoxide (DMSO). The DMSO was much less than 1% in total final volume, an amount reported to have no effect on the action potential properties of cardiac muscle (Rubart et al., 1998). This taxol dosage is approximately half of what can be observed clinically in serum levels after continuous intravenous infusion (Wiernik et al., 1987) and was previously shown to have no effect on cardiac function (Alloatti et al., 1998). The depolymerization of microtubules was accomplished with 100 μM colchicine, after a series of experiments with smaller dosages with no observed affects. At 30-min intervals throughout the experiment, we would repeat the measurements of the full pressure–volume curve and the $P$ value at either the low or midrange value. We measured full pressure–volume and stretch–response curve curves to complete the experiment. Upon completion of the experiment the left ventricle was weighed and prepared for pathological examination to confirm the effectiveness of the tubulin-binding agents. All data are reported as mean ± S.E. values. Student's $t$-test for paired observations was used to compare the data from the pre-drug and drug stages. Differences with a value of $p < 0.05$ (indicated with * in the figures) were considered statistically significant.

## 3. Results

### 3.1. Initiation of stretch-induced arrhythmias

Fig. 4 shows the extracted ventricular electrograms from 20 consecutive Stretch sequences, during Baseline and after 60 continuous min of 5 μM taxol administration. Before taxol, only three of 20 stretches produced an arrhythmia, whereas 12 of 20 stretches during administration of taxol produced an arrhythmia. Thus, in this example, taxol increased the probability of arrhythmia from 15% to 60%.

During Baseline, we ascertained the $P$–$\Delta V$ curve, an example of which is shown in Fig. 5. From the curve fitted points in the figure, we chose points designated as high ($P$ approximately 90%), medium (approximately 50%), and low (20–25%). Preliminary experiments indicated that values of $\Delta V$ on the Baseline $P$–$\Delta$V curve corresponding to $P > 0.5$ usually resulted in $P = 1.00$ during taxol administration. For the taxol experiments, our hypothesis predicted an increase in $P$, and to accommodate such an increase, we chose Baseline points that generally elicited an arrhythmia approximately 25% of the time for statistical analysis. During colchicine and control experiments, we chose a point close to 50% as little variation was hypothesized.

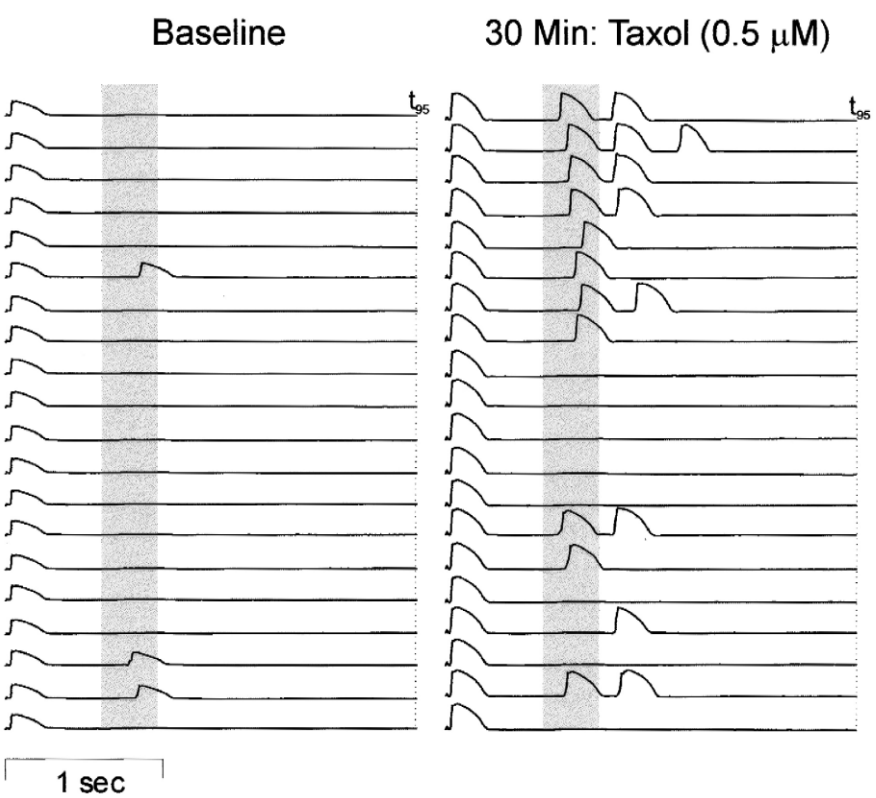


Fig. 4. Monophasic action potential recordings before (left tracings) and after (right tracings) 60 min of 5 μM taxol administration, demonstrating the increase in stretch-induced arrhythmias with microtubule hyperpolymerization. Each tracing begins with the last pacing stimulus of a stretch sequence. Vertical shaded bars show timing of left ventricular volume increase (Stretch).

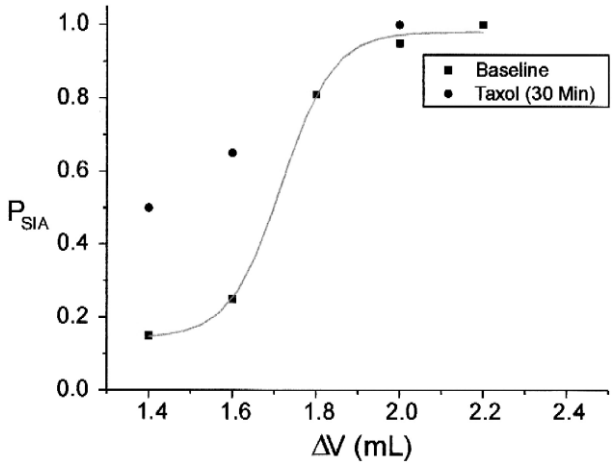


Fig. 5. The probability of stretch-induced arrhythmia vs. increase in left ventricular diastolic volume (Stretch) at Baseline and after 30 min of 5 μM taxol. After 30 min, only three points from the original curve are tested. Note the sigmoidal shape of the Baseline curve, typical for most hearts.

During the experiments, however, several points were tested at each data collection interval.

Fig. 6 illustrates the change in $P$ during the administration of taxol ($n = 6$ hearts), colchicine ($n = 5$), and during control experiments ($n = 5$). The Baseline points vary between groups, representing the variation in the properties of the hearts and the experimental design. In Fig. 6A, we see a statistically significant increase in stretch-induced arrhythmias after 30 min of taxol administration ($p = 0.001$) that was maintained throughout the course of the experiments (at 60 min, $p = 0.004$ vs. Baseline). In Fig. 6B data is presented from hearts ($n = 5$) treated with colchicine (100 μM). Ten previous experiments with varying dosages of colchicine (1, 5, and 10 μM) and no observed effect. As can be seen in the figure, no statistically significant change in $P$ was observed at either the 30 or 60 min time periods ($p = 0.359$ and 0.816, respectively) as compared to Baseline values. Similarly, in the control experiments shown in Fig. 6C, we observed no significant variation in $P$ during the time course of the experiments, ($n = 5$, $p = 0.078$ at 30 min and $p = 0.736$ at 60 min vs. Baseline $P$).

It is interesting to note that the hyperpolymerization of microtubules as induced by taxol was arrhythmogenic but that their depolymerization with colchicine had no effect on stretch-induced arrhythmia induction. The lack of an antiarrhythmic effect by colchicine is most likely due to the low number of microtubules in the adult cardiac myocyte (Rappaport and Samuel, 1988).

### 3.2. Hemodynamic effects of interventions

The hemodynamic effects of microtubule tubulin-binding, and control experiments are depicted in Fig. 7. In the figures, the mean values of both left ventricular end-diastolic and peak systolic pressure, indicators of compliance and contractility, respectively, are depicted. They are depicted as observed throughout the time course of the experiments. In Fig. 7A, we see that the compliance of the left ventricle throughout the experiment varies little, with no statistical significance at 30 min ($p = 0.322$) or 60 min ($p = 0.208$). Ventricular contractility shows no significant change after 30 min of taxol ($p = 0.177$), but by 60 min has shown a significant decrease ($p = 0.000$). However, in the colchicine experiments, as depicted in Fig. 7B, a statistically significant decrease in contractility was observed at 30 min ($p = 0.000$) and 60 min ($p = 0.001$). Compliance is relatively constant ($p = 0.860$ at 30 min

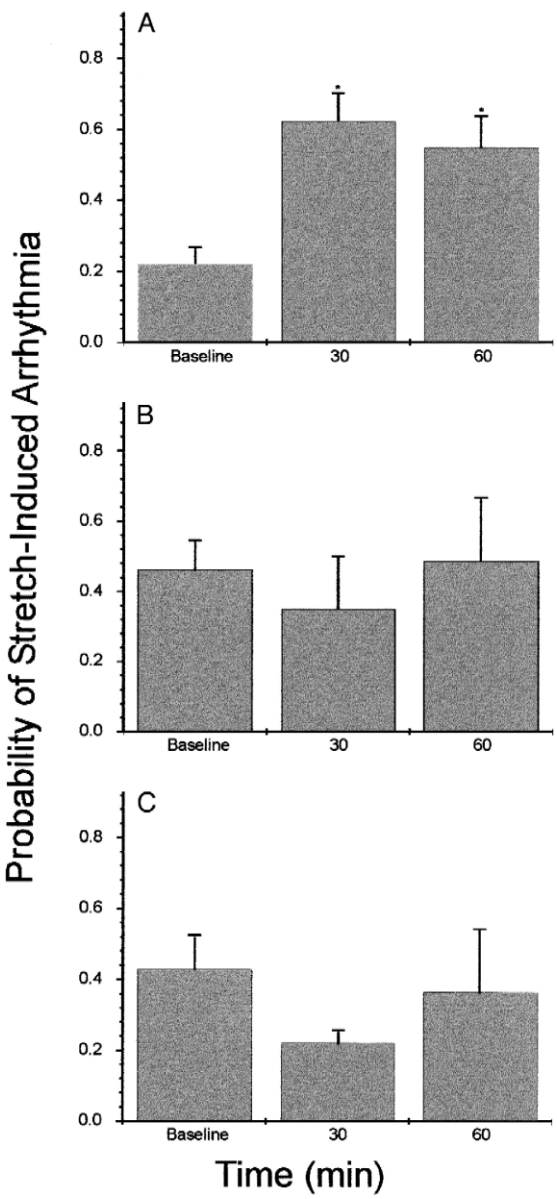


Fig. 6. The mean value ± S.E. of probability of stretch-induced arrhythmias during the time course of experiments: (A) microtubule polymerization with 5 μM taxol, (B) microtubule depolymerization with 100 μM colchicine, and (C) during control experiments.

and $p = 0.397$ at 60 min). During control experiments (Fig. 7C), we observed no statistically significant variation in contractility at 30 min ($p = 0.547$), but see a gradual, but significant, degradation of the contractile capability at 60 min ($p = 0.030$) with respect to Baseline. Again, as in Fig. 7A and 7B, we see no statistically significant variation in ventricular compliance ($p = 0.206$ at 30 min and $p = 0.351$ at 60 min as compared to Baseline). It can be assumed that the gradual degradation of contractile ability in the control hearts is due to the effects of time and long-term absence of hormonal influences in the contractile process of the ventricular myocytes. This same phenomenon was seen in Fig. 7A with the taxol-treated hearts.

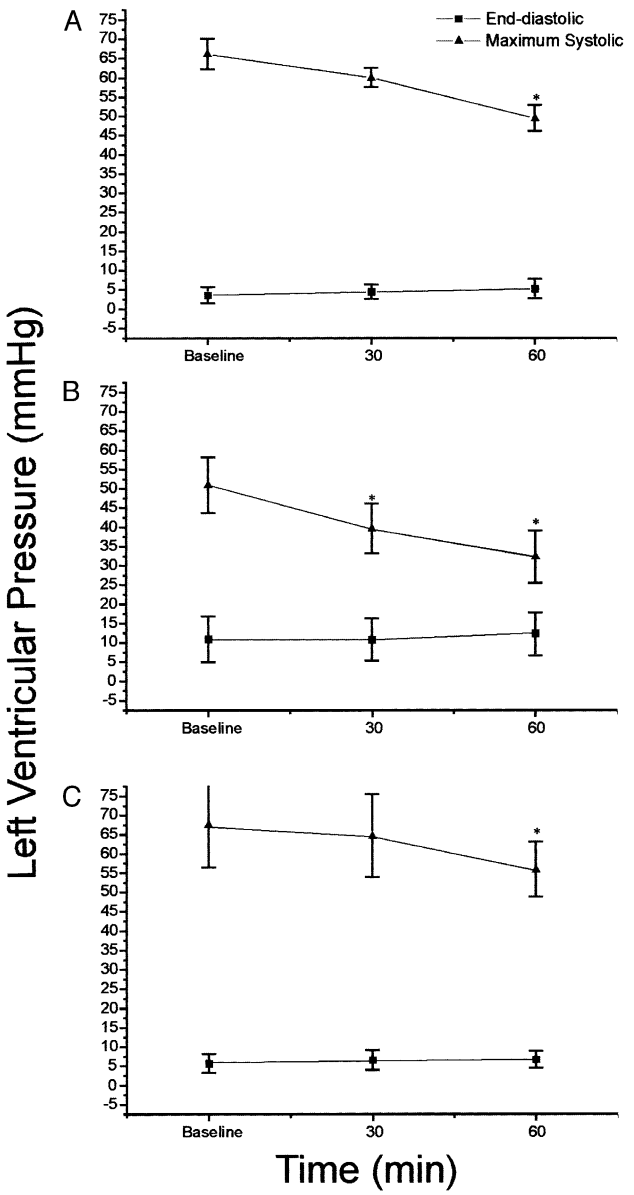


Fig. 7. The mean value ± S.E. of maximum left ventricular systolic pressure and left ventricular end diastolic pressure during the time course of experiments: (A) microtubule polymerization with 5 μM taxol, (B) microtubule depolymerization with 100 μM colchicine, and (C) during control experiments.

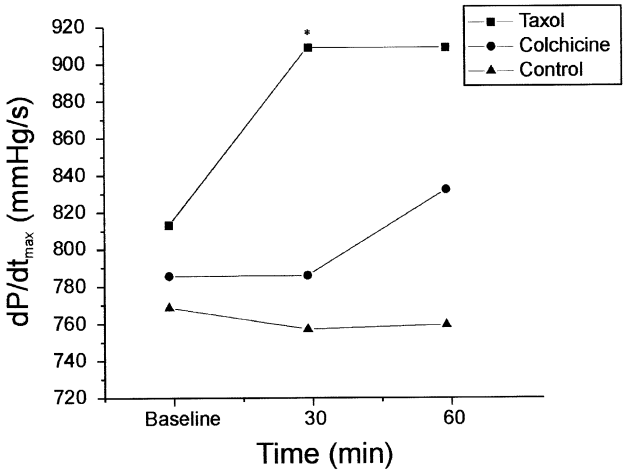


Fig. 8. Summary data for the effects of taxol, colchicine, and time on maximum rate of change of left ventricular pressure. The only statistically significant increase is during taxol administration.

Fig. 8 depicts the effects of microtubule proliferation and disruption as well as time on the mean values of maximum rate of left ventricular rise ($dP/dt_{max}$). The error bars are omitted for clarity. Again, Baseline values differ between data sets in accordance with the differences between the hearts. In the trace showing control values, we see little perturbation, as would be expected. In the trace for colchicine-treated hearts, we see a slight increase between 30 and 60 min, but it bears no statistical significance. The interesting part of the graph shows that a profound increase in $dP/dt_{max}$ occurs within 30 min of the onset of taxol administration ($p = 0.019$) coincident with the increase in $P$.

## 4. Discussion

The present study tested the mechanoarrhythmogenic effects of microtubule polymerization and depolymerization in the isolated, adult rabbit heart. We polymerized microtubules with taxol, a technique verified by Tagawa et al. (1997). After 30 min of taxol administration, a period of time sufficient for tubulin incorporation into microtubules (Schulze and Kirschner, 1986), we found an extraordinary increase in the probability of stretch-induced arrhythmias. This arrhythmogenic response was not temporally accompanied by perturbations in ventricular compliance indicators or contractile dysfunction attributed to the hyperpolymerization of microtubules in the ventricular myocyte (Tsutsui et al., 1993, 1994; Tagawa et al., 1996, 1997), but our experiments were half as long as the time required for their observed results. However, it was accompanied by an increase in the maximum rate of ventricular pressure rise.

Depolymerization of microtubules with colchicine did not alter the probability of stretch-induced arrhythmias, the maximum rate of ventricular pressure rise, or the ventricular compliance. Colchicine administration did, however,

degrade the ventricular contractility. Control experiments showed no change in the probability of stretch-induced arrhythmia and the effects of time on contractility of the Langendorff perfused hearts mimicked that of the taxol-treated hearts. No significant change in ventricular compliance or the maximum rate of ventricular pressure rise was observed. We would also point out that there were no observed arrhythmic effects of the decrease in ventricular contractility.

### *4.1. A model of arrhythmogenic effects of microtubule hyperpolymerization*

Like pressure-overloaded hypertrophy, taxol facilitates the hyperpolymerization of microtubules (Tagawa et al., 1997). There is increasing evidence that the architectural design of the cell cytoskeleton is that of a tensegrity structure (Ingber, 1993). Tensegrity structures are characterized by a unique balance of tensile and compressive forces and the structural elements that support them, and there is increasing evidence that cell homeostasis is a function of the cell's mechanical environment. Within the cell, compressive forces are hypothesized to be borne by microtubules and tensile forces are borne by actomyosin microfilaments (Ingber, 1993). We propose that this balance of forces is required for maintaining the routine electrophysiological response of the cardiocyte. Supporting this hypothesis is recent work showing that inhibiting the ability of microfilaments to bear tension would facilitate stretch-induced arrhythmias (Pitruzzello et al., 1998). Thus, hyperpolymerization of a structural element, such as the microtubule, or the inhibition of its ability to actively bear loads, as in the microfilament, would reorganize the distribution of mechanical stresses within the cell.

The cytoskeletal rearrangement resulting from the proliferation of microtubules may have one or more of three effects on L-type $Ca^{2+}$ channels: (1) restrict $Ca^{2+}$-dependent conformational changes in the cytoskeleton (Johnson and Byerly, 1993); (2) affect the concentration of inactivating ions near the mouths of the channels (Galli and DeFelice, 1994); or (3) increased, direct interaction between microtubules and the $Ca^{2+}$ channels (Galli and DeFelice, 1994). Regardless of the effects, the end result is an increased probability of the L-type $Ca^{2+}$ channels being found in the open state and an increased mean open time of the channels (Galli and DeFelice, 1994). This leads to an elevated level of intracellular $Ca^{2+}$, facilitating a subthreshold depolarization of the cell membrane and the increased availability of $Ca^{2+}$ for contraction. Previously, $Ca^{2+}$ overload has been demonstrated to potentiate stretch-induced arrhythmias (Johnson and Byerly, 1993). The slight depolarization of the cell membrane would probably lead to accommodation over time (Stacy et al., 1991), but initially might be arrhythmogenic should cation nonspecific stretch-activated channels open in response to mechanical perturbation. The opening of these mechanosensitive ion channels might be affected by redistribution of mechanical stresses within the cytoskeleton, or by increased direct interaction with tubulin. Contraction has been shown to be limited by the viscous forces presented by microtubule hyperpolymerization during pressure overloaded hypertrophy and taxol administration, with pronounced effects at 90 min of taxol administration (Tsutsui et al., 1994). Through 60 min of taxol administration, these effects were observed to build gradually, and are initially compensated for by increased intracellular $Ca^{2+}$ concentrations, which facilitate contraction. This combination of viscous resistance to contraction and higher intracellular $Ca^{2+}$ accounts for the apparent paradox observed between maximum left ventricular systolic pressure and the increased maximum rate of pressure development within the left ventricle.

While colchicine showed no effect on the probability of stretch-induced arrhythmia or maximum rate of pressure development, the data would tend to support the hypothesis. Depolymerization with colchicine might be expected to have two effects: a loss of cytoskeletal structural integrity, as seen with ischemic loss (Sperelakis, 1998), and a decreased probability of L-type $Ca^{2+}$ channels opening (Galli and DeFelice, 1994). The absence of mechanoarrhythmogenic consequences to their depolymerization might be an indication of the lack of interaction between tubulin and stretch-activated ion channels in the normal cardiocyte. More likely, the result is a reflection of the small amount of tubulin in adult cardiac myocytes (Rappaport and Samuel, 1988). Regardless, it has been demonstrated that colchicine increases the probability of finding L-type $Ca^{2+}$ channels in the closed state (Galli and DeFelice, 1994), and as such, would be expected to have a negative inotropic effect, as less $Ca^{2+}$ is available for calcium-induced, calcium release.

However, the loss of the cytoskeleton's ability to assume conformational states when microtubules are lost might indicate that ion channels, specifically those involved in the contractile process, could receive some feedback from the cytoskeleton which modulates their activity. If microtubules are hyperpolymerized, then the gain of this feedback might be increased. Should microtubules be lost, either through colchicine or ischemia, this feedback mechanism would be lost. This has been demonstrated by Galli and DeFelice (1994), who showed that normal L-type $Ca^{2+}$ channel kinetics were preserved when taxol was administered to the inside surface of cell-detached patches. That these channels exhibited no rundown nor sensitivity to taxol indicates that they are capable of functioning independent of cytoskeletal feedback, but, should it be present, their channel kinetics can be influenced. The existence of regulatory processes other than membrane voltage for calcium-induced, calcium release have been reported (Iwai et al., 1990) and leave open the possibility of mechanical input into the calcium-induced, calcium release feedback loop.

### 4.2. Calcium processing in hypertrophic hearts

In hypertrophied hearts, where one sees an over abundance of microtubules (Tsutsui et al., 1993; Rappaport and Samuel, 1988; Niggli and Lederer, 1990; Forbes and Sperelakis, 1983), abnormalities in $Ca^{2+}$ handling have also been observed (Samuel et al., 1986; Kiss et al., 1995) that include a second $Ca^{2+}$ transient attributed to the sarcoplasmic reticulum's inability to resequester intracellular $Ca^{2+}$. This second $Ca^{2+}$ transient is attributed to increased $Ca^{2+}$ entry into the cell and the retarded ability of the sarcoplasmic reticulum to resequester the $Ca^{2+}$ following the ensuing contraction.

However, if the relaxation of cardiocytes in diastole is curbed, as has been reported (Samuel et al., 1986), whether due to altered $Ca^{2+}$ resequestration, increased expression of titin and desmin (Meuse et al., 1992) or microtubules (Tagawa et al., 1997), or a combination thereof, the end result would be a myocyte not as mechanically compliant as the normal state. Based on previous results (Parker et al., 1997b), we believe that cytoskeletal load distributions have an effect on stretch-activated channels and are potentially arrhythmogenic.

In conclusion, hyperpolymerization of microtubules, as facilitated by taxol, increased the probability of stretch-induced ventricular arrhythmias. This increase was unaccompanied by any measurable contractile dysfunction. This increase was accompanied by an increase in the maximum rate of pressure development within the ventricle. We believe these phenomena are explainable given recent reports on the altered kinetics of $Ca^{2+}$ channels during exposure to taxol. Administration of colchicine did not facilitate arrhythmia, but reduced the maximum systolic pressure within the ventricles. Our results have clinical implications for chemotherapeutic patients and those with uncompensated ventricular hypertrophy, and suggest that the myocyte cytoskeleton may be a suitable target for pharmacological treatment of cardiac arrhythmias.

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## References

Alloatti, G., Penna, C., Gallo, M.P., Levi, R.C., Bombardelli, E., Appendino, G., 1998. Differential effects of paclitaxel and derivatives on guinea pig isolated heart and papillary muscle. J. Pharmacol. Exp. Ther. 284 (2), 561–567.

Califf, R., Burks, J., Behar, V., Marigolds, J., Wagner, G., 1987. Relationship among ventricular arrhythmias, coronary artery disease and angiographic and electrocardiographic indicators of myocardial fibrosis. Circulation 57, 725–732.

Dean, J.W., Lab, M.J., 1989. Arrhythmia in heart failure: role of mechanically induced changes in electrophysiology. Lancet 1, 1309–1312.

Forbes, M.S., Sperelakis, N., 1983. The membrane systems and cytoskeletal elements of mammalian myocardial cells. Cell Muscle Motil. 3, 89–155.

Francis, G.S., 1986. Development of arrhythmias in the patient with congestive heart failure: pathophysiology, prevalence and prognosis. Am. J. Cardiol. 57, 3B–7B.

Franz, M., 1996. Mechano-electrical feedback in the ventricular myocardium. Cardiovasc. Res. 32, 15–24.

Franz, M.R., Burkhoff, D., Spurgeon, H., Weisfeldt, M.L., Lakatta, E.G., 1986. In vitro validation of a new cardiac catheter technique for recording monophasic action potentials. Eur. Heart J. 7, 34–41.

Franz, M.R., Burkhoff, D., Yue, D.T., Sagawa, K., 1989. Mechanically induced action potential changes and arrhythmia in isolated and in situ canine hearts. Cardiovasc. Res. 23, 213–223.

Galli, A., DeFelice, L.J., 1994. Inactivation of L-type Ca channels in embryonic chick ventricle cells: dependence on the cytoskeletal agents colchicine and taxol. Biophys. J. 67, 2296–2304.

Ghali, J.K., Kadakia, S., Cooper, R.S., Liao, Y., 1991. Impact of left ventricular hypertrophy on ventricular arrhythmias in the absence of coronary artery disease. J. Am. Coll. Cardiol. 17, 1277–1282.

Halperin, B.D., Adler, S.W., Mann, D.E., Reiter, M.J., 1993. Mechanical correlates of contraction–excitation feedback during acute ventricular dilatation. Cardiovasc. Res. 27, 1084–1087.

Hansen, D.E., 1993. Mechanoelectrical feedback effects of altering preload, afterload, and ventricular shortening. Am. J. Physiol. 264, H423–H432.

Hansen, D.E., Borganelli, S.M., Stacy Jr., G.P., Taylor, L.K., 1991. Dose-dependant inhibition of stretched-induced arrhythmias by gadolinium in isolated canine ventricles: evidence for a unique mode of antiarrhythmic action. Circ. Res. 69, 820–831.

Hu, H., Sachs, F., 1997. Stretch-activated ion channels in the heart. J. Mol. Cell. Cardiol. 29, 1511–1523.

Ingber, D.E., 1993. Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. J. Cell. Sci. 104, 613–627.

Iwai, K., Hori, M., Kitabatake, A., Kurihara, H., Uchida, K., Inoue, M., Kamada, T., 1990. Disruption of microtubules as an early sign of irreversible ischemic injury. Circ. Res. 67 (3), 694–706.

Johnson, B.D., Byerly, L., 1993. A cytoskeletal mechanism for $Ca^{2+}$ channel metabolic dependance and inactivation by intracellular $Ca^{2+}$. Neuron 10, 797–804.

Kiss, E., Ball, N.A., Kranias, E.G., Walsh, R.A., 1995. Differential changes in cardiac phospholambin and the sarcoplasmic reticular $Ca^{2+}$-ATPase protein levels. Circ. Res. 77 (4), 759–764.

Lab, M.J., 1982. Contraction–excitation feedback in myocardium: physiological basis and clinical relevance. Circ. Res. 50 (6), 757–766.

Levy, D., Anderson, K., Savage, D.D., Balkus, S.A., Kannel, W.B., Castelli, W.P., 1987. Risk of ventricular arrhythmias in left ventricular hypertrophy: the Framingham heart study. Am. J. Cardiol. 60, 560–565.

Matsuda, N., Hagiwara, N., Shoda, M., Kasanuki, H., Hosoda, S., 1996. Enhancement of the L-type $Ca^{2+}$ current by mechanical stimulation in single rabbit cardiac myocytes. Circ. Res. 78 (4), 650–659.

Meuse, A.J., Perreault, C.L., Morgan, J.P., 1992. Pathophysiology of cardiac hypertrophy and failure of human working myocardium: abnormalities in calcium handling. Basic Res. Cardiol. 87, 223–233.

Niggli, E., Lederer, W.J., 1990. Voltage-independent calcium release in heart muscle. Science 250, 565–568.

Parker, K.K., Taylor, L.K., Wray, J., Wikswo Jr., J.P., Hansen, D.E., 1997a. Effects of osmotic stress on the probability of stretch-induced cardiac arrhythmias. Bull. Am. Phys. Soc. 42 (9), 1798, Abstract.

Parker, K.K., Taylor, L.K., Wray, J., Atkinson, J., Wikswo Jr., J.P., Hansen, D.E., 1997b. The effects of cytoskeletal reorganization on the

probability of stretch-induced arrhythmias in mature rabbit heart. Ann. Biomed. Eng. 25, S-63, Abstract.

Pitruzzello, A.M., Parker, K.K., Hansen, D.E., Wikswo Jr., J.P., 1998. Chemical excitation–contraction decoupling facilitates cardiac mechanoarrhythmogenesis. Ann. Biomed. Eng. 26, S-24, Abstract.

Rappaport, L., Samuel, J.L., 1988. Microtubules in cardiac myocytes. Int. Rev. Cytol. 113, 101–143.

Rowinsky, E.K., Donehower, R.C., 1995. Paclitaxel (taxol). New Engl. J. Med. 332, 1004–1014.

Rubart, M., Biermann, M., Wu, J., Moreno, A., Josiah-Dorant, A., Zipes, D.P., 1998. Differential effects of cytochalasin D and 2,3-butanedione monoxime on cardiac excitation–contraction coupling. Biophys. J. 74 (2), A55, Abstract.

Samuel, J.-L., Marotte, F., Delcayre, C., Rappaport, L., 1986. Microtubule reorganization is related to rate of heart myocyte hypertrophy in rat. Am. J. Physiol. 250, H1118–H1125.

Schiff, P.B., Fant, J., Horwitz, S.B., 1979. Promotion of microtubule assembly in vitro by taxol. Nature 277, 665–667.

Schulze, E., Kirschner, M.W., 1986. Microtubule dynamics in interphase cells. J. Cell Biol. 102, 1020–1031.

Schulze, R.A., Rouleau, J., Rigo, P., Bowers, S., Strauss, W., Pitt, B., 1975. Ventricular arrhythmias in the late hospital phase of acute myocardial infarction-related to left ventricular function detected by gated cardiac blood pool scanning. Circulation 52, 1006–1011.

Sigurdson, W.J., Ruknudin, A., Sachs, F., 1992. Calcium imaging of mechanically induced fluxes in tissue-cultured chick heart: role of stretch-activated ion channels. Am. J. Physiol. 1262, H1110–H1115.

Sperelakis, N., 1998. Electrogenesis of membrane excitability. In: Sperelakis, N. (Ed.), Cell Physiology Source Book. 2nd edn. Academic Press, San Diego, pp. 368–390.

Stacy Jr., G.P., Taylor, L.K., Alexander Jr., J., Hansen, D.E., 1991. Calcium overload potentiates stretch-induced arrhythmias. Circulation 84 (Suppl. II), II-668, Abstract.

Stacy Jr., G.P., Jobe, R.L., Taylor, L.K., Hansen, D.E., 1992. Stretch-induced depolarizations as a trigger of arrhythmias in isolated canine left ventricles. Am. J. Physiol. 263, H613–H621.

Tagawa, H., Rozich, J.D., Tsutsui, H., Narishige, T., Kuppuswamy, D., Sato, H., McDermott, P.J., Koide, M., Cooper IV, G., 1996. Basis for increased microtubules in pressure-hypertrophied cardiocytes. Circulation 93 (6), 1230–1243.

Tagawa, H., Wang, N., Narishige, T., Ingber, D.E., Zile, M.R., Cooper IV, G., 1997. Cytoskeletal mechanics in pressure-overload cardiac hypertrophy. Circ. Res. 80 (2), 281–289.

Terzic, A., Kurachi, Y., 1998. Cytoskeleton effects on ion channels. In: Sperelakis, N. (Ed.), Cell Physiology Source Book. 2nd edn. Academic Press, San Diego, pp. 532–546.

Tsutsui, H., Ishihara, K., Cooper IV, G., 1993. Cytoskeletal role in the contractile dysfunction of hypertrophied myocardium. Science 260, 682–686.

Tsutsui, H., Tagawa, H., Kent, R.L., McCollam, P.L., Ishihara, K., Nagatsu, M., Cooper IV, G., 1994. Role of microtubules in contractile dysfunction of hypertrophied cardiocytes. Circulation 90 (1), 533–555.

Wiernik, P.H., Schwartz, E.L., Einzig, A., 1987. Phase I trial of taxol given as a 24 hour infusion every 21 days: responses observed in metastatic melanoma. J. Clin. Oncol. 5, 1232–1239.

Yamashita, T., Oikwa, N., Murakawa, Y., Nakajima, T., Omata, M., Inoue, H., 1994. Contraction–excitation feedback in atrial reentry: role of velocity of mechanical stretch. Am. J. Physiol. 267, H1254–H1262.