Oscillations of Intracellular Calcium Induced by Vasopressin in Individual Fura-2-loaded Mesangial Cells

FREQUENCY DEPENDENCE ON BASAL CALCIUM CONCENTRATION, AGONIST CONCENTRATION, AND TEMPERATURE*

(Received for publication, January 25, 1991)

Roger J. Hajjar and Joseph V. Bonventre‡
From the Medical Services, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts 02114

Intracellular free calcium concentration ([Ca\(^{2+}\)\(_{i}\)]) was measured in fura-2-loaded single rat mesangial cells by dual wavelength spectrofluorometry. Stimulation with arginine vasopressin (AVP) caused an initial sharp rise of [Ca\(^{2+}\)\(_{i}\)], followed by repetitive spikes. The frequency of the oscillations was dependent on the concentration of AVP. At 0.1, 1.0, 10.0, and 100.0 nM AVP, the frequencies of oscillations were 0.17 ± 0.05 (n = 6), 0.32 ± 0.05 (n = 6), 0.49 ± 0.05 (n = 6), and 0.48 ± 0.05 min\(^{-1}\) (n = 5), respectively. Reduction in extracellular [Ca\(^{2+}\)] reduced the frequency of AVP-induced oscillations but did not abolish the oscillations. The frequency of calcium oscillations, upon stimulation with 1.0 nM AVP, was directly correlated with the basal [Ca\(^{2+}\)] prior to stimulation. Oscillation frequency increased with increasing temperature. An Arrhenius plot between 24 and 37 °C indicated a strong temperature dependency of the oscillations with a Q\(_{10}\) of 3.0.

Protein kinase C stimulation by active phorbol esters inhibited AVP-induced calcium oscillations but not the initial [Ca\(^{2+}\)] response to AVP. These observations are consistent with a model incorporating a feedback loop linking [Ca\(^{2+}\)]\(_{i}\) to the mechanism of [Ca\(^{2+}\)], increase. Ca\(^{2+}\)-induced Ca\(^{2+}\) release may be involved, whereby inositol 1,4,5-trisphosphate (inositol 1,4,5-P\(_{3}\)) formation releases Ca\(^{2+}\) from an inositol 1,4,5-P\(_{3}\)-sensitive pool, with subsequent Ca\(^{2+}\) uptake and release from an inositol 1,4,5-P\(_{3}\)-insensitive pool.

Since intracellular free calcium concentration ([Ca\(^{2+}\)]\(_{i}\)) is an important factor in the control of cell function, it is functional that mechanisms for feedback control of [Ca\(^{2+}\)] exist not only at rest but during activation (1). Agonist-evoked Ca\(^{2+}\) oscillations suggest the existence of such regulation. A variety of cells have been reported to display repetitive spikes of [Ca\(^{2+}\)] during hormonal stimulation (2). The oscillatory behavior of the [Ca\(^{2+}\)]\(_{i}\) response has been observed in excitable cells such as myocytes (3, 4), smooth muscle cells (5), neuronal cells (6), and in nonexcitable cells such as oocytes (7), hepatocytes (8, 9), pancreatic acini (10), parotid acini (11), and macrophages (12). Thus, in many cells, where the elevation of [Ca\(^{2+}\)]\(_{i}\) links receptor binding to cellular response, oscillations of [Ca\(^{2+}\)]\(_{i}\) are present and may play a central role in signal transduction. In order to understand the mechanisms involved in the generation of Ca\(^{2+}\) oscillations, it is important to know how the frequency of oscillations depends upon levels of cytosolic free [Ca\(^{2+}\)]. Also the temperature dependency of oscillation frequency can help to distinguish whether the feedback processes involved are enzymatic or diffusional in character. These issues have not been adequately resolved by the previous studies.

Glomerular mesangial cells provide a good model system in which the regulation of [Ca\(^{2+}\)], by receptor-mediated activation has been well characterized. Vasopressin increases [Ca\(^{2+}\)], in renal mesangial cells in culture (13). Vasopressin activates phosphatidylinositol-specific phospholipase C, which hydrolyzes phosphatidylinositol 4,5-bisphosphate to form diacylglycerol and inositol 1,4,5-trisphosphate (inositol 1,4,5-P\(_{3}\)). Inositol 1,4,5-P\(_{3}\) in turn releases Ca\(^{2+}\) from intracellular stores (14). The mobilization of Ca\(^{2+}\) by inositol 1,4,5-P\(_{3}\) is an important component of the models proposed to explain calcium oscillations. Some of these models are based on feedback processes that either generate oscillations of inositol 1,4,5-P\(_{3}\) or explain oscillatory behavior in the presence of steady levels of inositol 1,4,5-P\(_{3}\) by invoking Ca\(^{2+}\)-induced Ca\(^{2+}\) release (2).

We found that single mesangial cells respond to vasopressin with repetitive [Ca\(^{2+}\)] spikes. The presence of oscillations demonstrates the existence of feedback pathways in the control of elevated [Ca\(^{2+}\)]. In addition, the existence of such oscillations implies that information carried by second messengers may be frequency-encoded. We investigated 1) the effect of agonist concentration on the frequency of oscillations; 2) the temperature dependence of this frequency; 3) the effect of basal [Ca\(^{2+}\)], on the frequency of oscillations; and 4) the potential modulation of this frequency by prostaglandin E\(_{2}\) and phorbol esters. Our results are consistent with a model of agonist-induced calcium oscillations in which Ca\(^{2+}\)-induced Ca\(^{2+}\) release from intracellular storage sites may play a major role.

EXPERIMENTAL PROCEDURES

Isolation of Mesangial Cells—Isolated mesangial cells were prepared from Wistar rat glomeruli as previously described (13). The cells were
maintained in RPMI 1640 medium (GIBCO containing 20% fetal calf serum in an incubator at 37 °C, with 95% air and 5% CO₂. The cells were passaged using split ratios of 1:3 and used in passages 8-12. Two days prior to the experiments, cells were plated on glass coverslips, 3 mm in diameter and 0.07 mm in thickness (Biophysics Technologies, Inc., Baltimore, MD). Coverslips were plated with mesangial cells were incubated for 45 min in a buffer containing 20 mM HEPES, 1 mM CaCl₂, 5 mM KCl, 145 mM NaCl, 0.5 mM MgSO₄, 25 mM NaHCO₃, 1.4 mM KH₂PO₄, 10 mM glucose, and 0.5 mM fura-2/AM, at 37 °C at pH 7.4, aerated with 95% air and 5% CO₂. Cells loaded with fura-2 were washed with a modified Krebs-Henseleit buffer containing 20 mM HEPES, 1 mM CaCl₂, 5 mM KCl, 155 mM NaCl, 0.5 mM MgSO₄, 1 mM NaH₂PO₄, 5 mM glucose, pH 7.4. To check that leakage of the ester occurred, we sampled the excitation spectra after different times of fura-2/AM incubation. At 5 min after the start of incubation, the excitation spectrum peaked at approximately 380 nm, reflecting incomplete hydrolysis of fura-2/AM, whereas beyond 5 min the excitation spectrum peaked at 345 nm, which is characteristic of fura-2 free acid.

**Measurement of Fura-2 Fluorescence**—To measure fluorescence of individual cells, the cover slips were placed in a microscope tissue chamber (Biophysics Technologies, Inc.). The chamber consisted of a shallow sloped compartment made of Teflon with a silicone rubber seal. The cover slips were placed as the bottom of the chamber. A heater/cooler ring was encased in the silicone rubber that sealed the coverslip in place. Temperatures were varied between 22 and 37 °C by applying 0-4 °C to the ring. If the temperature is not specifically stated, then the experiment was performed at 27 °C. The chamber was mounted on the stage of an inverted microscope (Zeiss IM-35, Carl Zeiss, Inc., Thornwood, NY). Fura-2 fluorescence was excited with a 78-watt xenon arc lamp placed at the focal point of a condenser (Photon Technologies International (PTI) Inc., Princeton, NJ). Grating monochromators, alternated by a rotating chopper in which mirror vanes alternate with transmitting sectors, were used for selecting wavelengths. The monochromator outputs were combined to form a common optical path that exited the source housing through a rotating chopper in which light is divided into a series of wavelengths. The monochromator outputs were combined to an adjustable iris. The light then passed through quartz lenses and a 340 nm signal was reduced to baseline. A photomultiplier tube device detection system was used to measure the light output. Data analysis was performed using PTI software run on an IBM-compatible AT/286 computer using the MS-DOS operating system. Data were retained and manipulated in a packed binary format.

**[Ca²⁺]i Determination**—Intracellular calcium concentrations were calculated according to the formula [Ca²⁺]i = Kₛ[R(R - Rₘₚ)/(Rₘₚ - R)]B, where R is the ratio of fluorescence of the cell at 340 and 380 nm, Rₘₚ, and Rₘₚ represent the ratio of fura-2 fluorescence intensity at 340 and 380 nm excitation wavelengths in the presence and absence of 10 nM fura-2/AM, respectively; B is the ratio of fluorescence of fura-2 at 380 nm in zero calcium to that with saturating amounts of calcium; and Kₛ is the dissociation constant of fura-2 for calcium. To determine Rₘₚ, at the end of an experiment, ionomycin was added to the fura-2-loaded cells to increase intracellular Ca²⁺ concentration to high levels resulting in maximal Ca²⁺-dependent fura-2 fluorescence (15). To calculate Rₘₚ, 1 mM EGTA was then added to the bathing solution. Different dissociation constants were used at the different temperatures: 224 nM at 35-37 °C and 135 nM at 24-27 °C (16).

**Errors of [Ca²⁺]i, Estimation Associated with Fura-2—Fura-2 has limitations in its use as a Ca²⁺ indicator (17, 18). Incomplete dehydrolysis of fura-2/AM ester bonds might yield a fluorescent spectrum that can be calcium-insensitive (19). The extent of deesterrification of fura-2/AM is cell-specific. To determine whether mesangial cells, loaded with fura-2/AM, contain deesterified fura-2, we compared the spectrum of the fura-2-loaded cells with the spectrum of fura-2 in Ca²⁺-EGTA buffers at low free [Ca²⁺]. Even though complex spectra are difficult to deconvolute by eye, the spectra were not visually shifted with respect to each other. This does not assure the complete dehydrolysis of fura-2/AM, but offers some assurance that the intracellular fura-2 used to measure Ca²⁺ was predominantly in free acid form. Dye loss from the fura-2-labeled cells can also affect the estimation of [Ca²⁺]. Given the small volume of cells relative to bath, dye leakage was not a problem. Sequestration of fura-2 in noncytoplasmic compartments is another potential problem. Once inside the cells, fura-2/AM can cross the membranes of intracellular organelles if the level of cytosolic esterase is low. To evaluate the extent of fura-2 compartmentalization, we permeabilized fura-2-loaded cells with 10 μM digitonin. This concentration of digitonin would only affect the plasma membrane leaving intracellular organelles, including mitochondria, intact. The 340 nm signal was reduced by 87%. Further addition of 1% Triton, which destroys the mitochondria and other intracellular organelles, brought the signal to base line. This suggests that 87% of the loaded fura-2 is in the cytoplasm. For this reason, and since temperature changes might alter Ca²⁺ homeostasis, we did not attempt to prescore our cells to prevent intracellular organelle sequestration (15, 17). Excitation light can cause photo-bleaching and photochemical formation of harmful metabolites intracellularly. Since our experiments lasted 20 min, we compared the response of the fura-2-loaded cells to vasopressin after 2 min and 10 min. Our results indicate that the vasopressin stimulus did not alter Ca²⁺ responses. Finally, shifts in the absorption and emission spectra, as well as the dissociation constants, can occur as a function of viscosity and ionic strength. Recently, Pienno (20) showed that a 15% decrease or less in the Rₘₚ and Rₘₚ can account for viability effects. We have not incorporated a correction factor for viscosity into our data analysis.

**Measurements of [Ca²⁺], Oscillations**—We estimated the frequency of oscillations or repetitive spikes from the [Ca²⁺], signal by counting the number of repetitive spikes and then averaging over the period of time. Frequency is presented in units of number of spikes/minute or number of spikes/minute. The frequency is an average of at least three experiments. The frequency of repetitive fura-2 fluorescence is an average of at least three experiments. The frequency of repetitive fura-2 fluorescence is usually characterized by spikes combined with sustained increases in [Ca²⁺]. We considered [Ca²⁺]i signals that were characterized by a sustained elevation in [Ca²⁺], without any clear periodic changes after the initial spike as nonsinusoidal.

**Materials**—Fura-2/AM was purchased from Molecular Probes, Inc. (Eugene, OR), and ionomycin was obtained from Calbiochem. Pherbol 12-myristate 13-acetate (PMA), prostaglandin E₁, and arginine vasopressin, as well as the remaining chemicals used, were purchased from Sigma.

**Statistics**—Results were presented as means ± S.E. (standard error of the mean). Significance was evaluated by the Student’s t test, and differences were considered significant if p was less than 0.05. Unless otherwise stated, tracings of individual cell responses are representative of at least three experiments.

**RESULTS**

**[Ca²⁺], Response in Single Cells**— Representative changes in [Ca²⁺]i; in response to various concentrations of arginine vasopressin (AVP) at 27 °C are depicted in Fig. 1. The [Ca²⁺], transients had a characteristic initial large increase followed by repetitive [Ca²⁺], elevations. The mean resting [Ca²⁺], in single mesangial cells was 113 ± 3.4 nM (n = 81). Maximal values of [Ca²⁺], following 1 nM AVP stimulation averaged 920 ± 35.6 nM (n = 9), with a range of 525-1204 nM. These mean values are in reasonable accordance, although slightly higher, than previous measurements of basal and stimulated [Ca²⁺], in populations of mesangial and renal epithelial cells (13, 22, 23).

After stimulation with 0.1 nM of AVP, 6 of 11 of the cells studied showed oscillations. At 1.0 nM AVP, 6 of 9 displayed oscillations. [Ca²⁺], oscillations occurred in 6 of 9, and 5 of 8 cells at AVP concentrations of 10 and 100 nM, respectively. In Fig. 2, the frequency of Ca²⁺ oscillations is plotted as a function of AVP concentration. As shown in Figs. 1 and 2, increasing the concentration of AVP increased the frequency of the repetitive Ca²⁺ spikes. The frequency of oscillations increased with AVP concentration, reaching a plateau at approximately 10 nM AVP, with further increases with lower concentrations. The amplitude of the oscillations did not vary with the concentration of AVP. The relative change in amplitude of [Ca²⁺], for the repetitive spikes was in general smaller than the initial rise in [Ca²⁺], at all concentrations of AVP.

**Effect of Basal [Ca²⁺], on the Oscillations of [Ca²⁺],**—We examined whether the level of basal [Ca²⁺], determines the frequency of oscillations or repetitive spikes from the [Ca²⁺]. Figure 3 illustrates that the frequency of repetitive [Ca²⁺], spikes increases with the concentration of AVP to a maximum value, beyond which no further increases occur. In this experiment, the frequency of repetitive [Ca²⁺], spikes increased from 19 to 29 spikes/minute with increasing concentrations of AVP. The maximum frequency of repetitive [Ca²⁺], spikes was reached at approximately 10 nM AVP, with no further increases with higher concentrations. The amplitude of the oscillations did not vary with the concentration of AVP.
Basal [Ca\(^{2+}\)] Determines Ca\(^{2+}\) Oscillation Frequency

\[ [\text{AVP}] = 0.1 \text{ nM} \]

\[ [\text{AVP}] = 1.0 \text{ nM} \]

\[ [\text{AVP}] = 10.0 \text{ nM} \]

**FIG. 1.** [Ca\(^{2+}\)] responses in individual mesangial cells stimulated with 0.1 (A), 1.0 (B), and 10.0 (C) nM AVP in Krebs-Henseleit buffer at 27 °C. Each tracing represents the response of a different cell.

**FIG. 2.** Frequency of [Ca\(^{2+}\)] oscillations in individual mesangial cells as a function of four different vasopressin (AVP) concentrations: 0.1 nM (n = 6), 1.0 nM (n = 6), 10.0 nM (n = 6), and 100.0 nM (n = 5).

Frequency of the [Ca\(^{2+}\)] fluctuations observed after AVP exposure. When basal [Ca\(^{2+}\)], was decreased by incubating the cells in media with no added extracellular [Ca\(^{2+}\)] ([Ca\(^{2+}\)] = 1–5 μM), there was a resultant decrease in the frequency of AVP-induced Ca\(^{2+}\) oscillations, as compared with the stimulated frequency of oscillations in buffer containing 1.0 mM extracellular [Ca\(^{2+}\)], as shown in Fig. 3. In two out of four cells stimulated with AVP in the presence of 1–5 μM extracellular [Ca\(^{2+}\)] (including one of the cell responses depicted in Fig. 3), a cluster of spikes was observed after stimulation with no further [Ca\(^{2+}\)] transients, whereas in the remaining two, further spikes were observed between 7 and 13 min after stimulation. When extracellular [Ca\(^{2+}\)] was increased to 2 mM, resulting in an increase in basal [Ca\(^{2+}\)], there was an increase in the frequency of AVP-induced oscillations but no increase in the amplitude of the oscillations. The relationship between basal resting [Ca\(^{2+}\)], prior to stimulation with 1.0 nM AVP and the frequency of [Ca\(^{2+}\)] oscillations in the presence of 1.0 nM AVP is illustrated in Fig. 4. There is a highly significant correlation between the frequency of Ca\(^{2+}\) oscillations, or the mean recurrence time of a [Ca\(^{2+}\)] spike, and the level of basal [Ca\(^{2+}\)], in the cells prior to AVP addition.

**Effect of Temperature**—To determine whether [Ca\(^{2+}\)] oscillations are more likely to be due to an enzymatic or to a diffusional process in the cell, we examined the frequency of the oscillations at four different temperatures (24, 27, 34 and 37 °C). As shown in Fig. 5, the frequency of oscillations increased with increasing temperature. The time course of the individual [Ca\(^{2+}\)] transients was reduced at the higher temperatures. Although basal [Ca\(^{2+}\)], decreased with increased temperatures, there was an increase in the frequency of oscillations when temperature was increased between 24 and 37 °C. In order to determine the Q\(_{10}\) of the oscillation frequency, the log of the mean values of these frequencies were plotted as a function of 1/temperature in an Arrhenius plot (Fig. 6). The Q\(_{10}\) value obtained from a least square fit of the points was 3.0, within the range found for typical biochemical processes (24).

**Effects of PGE\(_2\) and Protein Kinase C Activation**—We examined whether PGE\(_2\), which has been reported to activate polyphosphoinositide lipid turnover in mesangial cells (25), altered the oscillatory pattern produced by AVP (Fig. 7). 1 μM PGE\(_2\) alone did not initiate Ca\(^{2+}\) oscillations. In the presence of 1 μM PGE\(_2\), the AVP-stimulated initial [Ca\(^{2+}\)] spike was followed by a sustained increase in [Ca\(^{2+}\)], with
large fluctuations from base line. No clear decrease or increase in AVP-induced [Ca\(^{2+}\)] responses identified with PGE2 pretreatment. Interestingly, the [Ca\(^{2+}\)] transient became broader with a significant plateau.

To examine the possible effects of protein kinase C activation on the [Ca\(^{2+}\)] transient, we tested whether [Ca\(^{2+}\)] oscillations are affected by phorbol esters. As shown in Fig. 8, pretreatment with 300 nM PMA inhibited AVP-induced [Ca\(^{2+}\)] oscillations but not the initial response to 1 nM AVP. Interestingly, the [Ca\(^{2+}\)] transient became broader with a significant plateau.

**DISCUSSION**

**Single Cells Versus Population of Cells**—In contrast to previous studies with populations of mesangial cells in suspension where vasopressin-induced Ca\(^{2+}\) transients have an initial peak response followed by a sustained elevation in [Ca\(^{2+}\)], (13), the response in single mesangial cells is periodic in nature, with recurrent transient increases in [Ca\(^{2+}\)]. These differences in agonist-induced Ca\(^{2+}\) transients between cell suspensions and single cells have been observed in hepatocytes (8) and parotid acinar cells (9). A possible explanation for these differences is that the asynchronous response of individual cells results in an integrated response that is biphasic. Rooney et al. (21) have shown that there is marked heterogeneity among cells in the latency of agonist-induced [Ca\(^{2+}\)] responses. Furthermore, other factors such as noise level, cell movement, and background autofluorescence can also hide the oscillatory nature of the individual cell responses when populations are studied.

**Mechanism of Ca\(^{2+}\) Oscillations**—Oscillations in [Ca\(^{2+}\)] have been explained by many models. Berridge and Galione (2) have proposed that oscillatory models can be divided into two classes: 1) a receptor-controlled oscillator that would result in periodic changes in cellular levels of a mediator such as inositol 1,4,5-P\(_3\), which mobilizes Ca\(^{2+}\) and 2) a second messenger-mediated oscillator that would explain oscillations due to periodic changes in the sensitivity of Ca\(^{2+}\) stores to a messenger such as inositol 1,4,5-P\(_3\) or through Ca\(^{2+}\)-induced Ca\(^{2+}\) release. In the receptor-controlled oscillator models, a possible mechanism for the periodic formation of inositol 1,4,5-P\(_3\) is via a negative feedback loop in which protein kinase C, activated by diacylglycerol, another product of phospholipase C action on phospholipids, decreases phospholipase C-induced hydrolysis of inositol lipids and hence decreases inositol 1,4,5-P\(_3\) levels. In our experiments, the presence of active phorbol esters inhibited AVP-induced oscillations. These results are comparable with those of other investigators (8, 21, 26). Protein kinase C activation has been proposed to inhibit, by phosphorylation, the CTP-binding protein that couples the receptor to phospholipase C (27). This would result in a decrease in phosphatidylinositol 4,5-bisphosphate breakdown and formation of inositol 1,4,5-P\(_3\).

One second messenger-controlled oscillator model is based on Ca\(^{2+}\)-induced Ca\(^{2+}\) release by the endoplasmic reticulum/sarcoplasmic reticulum (2). This model requires two different intracellular Ca\(^{2+}\) pools: one inositol 1,4,5-P\(_3\)-sensitive and one inositol 1,4,5-P\(_3\)-insensitive. Receptor activation induces an increase in inositol 1,4,5-P\(_3\), which then releases Ca\(^{2+}\) from the inositol 1,4,5-P\(_3\)-sensitive pool. Ca\(^{2+}\) is then taken up into the inositol 1,4,5-P\(_3\)-insensitive pool and is subsequently released through a Ca\(^{2+}\)-induced Ca\(^{2+}\) release process. In this model, [Ca\(^{2+}\)] oscillations depend solely on intracellular nonmitochondrial Ca\(^{2+}\) and should not be affected by extracellular Ca\(^{2+}\) under conditions where changes in extracellular [Ca\(^{2+}\)] do not alter intracellular stores or [Ca\(^{2+}\)]. This model has been supported by experiments in which injection of inositol 1,4,5-P\(_3\) induced Ca\(^{2+}\) oscillations (28). Our laboratory has recently established that only a portion of the Ca\(^{2+}\) stored in the intracellular nonmitochondrial pools of mesangial cells is released by inositol 1,4,5-P\(_3\) in the mesangial cell. The remaining Ca\(^{2+}\) resides in pools not regulated by inositol 1,4,5-P\(_3\) (29).

Goldbeter et al. (30) have described a two-variable model for signal-induced Ca\(^{2+}\) oscillations. In their analysis, they show that increasing intracellular inositol 1,4,5-P\(_3\) in a step-like manner triggers Ca\(^{2+}\) oscillations whose frequency depends on the steady-state level of inositol 1,4,5-P\(_3\). This model...
Basal $[\text{Ca}^{2+}]_{i}$ Determines $\text{Ca}^{2+}$ Oscillation Frequency

Fig. 5. Effect of varying temperature on the frequency of $[\text{Ca}^{2+}]_{i}$ oscillations in individual mesangial cells. As temperature is increased, there is an increase in the frequency of oscillations. AVP (1 nM) is added at the arrows.

Fig. 6. Arrhenius plot of frequency of oscillations after 1 nM AVP exposure *versus* the reciprocal of temperature. The line represents a least square fit of the data points. The calculated $Q_{10}$ is 3.0.

Fig. 7. Effect of 1.0 μM PGE2 on the $[\text{Ca}^{2+}]_{i}$ response to 1.0 nM AVP. PGE2 pretreatment did not induce oscillations and had no apparent modulatory effect upon the frequency of AVP-induced oscillations.

is consistent with our observation of an increase in the frequency of oscillations observed at increasing concentrations of AVP, since increasing doses of AVP might be expected to result in increasing inositol 1,4,5-P3 levels. A key feature in this model is the phenomenon of $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release. In cardiac muscle, $\text{Ca}^{2+}$ entry through voltage-sensitive $\text{Ca}^{2+}$ channels induces release of $\text{Ca}^{2+}$ from the sarcoplasmic reticulum through $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release (31). Fabiato (31) and Allen *et al.* (3) showed that conditions that result in $\text{Ca}^{2+}$ loading of intracellular stores lead to cyclical contractions and $[\text{Ca}^{2+}]_{i}$ oscillations. These are thought to be due to a loading of the sarcoplasmic reticulum since destruction of the sarcoplasmic reticulum abolished the oscillations, whereas raising $[\text{Ca}^{2+}]_{i}$ increased them. With such a mechanism, oscillation frequency would be expected to show dependence upon $[\text{Ca}^{2+}]_{i}$. In our studies, we found a positive correlation between basal $[\text{Ca}^{2+}]_{i}$ and frequency of oscillations.

A minimal model of an oscillating system requires a feedback mechanism with a delay that generates the periodicity
The delay can occur from either an enzymatic or diffusion-limited process. Our experiments, in which temperature was varied, yielded a Q10 value of 2.3 at 3.0 to close to those obtained in parotid acini (11) and oocytes. Since biochemical reactions have Q10 values between 2 and 4, whereas diffusion processes have Q10 values between 1.2 and 1.3 (24), our results would support an enzymatic rather than a diffusion-limited process. Such delays may occur in the interaction of Ca2+ with the Ca2+ release mechanism of the inositol 1,4,5-P3-insensitive pool. Also since, in this model, the frequency of oscillations is dependent on inositol 1,4,5-P3 levels, an increase in phospholipase C activity at higher temperatures would also result in higher oscillation frequencies. The decrease in oscillatory response associated with phorbol ester pretreatment suggests that protein kinase C activation may be important for the determination of the oscillatory response.

**Amplitude Modulation Versus Frequency Modulation**—Ca2+ plays a central role in signal transduction and it has generally been felt that the amplitude of the Ca2+ signal is the important parameter that modulates the cellular response to agonists linked to Ca2+ (27, 34, 35). A potentially more attractive hypothesis is that Ca2+ exerts its action through a frequency-dependent mode rather than an amplitude-dependent mode (2, 8, 28). Oscillation frequency in our study was found to be sensitive to agonist concentration. It has been proposed that Ca2+ oscillations can be translated into frequency-dependent cellular responses through protein phosphorylation (2, 30). Increases in the frequency of Ca2+ spikes could translate into rising levels of phosphorylated proteins if these proteins were products of Ca2+-activated kinases. In their model, Goldbeter et al. (30) showed that this encoding process can be effective when appropriate conditions hold for the kinetics of the kinase and phosphatase involved.

In conclusion, we have shown that the Ca2+ response of individual mesangial cells to vasopressin is characterized by repetitive spikes with a frequency that depends on the concentration of vasopressin. There is a direct correlation between the basal levels of Ca2+, prior to stimulation with 1.0 nM vasopressin and the frequency of stimulated oscillations. The stimulated frequency of Ca2+ oscillations was also dependent on the temperature, with the stimulated frequency increasing with a Q10 of 3.0 between 24 and 37 °C. This oscillatory behavior may be important for frequency encoding of intracellular signals.

**Acknowledgments**—We wish to thank Xia-Mei Liu for her assistance in preparing the cell cultures and Karen Delio for graphics art help. We would also like to thank Dr. Martha Gray for her critical appraisal of the manuscript.

**REFERENCES**