Governance of arteriolar oscillation by ryanodine receptors

Tsuneo Takenaka,1 Yoichi Ohno,2 Koichi Hayashi,2 Takao Saruta,2 and Hiromichi Suzuki1

1Department of Medicine, Saitama Medical College, Iruma, Saitama 350–0495; and
2Department of Medicine, School of Medicine, Keio University, Shinjuku, Tokyo 160-8582, Japan

Submitted 25 November 2002; accepted in final form 13 February 2003

Calcium oscillation occurs during fertilization, segmentation, gene expression, exocytosis, and contraction (1). In general, temporal changes in calcium influx due to fluctuations in membrane potential underlie calcium oscillation in excitable cells. Calcium oscillation is mediated by periodic calcium release from endoplasmic reticulum in nonexcitable cells. Calcium oscillation is also observed in vasculature (12). Vascular oscillation gives turbulence, thereby markedly elevating vascular resistance. Because vascular resistance depends on its diameter, arteriolar oscillations are of great physiological significance. Vascular tissue from hypertensive animals, which may show abnormal genotypes in sarcoplasmic reticulum Ca-ATPase, frequently exhibits oscillation (15, 20). However, there are scant data regarding calcium store in arteriolar oscillatory movements (9). In addition, relative contributions of calcium influx and calcium release mechanisms to arteriolar oscillation were not adequately determined.

Glomerular hemodynamics controls glomerular capillary pressure and renal function curve, playing an overdominant role in long-term regulation of systemic blood pressure (10, 18). Renal preglomerular vasculature constricts in response to elevations of arterial pressure, mediating excellent autoregulatory capacity (18, 24). Especially the afferent arteriole, typical excitable tissue, plays the most important role in autoregulatory adjustments of renal vascular resistance, responding to variations in blood pressure (26). Both myogenic and tubuloglomerular feedback (TGF-dependent) signals are required for full expression of afferent arteriolar autoregulatory behavior (24). TGF oscillates tubular pressure and/or flow, possibly amplifying signals to macula densa cells (30). Recent studies have demonstrated that calcium influx through receptor-activated or voltage-dependent calcium channels strengthens inositol trisphosphate (IP3)-induced calcium release (IICR) on AT1 receptor stimulation in glomerular arterioles (27). Calcium release occurs from endoplasmic reticulum through intracellular calcium channels, either ryanodine or IP3 receptors (2, 13). Although it is established that ryanodine receptors magnify membrane signals in cardiac myocytes, the physiological role of arteriolar ryanodine receptors remains unclear (7, 27).

In the present studies, we utilized an isolated perfused hydronephrotic kidney model that enables direct visualization of arteriolar behavior under controlled in vitro conditions (25). Our data indicate that voltage-dependent calcium channels are rich in afferent but not efferent arterioles. Furthermore, our results provide the first evidence that calcium influx through voltage-dependent calcium channels triggers periodic oscillations of arteriolar oscillatory movements (9). In addition, relative contributions of calcium influx and calcium release mechanisms to arteriolar oscillation were not adequately determined.

Glomerular hemodynamics controls glomerular capillary pressure and renal function curve, playing an overdominant role in long-term regulation of systemic blood pressure (10, 18). Renal preglomerular vasculature constricts in response to elevations of arterial pressure, mediating excellent autoregulatory capacity (18, 24). Especially the afferent arteriole, typical excitable tissue, plays the most important role in autoregulatory adjustments of renal vascular resistance, responding to variations in blood pressure (26). Both myogenic and tubuloglomerular feedback (TGF-dependent) signals are required for full expression of afferent arteriolar autoregulatory behavior (24). TGF oscillates tubular pressure and/or flow, possibly amplifying signals to macula densa cells (30). Recent studies have demonstrated that calcium influx through receptor-activated or voltage-dependent calcium channels strengthens inositol trisphosphate (IP3)-induced calcium release (IICR) on AT1 receptor stimulation in glomerular arterioles (27). Calcium release occurs from endoplasmic reticulum through intracellular calcium channels, either ryanodine or IP3 receptors (2, 13). Although it is established that ryanodine receptors magnify membrane signals in cardiac myocytes, the physiological role of arteriolar ryanodine receptors remains unclear (7, 27).

In the present studies, we utilized an isolated perfused hydronephrotic kidney model that enables direct visualization of arteriolar behavior under controlled in vitro conditions (25). Our data indicate that voltage-dependent calcium channels are rich in afferent but not efferent arterioles. Furthermore, our results provide the first evidence that calcium influx through voltage-dependent calcium channels triggers periodic oscillations of arteriolar oscillatory movements (9). In addition, relative contributions of calcium influx and calcium release mechanisms to arteriolar oscillation were not adequately determined.

Glomerular hemodynamics controls glomerular capillary pressure and renal function curve, playing an overdominant role in long-term regulation of systemic blood pressure (10, 18). Renal preglomerular vasculature constricts in response to elevations of arterial pressure, mediating excellent autoregulatory capacity (18, 24). Especially the afferent arteriole, typical excitable tissue, plays the most important role in autoregulatory adjustments of renal vascular resistance, responding to variations in blood pressure (26). Both myogenic and tubuloglomerular feedback (TGF-dependent) signals are required for full expression of afferent arteriolar autoregulatory behavior (24). TGF oscillates tubular pressure and/or flow, possibly amplifying signals to macula densa cells (30). Recent studies have demonstrated that calcium influx through receptor-activated or voltage-dependent calcium channels strengthens inositol trisphosphate (IP3)-induced calcium release (IICR) on AT1 receptor stimulation in glomerular arterioles (27). Calcium release occurs from endoplasmic reticulum through intracellular calcium channels, either ryanodine or IP3 receptors (2, 13). Although it is established that ryanodine receptors magnify membrane signals in cardiac myocytes, the physiological role of arteriolar ryanodine receptors remains unclear (7, 27).

In the present studies, we utilized an isolated perfused hydronephrotic kidney model that enables direct visualization of arteriolar behavior under controlled in vitro conditions (25). Our data indicate that voltage-dependent calcium channels are rich in afferent but not efferent arterioles. Furthermore, our results provide the first evidence that calcium influx through voltage-dependent calcium channels triggers periodic oscillations of arteriolar oscillatory movements (9). In addition, relative contributions of calcium influx and calcium release mechanisms to arteriolar oscillation were not adequately determined.

Glomerular hemodynamics controls glomerular capillary pressure and renal function curve, playing an overdominant role in long-term regulation of systemic blood pressure (10, 18). Renal preglomerular vasculature constricts in response to elevations of arterial pressure, mediating excellent autoregulatory capacity (18, 24). Especially the afferent arteriole, typical excitable tissue, plays the most important role in autoregulatory adjustments of renal vascular resistance, responding to variations in blood pressure (26). Both myogenic and tubuloglomerular feedback (TGF-dependent) signals are required for full expression of afferent arteriolar autoregulatory behavior (24). TGF oscillates tubular pressure and/or flow, possibly amplifying signals to macula densa cells (30). Recent studies have demonstrated that calcium influx through receptor-activated or voltage-dependent calcium channels strengthens inositol trisphosphate (IP3)-induced calcium release (IICR) on AT1 receptor stimulation in glomerular arterioles (27). Calcium release occurs from endoplasmic reticulum through intracellular calcium channels, either ryanodine or IP3 receptors (2, 13). Although it is established that ryanodine receptors magnify membrane signals in cardiac myocytes, the physiological role of arteriolar ryanodine receptors remains unclear (7, 27).

In the present studies, we utilized an isolated perfused hydronephrotic kidney model that enables direct visualization of arteriolar behavior under controlled in vitro conditions (25). Our data indicate that voltage-dependent calcium channels are rich in afferent but not efferent arterioles. Furthermore, our results provide the first evidence that calcium influx through voltage-dependent calcium channels triggers periodic oscillations of arteriolar oscillatory movements (9). In addition, relative contributions of calcium influx and calcium release mechanisms to arteriolar oscillation were not adequately determined.

Glomerular hemodynamics controls glomerular capillary pressure and renal function curve, playing an overdominant role in long-term regulation of systemic blood pressure (10, 18). Renal preglomerular vasculature constricts in response to elevations of arterial pressure, mediating excellent autoregulatory capacity (18, 24). Especially the afferent arteriole, typical excitable tissue, plays the most important role in autoregulatory adjustments of renal vascular resistance, responding to variations in blood pressure (26). Both myogenic and tubuloglomerular feedback (TGF-dependent) signals are required for full expression of afferent arteriolar autoregulatory behavior (24). TGF oscillates tubular pressure and/or flow, possibly amplifying signals to macula densa cells (30). Recent studies have demonstrated that calcium influx through receptor-activated or voltage-dependent calcium channels strengthens inositol trisphosphate (IP3)-induced calcium release (IICR) on AT1 receptor stimulation in glomerular arterioles (27). Calcium release occurs from endoplasmic reticulum through intracellular calcium channels, either ryanodine or IP3 receptors (2, 13). Although it is established that ryanodine receptors magnify membrane signals in cardiac myocytes, the physiological role of arteriolar ryanodine receptors remains unclear (7, 27).

In the present studies, we utilized an isolated perfused hydronephrotic kidney model that enables direct visualization of arteriolar behavior under controlled in vitro conditions (25). Our data indicate that voltage-dependent calcium channels are rich in afferent but not efferent arterioles. Furthermore, our results provide the first evidence that calcium influx through voltage-dependent calcium channels triggers periodic oscillations of arteriolar oscillatory movements (9). In addition, relative contributions of calcium influx and calcium release mechanisms to arteriolar oscillation were not adequately determined.

Glomerular hemodynamics controls glomerular capillary pressure and renal function curve, playing an overdominant role in long-term regulation of systemic blood pressure (10, 18). Renal preglomerular vasculature constricts in response to elevations of arterial pressure, mediating excellent autoregulatory capacity (18, 24). Especially the afferent arteriole, typical excitable tissue, plays the most important role in autoregulatory adjustments of renal vascular resistance, responding to variations in blood pressure (26). Both myogenic and tubuloglomerular feedback (TGF-dependent) signals are required for full expression of afferent arteriolar autoregulatory behavior (24). TGF oscillates tubular pressure and/or flow, possibly amplifying signals to macula densa cells (30). Recent studies have demonstrated that calcium influx through receptor-activated or voltage-dependent calcium channels strengthens inositol trisphosphate (IP3)-induced calcium release (IICR) on AT1 receptor stimulation in glomerular arterioles (27). Calcium release occurs from endoplasmic reticulum through intracellular calcium channels, either ryanodine or IP3 receptors (2, 13). Although it is established that ryanodine receptors magnify membrane signals in cardiac myocytes, the physiological role of arteriolar ryanodine receptors remains unclear (7, 27).

In the present studies, we utilized an isolated perfused hydronephrotic kidney model that enables direct visualization of arteriolar behavior under controlled in vitro conditions (25). Our data indicate that voltage-dependent calcium channels are rich in afferent but not efferent arterioles. Furthermore, our results provide the first evidence that calcium influx through voltage-dependent calcium channels triggers periodic oscillations of arteriolar oscillatory movements (9). In addition, relative contributions of calcium influx and calcium release mechanisms to arteriolar oscillation were not adequately determined.
calcium release through ryanodine receptors in afferent arterioles. Finally, the present findings implicate that calcium-induced calcium release occurs through ryanodine receptors in glomerular arterioles.

METHODS

BAYK-8644, caffeine, EGTA, ouabain, ryanodine, tetraethylammonium (TEA), and thapsigargin were obtained from Sigma (St. Louis, MO). 2-Aminoethoxydiphenyl borate (APB) and nifedipine were provided from Ono Pharmaceuticals (Tokyo, Japan) and Bayer (Osaka, Japan), respectively. All the protocols were approved by our institutional ethical committees (26).

Experiments were performed as described previously (25, 26). In brief, hydronephrosis was induced in 37 adult male Sprague-Dawley rats (Charles River Japan, Atsugi, Kanagawa) by ligating the right ureter under ether anesthesia. After 8–10 wk from the surgery when renal tubular atrophy had progressed to a stage that allowed direct microscopic visualization of glomerular arteriolar behavior, the right kidney was harvested for perfusion study. The perfusion media consisted of PSS (in mM: 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 5 HEPES, and 5 glucose, pH 7.4). Driving force for perfusion was obtained from the inflow of warm hydrated gas of 95% O₂-5% CO₂. Perfusion pressure at the level of the renal artery was maintained constant at 80 mmHg throughout experiments to avoid pressure-induced arteriolar constriction (26). Video images of renal microvessels were obtained by a CCD camera (model ICD-42AC, Ikegami, Tokyo, Japan) and recorded with a videocassette recorder (model EVO-9850, Sony). The video recordings were transmitted to IBM-AT computer equipped with a display board (model TARGA++, Truevision, Indianapolis, IN). Arteriolar diameters were estimated by an automated program custom-designed to determine the mean distance between parallel edges. The renal microvessel diameters were obtained during the plateau of the response, unless otherwise stated (15, 25).

In the first series of experiments, the kidneys (n = 5) were initially exposed to increasing doses of BAYK-8644, a calcium agonist (11), from 100 to 300 nM. In the presence of BAYK-8644 (300 nM), nifedipine (1 μM) was administered directly to the perfusate (25). In the second series of experiments, the kidneys (n = 4) were treated with 1 μM of thapsigargin (25). Then BAYK-8644 was added. Subsequently, the perfusate was replaced by calcium-free media containing 2 mM of EGTA, in which thapsigargin and BAYK-8644 had been added (27). In the third series of studies, the kidneys (n = 5) were treated with BAYK-8644 (300 nM). Then 100 μM of APB, an inhibitor of both IICR and store-operated calcium entry (17,
At 300 nM, BAYK-8644 constricted afferent (19.6 ± 1 μm and interval of 20 ± 2 s (Fig. 2). Elevations of renal arterial pressure increased the frequency of arteriolar vasomotion. The exposure to nifedipine (1 μM) reversed both afferent arteriolar persistent constriction and rhythmic contraction by BAYK-8644 (to 19.4 ± 0.5 μm).

In the fourth series of studies (4 kidneys), ryanodine (10 μM) was initially administered (2, 27). Subsequently, BAYK-8644 (300 nM) was added. Finally, extracellular calcium was removed by replacing the media with calcium-free one.

In the fifth group (4 kidneys), basal arteriolar diameter was initially observed, and then ouabain (1 mM) was administered to suppress Na-K-ATPase (9). BAYK-8644 (300 nM) was added. Finally, nifedipine (1 μM) was administered. In the sixth group, the kidneys (n = 4) were initially treated with 1 mM of TEA to inhibit calcium-activated potassium channels (6, 19). Then BAYK-8644 (300 nM) was added.

In the seventh group, the kidneys (n = 8) were exposed to 1 mM of caffeine (2, 8). Then extracellular potassium concentration was increased to 10 and 20 mM (26). Potassium level was elevated isosmotically by adding potassium-rich PSS, in which NaCl was replaced with KCl. Subsequently, ryanodine (10 μM) was added. Finally, nifedipine (1 μM) was administered. For the additional three kidneys, the effects of high-potassium media were observed in the absence of caffeine.

Data are expressed as means ± SE. Statistical significance was determined by Student’s t-tests or ANOVA followed by Newman-Keuls test. P values < 0.05 were considered to be statistically significant.

RESULTS

As shown in Fig. 1, at 100 nM, BAYK-8644 failed to alter both afferent and efferent arteriolar diameters. At 300 nM, BAYK-8644 constricted afferent (19.6 ± 0.6 to 17.6 ± 0.5 μm, n = 6, P < 0.01) but not efferent arterioles.1 Furthermore, BAYK-8644 (300 nM) let the afferent arteriole oscillate with an amplitude of 10 ± 1 μm and interval of 20 ± 2 s (Fig. 2). Elevations of renal arterial pressure increased the frequency of arteriolar vasomotion. The exposure to nifedipine (1 μM) reversed both afferent arteriolar persistent constriction and rhythmic contraction by BAYK-8644 (to 19.4 ± 0.5 μm).

Figure 3 depicted the influence of thapsigargin on afferent arteriolar responses to BAYK-8644. Although thapsigargin by itself did not alter afferent arteriolar diameters, it prevented afferent arteriolar oscillation by BAYK-8644. Thus BAYK-8644 (300 nM) constricted afferent arterioles (19.7 ± 0.6 to 16.8 ± 0.6 μm, n = 5, P < 0.05) without substantial vasomotions in the presence of thapsigargin. Extracellular calcium removal restored the decrements in afferent arteriolar diameters (to 20.0 ± 0.5 μm).

BAYK-8644 constricted the afferent arteriole (19.6 ± 0.6 to 17.8 ± 0.6 μm, n = 6, P < 0.01) and elicited rhythmic contraction (interval 21 ± 2 s, amplitude 11 ± 1 μm). As shown in Fig. 4, afferent arteriolar rhythmic contractions induced by BAYK-8644 were suppressed with ryanodine but not APB. Because APB inhibits store-operated calcium entry as well as IICR (27), the above results suggest that capacitative calcium entry plays a small role in arteriolar oscillation. Subsequent addition of nifedipine reversed afferent arteriolar constriction by BAYK-8644 (to 19.9 ± 0.6 μm).

Figure 5 described the effect of ryanodine by itself on afferent arteriolar responses to BAYK-8644. Although ryanodine itself failed to alter arteriolar tone, BAYK-8644 constricted afferent arterioles (20.6 ± 0.7 to 17.9 ± 0.8 μm, n = 5, P < 0.05) without considerable oscillatory movements in the presence of ryanodine.

1Supplemental figures (a–d) and a movie (e), available at http://ajpregu.physiology.org/cgi/content/full/00711.2002/DC1, depict the effects of BAYK-8644 on glomerular arterioles. BAYK-8644 constricted afferent (supplemental figures a and b) but not efferent arterioles (supplemental figures c and d). BAYK-8644 also elicited afferent arteriolar vasomotion [supplemental movie (e)].
The removal of extracellular calcium abolished afferent arteriolar constriction by BAYK-8644 (to 21.0 ± 0.7 μm).

Exposure to ouabain gradually reduced afferent arteriolar diameters (19.8 ± 1.1 to 17.7 ± 0.9 μm, n = 5, P < 0.05). These arterioles were constricted (to 15.0 ± 0.8 μm, P < 0.01) and oscillated by BAYK-8644 (Fig. 6). The interval and amplitude of arteriolar vasomotions were 20 ± 2 s and 9 ± 1 μm, respectively.

As seen in Fig. 7, the administration of TEA gradually decreased afferent arteriolar diameters (20.3 ± 1.0 to 16.9 ± 0.9 μm, n = 5, P < 0.01). Subsequent addition of BAYK-8644 elicited further afferent arteriolar constriction (to 15.1 ± 0.7 μm, P < 0.05) and substantial oscillatory movements (interval 19 ± 2 s, amplitude 10 ± 1 μm). Similar results were observed when ibetirtoxin was used instead of TEA (19).

Figure 8 showed the impact of ryanodine on caffeine-induced arteriolar rhythmic contraction. In the absence of caffeine, high-potassium media (20 mM) constricted afferent arterioles (20.7 ± 1.0 to 17.6 ± 0.5 μm, n = 4, P < 0.05) without apparent vasomotions. Although the exposure to caffeine by itself did not alter afferent arteriolar diameter, subsequent membrane depolarization with isosmotic elevations of potassium concentration of 5 ± 1 to 11 ± 1 mM elicited arteriolar oscillatory movements. Further increase in potassium to 21 ± 2 mM shortened the interval of oscillation (77 ± 7 to 28 ± 3 s) without significant changes in amplitude (7 ± 1 vs. 7 ± 1 μm). In addition, the higher potassium concentration caused the greater arteriolar constriction (19.5 ± 0.5 to 18.4 ± 0.5 and then 16.5 ± 0.6 μm, n = 8, P < 0.05). Administration of ryanodine inhibited afferent arteriolar rhythmic contraction. Subsequent addition of nifedipine reversed residual afferent arteriolar constriction to 19.5 ± 0.6 μm.

**DISCUSSION**

Calcium is a versatile multitarget intracellular second messenger in eukaryotic cells (2). Calcium oscillation is mediated by various mechanisms in diverse cells. In addition to calcium influx, calcium release constitutes one of the main pathways by which cytosolic calcium is regulated (13). IICR underlies calcium oscillation after vascular receptor stimulation (12). Temporal fluctuations in calcium influx are involved in calcium oscillation of sympathetic neurons (8). Thus the mechanisms mediating oscillation may differ even among excitable cells and need to be determined in each situation.
The present observations that BAYK-8644 elicited afferent but not efferent arteriolar constriction are consistent with the notion that voltage-dependent calcium channels are rich in preglomerular but not postglomerular microvessels (25, 27). Although BAYK-8644 elicited oscillatory movements in afferent arterioles, the oscillatory frequency appears different from that observed during oscillation dependent on IP3 and/or protein kinase C (15, 23, 28). A mathematical model indicates that a typical calcium oscillation dependent on IP3 (0.15 Hz) is faster than that observed in the present study (28). Indeed, we previously reported that endothelin, which involves IP3 and protein kinase C as constrictor mechanisms, induced afferent arteriolar oscillation with intervals of 3 s (15, 23). However, the interval and frequency of afferent arteriolar rhythmic contractions by BAYK-8644 extended to 20 s and 0.05 Hz, respectively. The discrepancies suggest that the other mechanisms mediate this relatively slow arteriolar oscillation.

The present findings that nifedipine reversed afferent arteriolar vasomotion indicated that calcium influx through L-type calcium channels triggers arteriolar oscillation. In addition, our data that thapsigargin
prevented afferent arteriolar oscillatory movements by BAYK-8644 provided the evidence that calcium release mediates arteriolar oscillation. Furthermore, the present results that ryanodine, but not APB, inhibited afferent arteriolar rhythmic contraction by BAYK-8644 suggested that ryanodine receptors, but not IP$_3$ receptors, are involved. BAYK-8644 renders the opening of L-type calcium channels longer, presumably allowing the greater increase in subplasmalemmal calcium levels (11). Ryanodine receptors are located also in the periphery of vascular myocytes (14). In the absence of IP$_3$, ryanodine receptors open in response to smaller increases in cytosolic calcium than IP$_3$ receptors (4), mediating a periodic calcium surge from sarc(endo)plasmic reticulum.

Complementary studies were performed using caffeine to sensitize ryanodine receptors (2, 8). In the presence of caffeine, normal activation of voltage-dependent calcium channels by high-potassium media made the afferent arteriole oscillate, supporting the notion that ryanodine receptors play an important role in slow arteriolar vasomotion. Calcium entry through L-type calcium channels, which is estimated from the changes in membrane potential (26), would increase subplasmalemmal calcium level to have ryanodine receptors open. Indeed, graded increases in potassium concentrations elicited more frequent oscillation in afferent arterioles treated with caffeine, supporting the proposal that calcium influx through voltage-dependent calcium channels triggers repeated calcium release through ryanodine receptors, mediating afferent arteriolar rhythmic contraction.

Possible mechanisms on membrane property should be considered. First, calcium spark gates calcium-activated potassium channels and hyperpolarizes cerebral arteries (19). Thus it could swing arteriolar membrane potential. However, recent data demonstrate that calcium-activated potassium channels on afferent arterioles provide physiological brake to myogenic responses but not depolarizing agonists (6, 16). Our data that BAYK-8644 still induced afferent arteriolar vasomotion in the presence of TEA are consistent with the latter and mitigated against a mediatory role of calcium-activated potassium channels in oscillation by BAYK-8644. Because calcium release through ryanodine receptors induces calcium wave (1, 3), it appears to overflow subplasmalemmal space and elicit arteriolar contraction. Second, the alterations in Na-K-ATPase activity and subsequent changes in membrane potential evoke oscillation in mesenteric small arteries (9). In contrast, BAYK-8644 elicited oscillatory movements in afferent arterioles treated with ouabain. Third, Chilton and Loutzenhiser (5) reported that the media of 15 mM potassium dilated afferent arterioles pretreated with ibuprofen. However, we did not observe afferent arteriolar dilation (20.7 ± 1.0 to 19.7 ± 0.7 μm) by 10 mM of potassium. Although precise reasons for the discrepancy are not clear, they may relate to differing underlying arteriolar tones. The removal of endogenous prostaglandins renders ATP-sensitive potassium channels quiescent (16, 21). Thus inwardly rectifying potassium channels may exert greater contribution to membrane potential in their condition. Collectively, it is unlikely that fluctuations of calcium influx through L-type calcium channels underlie renal arteriolar oscillation.

A caveat is in order. Because of marked tubular atrophy, TGF does not efficiently work in the present experimental settings (25). There seems no way for TGF to influence this model by its oscillations. However, caffeine-induced oscillatory frequency (0.01–0.03 Hz) is in the range of physiological oscillations of tubular pressure (30). Of interest, recent investigations have revealed that purinergic agonists, the putative chemical mediator of TGF (18, 22), affect ryanodine channels (29). Thus these observations prompt one to speculate that TGF prepares afferent arteriolar ryanodine receptors to oscillate tubular pressure and/or flow, as well as activates voltage-dependent calcium channels (24). However, further studies using either micropuncture or juxtamedullary nephron methods are required to elucidate this issue.

In summary, our results indicate that L-type calcium channels are rich in afferent arterioles. Furthermore, the present findings suggest that calcium influx through L-type calcium channels triggers afferent arteriolar vasomotion. Finally, our data suggest that calcium release through ryanodine receptors is required for slow afferent arteriolar rhythmic contraction.

We thank Dr. K. Kumagai for producing a video file from the recorded tape. We also appreciate Ono Pharmaceuticals and Bayer for providing 2-aminoethoxydiphenyl borate and nifedipine, respectively.

Part of the data presented in this manuscript was presented at the 32nd Annual Meeting of the American Society of Nephrology, Miami Beach, FL, November 1999, and has been published as an abstract (J Am Soc Nephrol 10: 368A, 1999).

REFERENCES