

Max Salomonsson and William J. Arendshorst

Am J Physiol Renal Physiol 286:866-874, 2004. First published Jan 13, 2004;
doi:10.1152/ajprenal.00213.2003

You might find this additional information useful...

This article cites 53 articles, 23 of which you can access free at:

<http://ajprenal.physiology.org/cgi/content/full/286/5/F866#BIBL>

This article has been cited by 1 other HighWire hosted article:

Involvement of BKCa {alpha} subunit tyrosine phosphorylation in vascular hyporesponsiveness of superior mesenteric artery following hemorrhagic shock in rats

R. Zhou, L. Liu and D. Hu

Cardiovasc Res, November 1, 2005; 68 (2): 327-335.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Updated information and services including high-resolution figures, can be found at:

<http://ajprenal.physiology.org/cgi/content/full/286/5/F866>

Additional material and information about *AJP - Renal Physiology* can be found at:

<http://www.the-aps.org/publications/ajprenal>

This information is current as of November 27, 2009 .

Effect of tyrosine kinase blockade on norepinephrine-induced cytosolic calcium response in rat afferent arterioles

Max Salomonsson¹ and William J. Arendshorst²

¹Department of Medical Physiology, Division of Renal and Cardiovascular Research, The Panum Institute, University of Copenhagen, 2200 Copenhagen N, Denmark; and ²Department of Cell and Molecular Physiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7545

Submitted 6 June 2003; accepted in final form 31 December 2003

Salomonsson, Max, and William J. Arendshorst. Effect of tyrosine kinase blockade on norepinephrine-induced cytosolic calcium response in rat afferent arterioles. *Am J Physiol Renal Physiol* 286: F866–F874, 2004. First published January 13, 2004; 10.1152/ajprenal.00213.2003.—We used genistein (Gen) and tyrphostin 23 (Tyr-23) to evaluate the importance of tyrosine phosphorylation in norepinephrine (NE)-induced changes in intracellular free calcium concentration ($[Ca^{2+}]_i$) in rat afferent arterioles. $[Ca^{2+}]_i$ was measured in microdissected arterioles using ratiometric photometry of fura 2 fluorescence. The control $[Ca^{2+}]_i$ response to NE (1 μ M) consisted of a rapid initial peak followed by a plateau phase sustained above baseline. Pretreatment with the tyrosine kinase inhibitor Tyr-23 (50 μ M, 10 min) caused a slow 40% increase in baseline $[Ca^{2+}]_i$. Tyr-23 attenuated peak and plateau responses to NE, both by \sim 70%. In the absence of extracellular Ca^{2+} (0 Ca), Tyr-23 reduced the immediate $[Ca^{2+}]_i$ response to NE by \sim 60%, indicative of mobilization of internal stores, and abolished the plateau phase. In other arterioles, the $[Ca^{2+}]_i$ response to depolarization induced by KCl (50 mM) was not attenuated by Tyr-23, indicating no direct effect on L-type Ca^{2+} channels activated by depolarization. The Ca^{2+} channel blocker nifedipine (1 μ M) inhibited the NE response by \sim 50%; the effects of nifedipine and Tyr-23 were not additive. Nifedipine had no inhibitory effect after Tyr-23 pretreatment, indicating Tyr-23 inhibition of Ca^{2+} entry. Another tyrosine kinase inhibitor, Gen (5 and 50 μ M), did not affect baseline $[Ca^{2+}]_i$. High-dose Gen inhibited the peak and plateau response to NE by 87 and 75%, respectively; low-dose Gen attenuated both responses by \sim 20%. In 0 Ca, Gen (50 μ M) abolished the immediate $[Ca^{2+}]_i$ mobilization response. Combined nifedipine and Gen (50 μ M) inhibited the rapid NE response by \sim 90% in the presence of extracellular Ca^{2+} . Gen (50 μ M) also inhibited by 60% the $[Ca^{2+}]_i$ response to 50 mM KCl, indicating a direct interaction with voltage-sensitive, L-type Ca^{2+} entry channels. These results indicate that tyrosine phosphorylation is an important link in the chain of events leading to α -adrenoceptor-induced Ca^{2+} recruitment (both entry and release) in afferent arteriolar smooth muscle cells. Furthermore, different blockers of tyrosine kinase appear to have different modes of action in renal microvessels.

α -adrenoceptor; renal circulation; vascular smooth muscle cells; genistein; tyrphostin 23; Ca^{2+} mobilization; Ca^{2+} entry; L-type Ca^{2+} channels

SYMPATHETIC NERVES RICHLY innervate the renal vasculature (3), releasing norepinephrine (NE) to exert its contractile effect by activation of cell-surface α_1 -adrenoceptors on vascular smooth muscle cells (VSMC) to increase cytosolic calcium concentration ($[Ca^{2+}]_i$) and constrict renal resistance vessels (6, 32, 34,

37). In this regard, the sympathetic autonomic system plays a crucial role in the regulation of renal hemodynamics and glomerular filtration, in addition to direct effects on tubular reabsorption, and thus in the short- and long-term regulation of extracellular fluid volume and arterial blood pressure (6).

The main vascular action of NE is exerted via G protein-coupled α_1 -adrenoceptors and their activation of phospholipase C with subsequent hydrolysis of membrane phosphoinositides generating inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (46). This is followed by an increase in $[Ca^{2+}]_i$ mediated by at least one of several mechanisms. Ca^{2+} is recruited either as mobilization from intracellular stores via IP_3 activation of release channels on the sarcoplasmic reticulum or as entry from the extracellular space through voltage-dependent and/or receptor-activated calcium channels located in the cell membrane. We previously demonstrated that the increase in $[Ca^{2+}]_i$ induced by activation of cell-surface α_1 -adrenoceptors in rat preglomerular vessels derives from both intra- and extracellular sources (32, 33). The exact mechanisms and intermediate pathways responsible for Ca^{2+} signaling on adrenoceptor activation await further investigation.

Tyrosine kinases are widely recognized for their function as cell-surface receptors for growth factors such as epidermal growth factor (EGF) and platelet-derived growth factor (38). Activation of these mitogenic receptors leads to stimulation of gene expression and growth that requires multiple phosphorylation steps involving protein tyrosine kinases. In addition, there are examples of ligand activation of receptor tyrosine kinases that elevate $[Ca^{2+}]_i$ (9, 40). Participation of tyrosine kinases in signaling events gains complexity as it is recognized that other families of tyrosine kinases are located in the cytosol and are devoid of extracellular binding sites for ligands (16). An example of cytosolic tyrosine kinases is the c-src family that has been detected in several tissues, including VSMC (6, 11). There is emerging evidence that cytosolic tyrosine kinase activity can be regulated by cell-surface G protein-coupled receptors during certain conditions (41). α -Adrenoceptor agonists such as NE and phenylephrine may stimulate protein tyrosine phosphorylation in VSMC (45, 47, 48). In addition to the well-characterized effects on growth and proliferation, some evidence suggests tyrosine kinases may play a role in the regulation of adrenoceptor-induced increase in $[Ca^{2+}]_i$ and vascular tone (22).

Relatively few studies have addressed the role of tyrosine kinases in agonist-induced renal vasoconstriction. In the rat

Address for reprint requests and other correspondence: W. J. Arendshorst, Dept. of Cell and Molecular Physiology, CB #7545, School of Medicine, Rm. 6341B Medical Biomolecular Research Bldg., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7545 (E-mail: arends@med.unc.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

juxtamedullary nephron preparation, afferent and efferent arteriolar contractile responses to ANG II are reported to be attenuated by tyrosine kinase inhibitors such as tyrphostin 23 (Tyr-23) and tyrphostin AG 1478 (4). It appears that ANG II transactivates a cell-surface growth factor receptor tyrosine kinase to increase $[Ca^{2+}]_i$ in rat afferent arterioles (5). Also, deendothelialized rat juxtamedullary arterioles respond to ANG II with an increase in $[Ca^{2+}]_i$ that can be blunted by genistein (Gen), a blocker of tyrosine kinase (29). NE-induced constriction of isolated rat interlobular arteries appears to rely, at least in part, on activation of tyrosine kinases (13). However, the importance of tyrosine kinases in mediating catecholamine-induced Ca^{2+} recruitment and vasoconstriction of the glomerular arterioles is not clear.

The aim of the present study was to evaluate the role of tyrosine kinases in adrenoceptor-induced changes in $[Ca^{2+}]_i$ in VSMC of rat renal resistance vessels isolated by microdissection. The pharmacological agents Gen and Tyr-23 were used to block tyrosine kinases on NE stimulation as $[Ca^{2+}]_i$ in afferent arterioles was measured using ratiometric fluorescence of the indicator fura 2. Furthermore, we sought to identify tyrosine kinase effects on NE-induced Ca^{2+} mobilization and/or entry and on depolarization-induced Ca^{2+} entry through L-type channels.

METHODS

Measurements of $[Ca^{2+}]_i$. Glomeruli with an attached isolated afferent arteriole were microdissected from 54 Wistar-Kyoto rats (weight 229 ± 5 g) of the Chapel Hill colony, Møllegaard (Ejby, Denmark) or Charles River (Sulzfeld, Germany). No differences were noted between strains. Thin slices (thickness 0.5–1 mm) were cut from the midregion of the kidney and transferred to a dissection dish containing ice-cold physiological salt solution (PSS) solution with bovine serum albumin (0.5 g/dl; Sigma). Sharpened forceps were used for the isolation procedure under microscopic visualization (magnification $\times 12$ –100) as described previously (32). A single afferent arteriole was cut using a sharp knife blade as close as possible to the bifurcation arising from an interlobular artery. A homogenous population of arterioles was obtained from the outer one-third of the cortex. If no preparation was obtained during the first 90 min of dissection, the kidney was discarded.

After dissection, an arteriole was loaded with fura 2-AM for 45–60 min in the dark at room temperature as previously described (18, 36). Fura 2-AM (Molecular Probes) was dissolved in DMSO as a stock solution (1 mM) and mixed with PSS to a final concentration of 2 μ M plus Pluronic F127 (Molecular Probes; 0.01%) immediately before use. A fura 2-loaded arteriole was transferred to a chamber containing PSS on the stage of an inverted microscope (Olympus IX 70 or IX 50) using an Eppendorf micropipette. The proximal end of the arteriole and attached glomerulus were aspirated into concentric glass-holding pipettes using negative pressure generated by a syringe connected to the back of the pipette. For measurements of $[Ca^{2+}]_i$, the arteriole was centered in the optical field of $\times 40$ quartz oil-immersion objective.

The first part of this study was performed using a photometer system. The preparation was visualized using a video camera (Sony) and monitor. Variable shutters were adjusted to center an arteriole in a sampling window. This made possible continuous control of the position of the preparation throughout an experiment. The arteriole was excited alternatively with UV light of 340- and 380-nm wavelengths from a dual-excitation wavelength DeltaScan equipped with dual monochromators and a light pathway chopper (Photon Technology International). Fluorescent light signals were directed through a 510-nm band pass filter and detected by a photometer. The fluorescence signal intensity was processed using Felix software (Photon

Technology International) and stored on an IBM-compatible computer. For the second part, an intensified video camera and ImageMaster software (Photon Technology International) were used for experiments conducted in Chapel Hill. Copenhagen studies used a digital video camera (SensiCam) and the Image Workbench software (Axon). The following experimental procedure was similar for the studies conducted in the two laboratories. A vessel was visualized on a computer monitor, and the region of interest for $[Ca^{2+}]_i$ was encircled using a software-based routine. A monochromator produced alternative excitation of UV light of 340- and 380-nm wavelengths, and fluorescent emission at 510 nm was recorded. The $[Ca^{2+}]_i$ was calculated using the Grynkiewicz equation (15): $[Ca^{2+}]_i = K_d \times [(R - R_{min})/(R_{max} - R)] \times (S_f/S_b)$, where K_d is the dissociation constant of fura 2 for calcium; S_f and S_b are the 380-nm fluorescence at zero and saturating calcium concentrations, respectively. R_{min} and R_{max} are values of R (fluorescence ratio 340/380) at zero and at saturating (39 μ M) calcium concentration, respectively. Values for K_d , R_{min} , R_{max} , S_f , and S_b were determined in vitro for each experimental set-up as previously described (18, 35, 36).

Artificial solutions. The PSS solution had the following composition (in mM): 135 NaCl, 5.0 KCl, 1.0 $CaCl_2$, 1.0 $MgCl_2$, 10 HEPES, and 5.0 D-glucose. A nominally calcium-free solution (0 Ca) was made by adding 2 mM EGTA (Sigma) to PSS and replacing $CaCl_2$ with NaCl. In the 50 mM KCl solution (K50), 45 mM of NaCl was replaced with an equal concentration of KCl.

Drugs. Nifedipine (Sigma) was dissolved in DMSO and diluted in PSS to a final concentration of 1 μ M, a concentration previously shown to completely inhibit L-type calcium channels (14, 32). Gen, Tyr-23, and tyrphostin 1 (Tyr-1; Sigma) were dissolved in DMSO and added to the PSS to produce the stated final concentrations.

Experimental protocol. All experimental solutions were added in a volume large enough to allow total exchange of the fluid in the experimental chamber several times. The fluid level in the experimental chamber was constantly maintained by means of a vacuum suction system. The viability of the preparation was assessed by $[Ca^{2+}]_i$ and contractile stimulation with 1 μ M NE or K50. Increases in $[Ca^{2+}]_i$ typically accompanied visual contraction of the vessel. Discarded nonresponding preparations were almost always visibly damaged by physical dissection procedures, and/or the cells of the vessel were clearly swollen. Earlier studies revealed that 1 μ M NE elicited roughly one-half of maximal response in $[Ca^{2+}]_i$; this concentration was used throughout the present studies (32, 33). We established that pretreatment for 10 min with the highest concentration of DMSO used to dissolve nifedipine, Gen, Tyr-23, and Tyr-1 did not significantly affect agonist-induced $[Ca^{2+}]_i$ responses.

The responses to NE (1 μ M) and K50 (50 mM) obtained during control conditions were compared in paired fashion with experimental responses recorded during pretreatment with blockers or 0 Ca. Arterioles were exposed to Tyr-23 (50 μ M), Tyr-1 (50 μ M), Gen (5 or 50 μ M), or nifedipine (1 μ M) for 2 or 10 min before and during NE stimulation. The mean prestimulation $[Ca^{2+}]_i$ values were obtained between 10 and 15 s before addition of NE or K50. After stimulation, $[Ca^{2+}]_i$ was measured as an immediate peak response (maximal peak value between 0 and 15 s) and a sustained plateau phase recorded between 30 and 35 s. In preliminary studies, we established that the response at 30–35 s was commonly maintained for several minutes.

The experiments were performed in random order to establish reversibility and to exclude possible effects of a prolonged action of a particular treatment. Also, to secure reversibility, a control response to NE or K50 was usually obtained both before and after the response in presence of blocker or 0 Ca. In poststimulation experiments, the responses to NE were recorded until a stable plateau level was reached (typically < 30 s) and then during nifedipine inhibition during continued exposure to NE and pretreatment solution. For this series, we present the plateau values of $[Ca^{2+}]_i$ before and after inhibition.

Statistical analysis. Data are presented as means \pm SE. SigmaStat software (SPSS) was used for statistical analysis. Statistical signifi-

cance was evaluated by analysis of variance or by analysis of variance for repeated measurements and the Newman-Keuls test. Student's *t*-test was used for paired and unpaired observations. When data did not exhibit normality, the data were transformed to natural logarithms before testing. A *P* value of <0.05 is considered statistically significant.

RESULTS

$[Ca^{2+}]_i$ responses to NE and K50. Baseline $[Ca^{2+}]_i$ averaged 74 ± 4 nM in a total of 76 afferent arterioles from 54 rats. Addition of NE (1 μ M) to the bath caused an abrupt, sustained increase in $[Ca^{2+}]_i$. The initial peak was usually slightly greater than the sustained plateau. On average, NE caused $[Ca^{2+}]_i$ to increase from a basal value of 78 ± 5 to an initial peak of 199 ± 10 nM, followed by a sustained plateau of 157 ± 7 nM at 30–35 s ($n = 68$). Stimulation with KCl (50 mM) solution caused an immediate increase in $[Ca^{2+}]_i$ from 60 ± 7 to 118 ± 9 nM ($n = 25$), which was maintained at 112 ± 8 nM at 30–35 s. Contraction of arterioles visualized on a video monitor normally correlated temporally with the increases in $[Ca^{2+}]_i$.

Effect of Gen on NE responses. Administration of NE (1 μ M) in the control period increased $[Ca^{2+}]_i$ from 97 ± 8 to an immediate peak of 269 ± 31 nM, followed by a sustained plateau value of 187 ± 14 nM ($n = 8$). Subsequent 2-min exposure to Gen (50 μ M) caused a small decrease in basal $[Ca^{2+}]_i$ from 92 ± 8 to 81 ± 7 nM ($P < 0.01$). Gen pretreatment reduced peak and plateau responses to NE by $\sim 70\%$. NE challenge in the presence of Gen elicited a $[Ca^{2+}]_i$ peak increase to 107 ± 10 nM and a sustained plateau of 106 ± 8 nM at 30–35 s (Figs. 1 and 2).

A lower concentration of Gen (5 μ M) attenuated the $[Ca^{2+}]_i$ responses to NE by ~ 10 –20%, which was significantly less than with 50 μ M Gen. Under control conditions, NE increased $[Ca^{2+}]_i$ from 86 ± 7 to 162 ± 17 nM, with a plateau phase of 132 ± 13 nM at 30–35 s ($n = 10$). Five micromolar Gen did not affect basal $[Ca^{2+}]_i$. NE stimulation in the presence of Gen increased $[Ca^{2+}]_i$ from 80 ± 7 to a peak of 143 ± 12 nM; the sustained plateau was at 118 ± 11 nM.

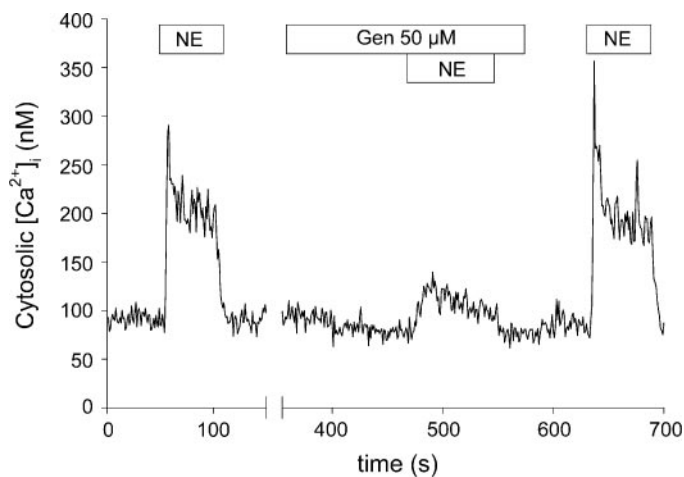


Fig. 1. Original recording of cytosolic calcium concentration ($[Ca^{2+}]_i$) in microdissected afferent arteriole depicting the stimulatory effect of norepinephrine (NE; 10^{-6} M) before and after treatment with genistein (Gen; 50 μ M) for 2 min. Note the attenuating effect of Gen on the NE response and that this effect is fully reversible.

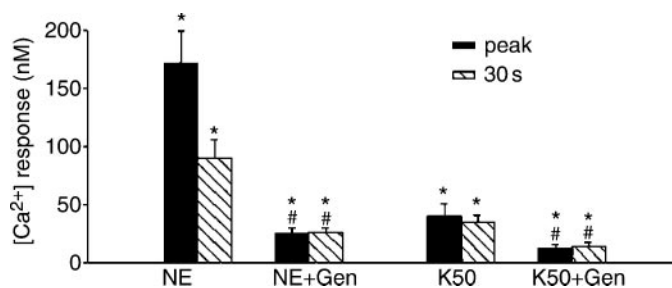


Fig. 2. Summarized data showing the peak (0–15 s) and sustained plateau (30–35 s) changes in $[Ca^{2+}]_i$ following stimulation with NE (10^{-6} M) or 50 mM KCl solution (K50) before and after 2-min treatment with Gen (50 μ M). The values are NE- and K50-induced absolute increases in $[Ca^{2+}]_i$ from baseline in the absence or presence of Gen solution. * $P < 0.05$ vs. baseline. # $P < 0.05$ vs. NE without treatment ($n = 8$ and 4 for NE and K50, respectively).

Longer exposure to the low concentration of Gen (10 min) had no greater inhibitory effect than that at 2 min. Before exposure to Gen, NE stimulated afferent arteriolar $[Ca^{2+}]_i$ from 58 ± 20 to a peak of 260 ± 40 nM and a plateau of 194 ± 19 nM ($n = 7$). Ten-minute Gen (5 μ M) exposure had no discernable effect on baseline $[Ca^{2+}]_i$ but inhibited responses to NE. NE increased $[Ca^{2+}]_i$ immediately from 46 ± 18 to 205 ± 17 nM and then remained at 155 ± 17 nM at 30–35 s (both $P < 0.001$ vs. baseline; $n = 7$). Although Gen tended to reduce the peak, it was not statistically different from the control peak ($P > 0.15$). Gen attenuated the $[Ca^{2+}]_i$ plateau to $\sim 80\%$ of the control response to NE.

In other experiments, we evaluated whether Gen affected Ca^{2+} mobilization from intracellular stores, using the higher concentration of Gen (50 μ M) and the 2-min pretreatment time. After the arterioles had exhibited a normal control response to NE, they were exposed to a nominally Ca^{2+} -free EGTA-containing solution (0 Ca) and Gen. This bathing solution caused a significant reduction in resting $[Ca^{2+}]_i$ from 87 ± 16 to 61 ± 8 nM ($n = 5$). NE had no stimulatory effect on arterioles pretreated with 0 Ca + Gen. In the presence of Gen, the average $[Ca^{2+}]_i$ peak was 64 ± 10 nM and the plateau at 30–35 s was 63 ± 10 nM (Fig. 3). Control experiments without Gen when the bath was 0 Ca showed that the 0 Ca caused basal $[Ca^{2+}]_i$ to decline from 95 ± 10 to 74 ± 7 nM ($n = 11$). Subsequent challenge with NE caused a rapid peak to 127 ± 16 nM, whereas the plateau value of 80 ± 6 nM at 30–35 s did not differ from baseline ($P = 0.2$). These findings indicate that Gen affects the peak phase of mobilization. The absence of the plateau phase when extracellular Ca^{2+} was absent indicates that the $[Ca^{2+}]_i$ plateau response at 30–35 s represents Ca^{2+} entry. Thus it is reasonable to conclude that Gen influences Ca^{2+} entry as the plateau phase is attenuated by Gen when extracellular Ca^{2+} is normal.

This led us to investigate whether the effects of Gen might be exerted via blockade of L-type channels. We previously showed that $\sim 50\%$ of the Ca^{2+} entry during the plateau response to NE is mediated via these dihydropyridine-sensitive channels and 50% by other entry pathways (32, 33). These observations were confirmed by our present results. We found that in the presence of 1 μ M nifedipine, NE caused an increase in $[Ca^{2+}]_i$ from 80 ± 6 nM to a peak value of 132 ± 21 nM and a plateau of 110 ± 13 nM ($n = 5$; Fig. 4). The combination of nifedipine (1 μ M) and Gen (50 μ M) inhibited the NE

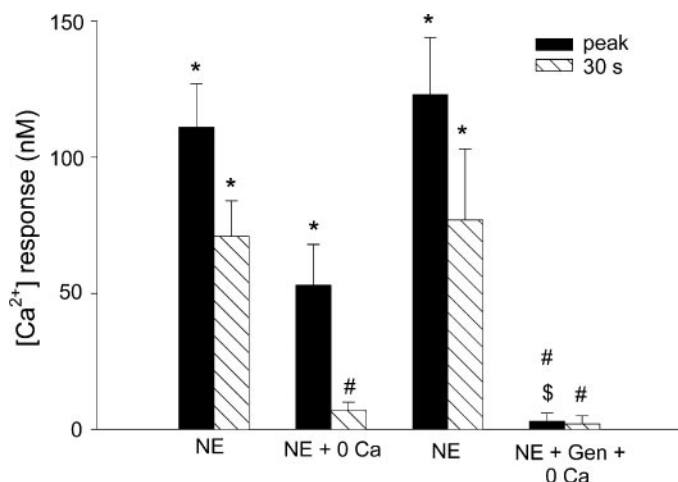


Fig. 3. Peak and sustained plateau changes in $[Ca^{2+}]_i$ following stimulation with NE (10^{-6} M) before and after treatment with a nominally calcium-free solution containing 1 mM EGTA (0 Ca) or Gen (50 μ M) + 0 Ca. Values are NE-induced absolute increases in $[Ca^{2+}]_i$ from baseline in the presence or absence of the pretreatment solution. Each group consisted of 5 or more arterioles. * $P < 0.05$ vs. baseline. # $P < 0.05$ vs. NE without treatment. \$ $P < 0.05$ vs. 0 Ca only.

responses even more. The corresponding values were 69 ± 11 nM for baseline and 73 ± 10 nM for both peak and 73 ± 9 nM for plateau ($n = 5$). The small increases in $[Ca^{2+}]_i$ above baseline were not statistically significant. The combination of Gen and nifedipine attenuated the plateau response significantly more than pretreatment with Gen only. The difference in peak response did not, however, reach the level of statistical significance ($P = 0.13$). Because the effects of the L-type Ca^{2+} channel antagonist nifedipine and Gen are additive, one might conclude that tyrosine kinases are involved in activating the ill-defined Ca^{2+} entry pathway distinct from voltage-gated channels.

Effect of Gen on K50 responses. From the data presented above, it is not clear whether Gen has a direct effect on voltage-gated L-type Ca^{2+} channels, as has been reported by

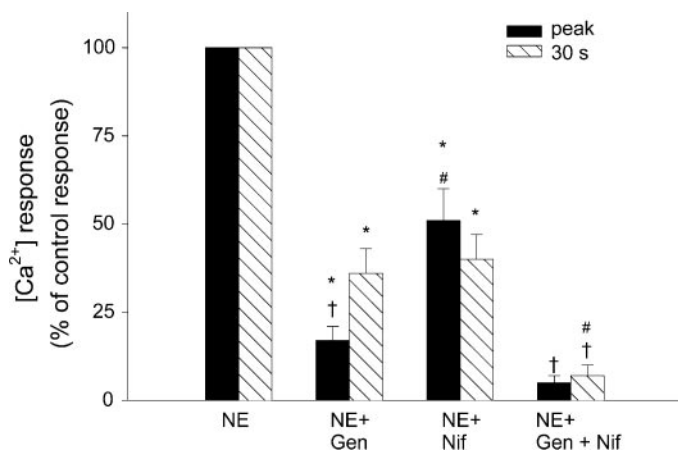


Fig. 4. Peak and sustained plateau changes in $[Ca^{2+}]_i$ following stimulation with NE (10^{-6} M) after 10-min pretreatment with nifedipine (1 μ M), Gen (50 μ M), or a combination thereof. Values are expressed as a percentage of control responses (without treatment). All responses are significantly smaller than corresponding control. Each group consisted of 5 or more arterioles. * $P < 0.05$ vs. baseline. # $P < 0.05$ vs. genistein. † $P < 0.05$ vs. nifedipine.

others for nonrenal vessels (25, 49). We therefore performed experiments to more definitively investigate the issue of direct vs. indirect action. Administration of K50 in the control period increased $[Ca^{2+}]_i$ from 88 ± 21 to 128 ± 22 nM in the initial 15 s and to 123 ± 22 nM at 30–35 s ($n = 4$; Fig. 2). After 2-min exposure to Gen (50 μ M), K50-induced depolarization caused a smaller, but significant, increase in $[Ca^{2+}]_i$ initially and during the plateau phase (from 75 ± 23 nM to 87 ± 23 and 89 ± 25 nM, for peak and plateau, respectively, $n = 4$).

A lower concentration of Gen (5 μ M) had a smaller inhibitory effect. In the control period, K50 elevated $[Ca^{2+}]_i$ from baseline 80 ± 8 to 118 ± 16 and 116 ± 14 nM for peak and plateau, respectively ($n = 7$). After pretreatment with Gen, the corresponding values were 78 ± 7 , 108 ± 12 , and 106 ± 12 nM. The plateau value was significantly attenuated, whereas the mean peaks did not differ from the control response ($P > 0.1$). Thus it is clear that Gen, at least at the higher concentration tested, exerts a direct inhibitory influence on the L-type channels.

Effect of Tyr-23 and Tyr-1 on NE responses. We tested the effect of a different blocker of tyrosine kinase, Tyr-23, that acts by binding to the substrate binding site of tyrosine kinase, in contrast to Gen which binds to the ATP site. Tyr-23 is reported to be a more specific blocker of tyrosine kinases than Gen (16). Although long-term exposure (up to 16 h) has been reported to be required for optimal effects of tyrphostins (26), more recent reports using different VSMC preparations, among them renal vasculature, indicate that 10 min of preincubation are sufficient to produce effective inhibition (4, 42, 44). Because tyrphostins may degrade to products with different potencies, we wanted to minimize the duration of preincubation (30).

In initial experiments, we tested the inhibitory effect of Tyr-23 (50 μ M) on the $[Ca^{2+}]_i$ response to NE after 3-min pretreatment. In the absence of the inhibitor, NE (1 μ M) elicited a rapid peak rise in $[Ca^{2+}]_i$ from 83 ± 8 to 156 ± 13 nM ($n = 14$). After 30–35 s, the $[Ca^{2+}]_i$ plateau value was 130 ± 12 nM. After 3-min pretreatment with Tyr-23, the peak response to NE was significantly blunted (from a baseline of 101 ± 11 to 144 ± 18 nM). The plateau response, however, was not statistically lower (to 139 ± 16 nM). When we extended the treatment period to 10 min, the responses to NE were more markedly attenuated ($P < 0.01$). In the control period, NE stimulation elevated $[Ca^{2+}]_i$ from 55 ± 6 to a peak of 169 ± 23 and a plateau of 132 ± 16 nM ($n = 18$; Fig. 5).

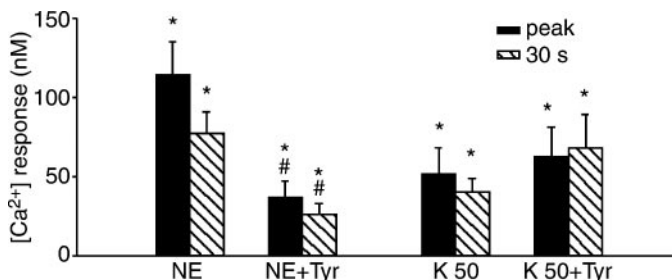


Fig. 5. NE (10^{-6} M)- and K50-induced peak and sustained plateau changes in $[Ca^{2+}]_i$ before and after pretreatment with tyrphostin 23 (Tyr; 50 μ M). Note that the blocking of the NE response is similar to the effect of 50 μ M Gen and that Tyr does not attenuate the response to K50. Values are NE- and K50-induced absolute increases in $[Ca^{2+}]_i$ from baseline in the presence or absence of the pretreatment solution. * $P < 0.05$ vs. baseline. # $P < 0.05$ vs. NE without treatment ($n = 18$ and 6 for NE and K50, respectively).

After 10-min pretreatment with Tyr-23 (50 μ M), baseline $[Ca^{2+}]_i$ rose from 55 ± 5 to 84 ± 7 nM. NE stimulation caused an increase to 121 ± 12 nM within the initial 15 s and to 110 ± 10 nM at 30–35 s. Thus Tyr-23 reduced the NE-induced peak and plateau responses to 30 ± 6 and $30 \pm 5\%$ of those during control conditions, respectively. This degree of inhibition is similar to what we observed with 50 μ M Gen. Preliminary experiments revealed that 250 μ M Tyr-23 exerted no stronger inhibition than did the 50 μ M dose. Thus, for the remaining studies involving Tyr-23, we elected to use a concentration of 50 μ M and a pretreatment time of 10 min. We also tested the effect of an inactive analog Tyr-1 on the NE-induced response in paired experiments. We found that the responses after 10-min pretreatment with Tyr-1 (50 μ M) were 112 ± 17 and $79 \pm 8\%$ ($n = 6$) of the control response for the peak and plateau, respectively. Both the peak and plateau responses are significantly greater than the corresponding values after Tyr-23 treatment (30 ± 6 and $30 \pm 5\%$; see above).

We also evaluated the effect of Tyr-23 on NE-induced Ca^{2+} mobilization and entry. NE stimulation in the presence of the 0 Ca solution was compared with that during 0 Ca + Tyr-23 (50 μ M). NE application in the presence of the 0 Ca solution caused the expected transient $[Ca^{2+}]_i$ peak, from 38 ± 12 to 75 ± 14 nM ($n = 7$) that returned to baseline (Fig. 6). Pretreatment with 0 Ca + Tyr-23 further attenuated the peak $[Ca^{2+}]_i$ response to NE, from the basal level of 42 ± 9 to 56 ± 13 nM ($n = 7$). In both 0 Ca experiments, $[Ca^{2+}]_i$ returned to baseline by 30–35 s, indicating the absence of a plateau phase that normally represents Ca^{2+} entry.

Because we found that Gen and nifedipine had additive inhibitory effects on the $[Ca^{2+}]_i$ response to NE, we investigated the combined effect of Tyr-23 (50 μ M) and nifedipine (1 μ M). Ten-minute pretreatment with nifedipine blocked the peak and plateau responses to NE to 69 ± 7 and $66 \pm 9\%$ of the control response, respectively. In the absence of nifedipine, NE increased the $[Ca^{2+}]_i$ from 60 ± 11 to peak and plateau values of 201 ± 23 and 154 ± 16 nM ($n = 9$). Nifedipine reduced the NE responses as basal 59 ± 14 nM rose to a peak

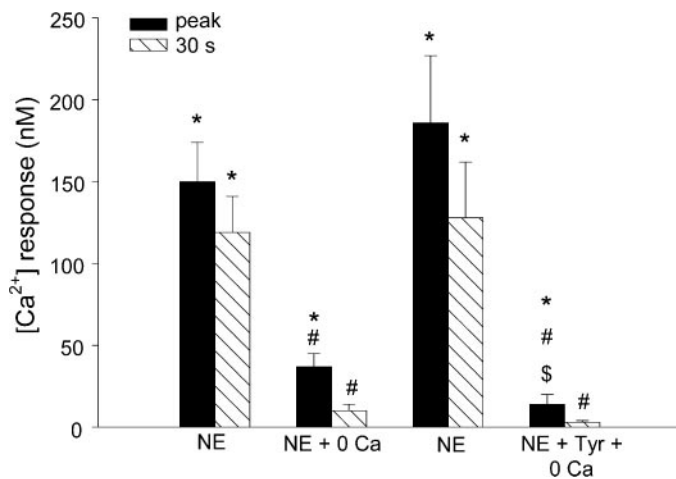


Fig. 6. Peak and sustained plateau changes in $[Ca^{2+}]_i$ following stimulation with NE (10^{-6} M) before and after pretreatment with the nominally Ca^{2+} -free solution 0 Ca or Tyr (50 μ M) + 0 Ca. Values are NE-induced absolute increases in $[Ca^{2+}]_i$ from baseline in the presence or absence of the pretreatment solution. Each group consisted of 7 arterioles. * $P < 0.05$ vs. baseline. # $P < 0.05$ vs. NE without treatment. \$ $P < 0.05$ vs. 0 Ca only.

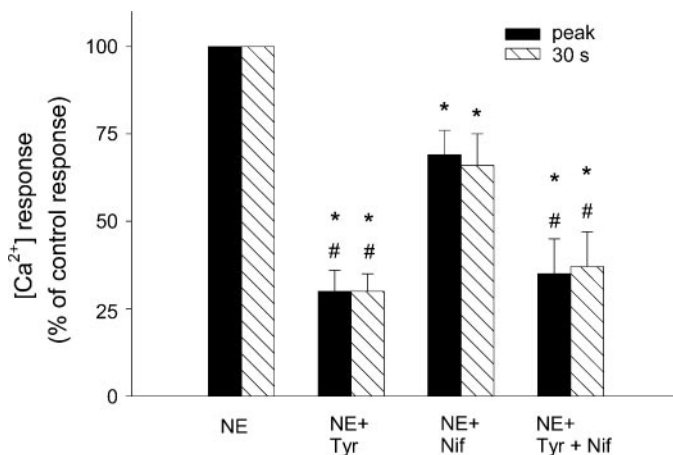


Fig. 7. Summary of experiments showing the peak and sustained plateau changes in $[Ca^{2+}]_i$ following stimulation with NE (10^{-6} M) after pretreatment with nifedipine (1 μ M), Tyr (50 μ M), or a combination thereof for 10 min. Values are expressed as a percentage of control responses (without pretreatment). All responses are significantly smaller than corresponding control. Each group consisted of 9 or more arterioles. * $P < 0.05$ vs. baseline. # $P < 0.05$ vs. nifedipine.

of 161 ± 27 and a plateau of 121 ± 18 nM ($n = 9$; Fig. 7). When the preparations were treated with the combination of Tyr-23 and nifedipine, greater inhibition was observed ($P < 0.02$). Peak and plateau responses were 35 ± 7 and $37 \pm 10\%$ (from 81 ± 9 to 129 ± 16 and 123 ± 14 nM; $n = 9$) of the control response (from 73 ± 11 to 212 ± 26 and 179 ± 16 nM; $n = 9$). On the other hand, the combined effect of Tyr-23 + nifedipine was no greater than the effect of Tyr-23 alone (30 ± 6 and $30 \pm 5\%$ of control response for peak and plateau, respectively; $P > 0.5$). This indicates that Tyr-23 blocks most of the Ca^{2+} entry via L-type Ca^{2+} channels.

We therefore determined the effect of addition of nifedipine on the NE-induced plateau phase in vessels exposed to Tyr-23. After 10-min Tyr-23 pretreatment, baseline $[Ca^{2+}]_i$ was 65 ± 11 nM. NE caused a sustained $[Ca^{2+}]_i$ plateau of 85 ± 11 nM ($n = 3$). Subsequent addition of 1 μ M nifedipine had no effect on the plateau level (83 ± 12 nM) in the continued presence of NE and Tyr-23. This finding further strengthens the notion that Tyr-23 blocks Ca^{2+} entry via L-type channels.

Effect of Tyr-23 on K50 responses. Additional studies assessed whether Tyr-23 inhibits L-type Ca^{2+} channels via a direct or indirect action. We examined effects of Tyr-23 on KCl-induced depolarization. The K50 solution caused a peak $[Ca^{2+}]_i$ increase from 54 ± 7 to 109 ± 12 nM and a sustained plateau of 98 ± 4 nM ($n = 6$; Fig. 5). Pretreatment with Tyr-23 (50 μ M) for 10 min did not inhibit the peak and plateau responses to K50 as in the presence of Tyr-23; K50 increased $[Ca^{2+}]_i$ from 86 ± 8 to 152 ± 19 and 158 ± 22 nM. Thus we did not find any indication that Tyr-23 exerted any direct blocking effect on L-type channels.

Effect of Tyr-23 and Tyr-1 on resting baseline $[Ca^{2+}]_i$ levels. As mentioned above, Tyr-23 for 10 min increased baseline $[Ca^{2+}]_i$. Tyr-1, on the other hand, did not increase the $[Ca^{2+}]_i$ baseline. On the contrary, after 10-min pretreatment with 50 μ M Tyr-1, there was a tendency for baseline $[Ca^{2+}]_i$ to decrease (from 89 ± 23 to 70 ± 20 nM; $n = 6$). This difference was not, however, statistically significant ($P > 0.08$). To evaluate whether the Tyr-23-induced increase was

due to Ca^{2+} entry via L-type channels, we pooled the paired experiments to compare Tyr-23 effects on basal $[Ca^{2+}]_i$ in the absence and presence of nifedipine. Pretreatment with Tyr-23 (50 μ M) for 10 min elevated $[Ca^{2+}]_i$ from 66 ± 8 to 90 ± 10 nM ($n = 9$). In the presence of nifedipine, Tyr-23 increased the baseline $[Ca^{2+}]_i$ from 63 ± 9 to 81 ± 9 nM. This increase was not significantly different from the increase obtained with Tyr-23 only ($P > 0.5$). Next, we pooled all paired pretreatment experiments using the 0 Ca solution and the 0 Ca + Tyr-23 combination. Treatment with 0 Ca for 10 min caused baseline $[Ca^{2+}]_i$ to decrease by 38 ± 4 nM ($n = 18$). When treated with a combination of 0 Ca + Tyr-23, the decrease was 22 ± 4 nM, a smaller drop than that recorded using 0 Ca only ($P < 0.01$). These findings suggest that blocked extrusion of Ca^{2+} from the cytoplasm rather than increased entry might be the reason $[Ca^{2+}]_i$ was increased by Tyr-23 treatment.

DISCUSSION

It is clear that an increase in $[Ca^{2+}]_i$ is a major link in the chain of events leading to contraction of VSMC (46). Previous studies characterized NE effects on $[Ca^{2+}]_i$ in isolated renal vessels (21, 28, 32–34, 51). We previously showed that the $[Ca^{2+}]_i$ response to adrenoceptor stimulation is dependent on both entry from extracellular fluid and mobilization from intracellular Ca^{2+} stores (32, 33). There is a paucity of information concerning the possible role of tyrosine kinases in the intracellular signaling mechanisms that mediate adrenoceptor-induced action on renal resistance vessels. To gain insight into this issue, our aims were to evaluate the role of tyrosine phosphorylation in NE-induced Ca^{2+} mobilization, entry, or both.

Tyrosine kinases are thought to act at several levels of intracellular signaling leading to VSMC contraction (16). This includes modulation of Ca^{2+} and K^+ channels, intracellular Ca^{2+} stores, Ca^{2+} sensitivity of the contractile apparatus, and interaction between the contractile apparatus and the cytoskeleton (16, 39). Several studies of nonrenal vascular beds report attenuation of adrenoceptor-induced vasoconstriction by inhibitors of tyrosine kinases (1, 8, 10, 13, 22, 43, 53). Although tyrosine kinases may affect contractility of VSMC unrelated to $[Ca^{2+}]_i$ (12), other studies suggest that tyrosine kinases act, at least in part, via control of $[Ca^{2+}]_i$ (22, 43). In addition, tyrosine kinase inhibitors impact on control of $[Ca^{2+}]_i$ to attenuate ANG II- and vasopressin-induced vasoconstriction (20, 44). In several VSMC preparations, it has been shown that activation of adrenoceptors causes tyrosine phosphorylation (20, 45, 47, 48). The phosphorylation is inhibited by tyrosine kinase inhibitors such as Gen and Tyr-23 (20, 47, 48). It seems that many proteins can be phosphorylated by NE activation of adrenoceptors in VSMC. In cultured aortic smooth muscle cells, at least nine different proteins are phosphorylated on NE stimulation (45). Other observations include NE-induced phosphorylation of paxillin (47) and mitogen-activated protein kinase (20). The diversity of proteins phosphorylated raises the possibility of several different effector mechanisms.

In the present study, we observed that two inhibitors of tyrosine kinase, Gen and Tyr-23, differing structurally and in mechanism of action, attenuated the $[Ca^{2+}]_i$ response to NE in isolated afferent arterioles. We found that Gen (50 μ M) and Tyr-23 (50 μ M) elicited similar buffering effects on the NE-induced $[Ca^{2+}]_i$ response, each blocking ~ 70 to 80%. It

appears that IC_{50} doses for these blockers vary among different preparations and subtypes of tyrosine kinases (16). In one study on large renal arteries (diameter ~ 150 – 300 μ m), Tyr-23 (50 μ M) is reported to have no effect on NE potency or maximal contraction (13). In the same study, Gen (50 μ M) had no effect on NE potency and only a minor effect on maximal contraction. Tyr-23 (100 μ M), however, attenuated maximal contraction to an extent similar to that found in the present study. Thus it is possible that the smaller renal resistance vessels are more sensitive or reactive than larger arteries. Tyr-23 is considered to be a more specific inhibitor of tyrosine phosphorylation than Gen (16). We also found that the inactive tyrphostin analog Tyr-1 had no attenuating effect on the peak response to NE and tended to have a small ($\sim 20\%$) attenuating effect on the plateau (at 30 s). Although Tyr-1 may have had a minor effect on the sustained phase, it should be appreciated that it was considerably smaller than the $\sim 70\%$ inhibition of both peak and plateau phases elicited by Tyr-23.

The action of another agonist of G protein-coupled receptors, ANG II, is thought to be partially dependent on tyrosine kinases in renal resistance vessels (4). Tyr-23 (100 μ M) was found to block $\sim 35\%$ of ANG II-induced contraction of afferent arterioles in the rat juxtamedullary nephron preparation. A subsequent study indicated that the EGF receptor tyrosine kinase mediates the response to ANG II by inhibiting Ca^{2+} influx but not mobilization (5). When the preparation was bathed in a solution containing a low concentration of Ca^{2+} (100 nM), the response to 100 nM ANG II consisted of a transient peak that was not affected by tyrosine kinase blockade with Tyr-AG 1748.

Our results indicate that tyrosine kinase inhibition attenuates the Ca^{2+} entry response to NE in renal arterioles. As we previously reported and as indicated by our present results, there is no elevation of sustained $[Ca^{2+}]_i$ during NE stimulation when Ca^{2+} entry is prevented by the absence of extracellular Ca^{2+} (32, 33). This finding indicates that Ca^{2+} recruitment during the sustained plateau phase occurs solely via Ca^{2+} entry from the extracellular fluid. Thus the attenuation of the $[Ca^{2+}]_i$ plateau by tyrosine kinase inhibition reflects blockade of Ca^{2+} entry. We have in this and other studies shown that 40–70% of the Ca^{2+} entry in response to NE is mediated via dihydropyridine-sensitive L-type Ca^{2+} channels (32, 33). We therefore sought to identify the role of tyrosine kinases in the NE-induced activation of L-type Ca^{2+} channels.

We found that 2 min of combined treatment with Gen (50 μ M) and nifedipine (1 μ M) caused an additive attenuation of the plateau response to NE. Under these conditions, there was no significant response to NE. This contrasts with the 35–40% of control responses observed when Gen or nifedipine was a single treatment. Thus it is clear that a substantial fraction of the non-L-type Ca^{2+} entry component is blocked by Gen.

Less certain, however, is at what level this inhibition occurs. Gen blocks a larger part of the response than can be accounted for by the non-L-type pathway, implicating an effect on inhibition of entry via the L-type channels. As other investigators reported that Gen may inhibit these channels (25, 49), we tested whether this occurred in afferent arterioles. It has also been suggested that pp60c-src, a cytosolic tyrosine kinase, stimulates L-type Ca^{2+} channel currents in VSMC (50). Indeed, we found that Gen blocks $\sim 60\%$ of the Ca^{2+} entry through voltage-gated Ca^{2+} channels in response to K50. This

observation supports the notion that part of the Gen effect on the total NE response is due to direct inhibition of L-type channels. This, however, does not exclude the possibility that Gen in addition exerts this effect by an action on upstream events that subsequently trigger activation of L-type channels.

Because Gen is thought to exert an indirect effect on L-type channels, we also used a more specific tyrosine kinase inhibitor Tyr-23 (52). As mentioned above, Tyr-23 (50 μM) attenuates the $[\text{Ca}^{2+}]_i$ response to NE to a similar extent as Gen (50 μM). When pretreatment with nifedipine and Tyr-23 was combined, there was no additive effect, in contrast to the effect observed with Gen. The combination of nifedipine and Tyr-23 produced no stronger inhibition of responses to NE than did Tyr-23 alone. On the other hand, the response was attenuated to a larger degree than after nifedipine pretreatment alone. Thus Tyr-23 appears to abolish NE-induced $[\text{Ca}^{2+}]_i$ entry via L-type channels and possibly inhibits some entry via an alternative pathway. This notion is further strengthened by results from posttreatment experiments in which addition of nifedipine failed to further inhibit the plateau response to NE after 10-min pretreatment with Tyr-23. We previously showed that the response to K50 is completely blocked by nifedipine (1 μM) (32). It has been reported that Tyr-23 directly inhibits Ca^{2+} channels in VSMC (43, 49). We found, however, in accord with findings from a recent study, that the response to depolarization with high extracellular $[\text{K}^+]$ was not attenuated by Tyr-23 (4).

Collectively, our results suggest different modes of action of Gen and Tyr-23. Both compounds appear to inhibit Ca^{2+} entry via L-type channels. Tyr-23 seems to effectively block this component of the NE-induced $[\text{Ca}^{2+}]_i$ response without interacting with the channel directly. Instead, Tyr-23 probably exerts a primary action on an upstream mechanism that secondarily activates L-type channels. Gen appears to directly inhibit L-type channel activity, but, paradoxically, the blockade of the sustained phase of the NE-induced response is less complete than with Tyr-23. Not clear is whether the effect of Gen on the Ca^{2+} entry component is solely due to direct action on the L-type channel or an indirect action on an upstream mechanism similar to that affected by Tyr-23. However, in the case of Gen, the direct action may predominate over the indirect. Further studies are required to resolve these differences. It is noteworthy that Tyr-23 and Gen inhibit tyrosine kinases by different mechanisms. Gen acts on ATP binding, whereas tyrphostins bind to the substrate-sensitive site and are accordingly considered to be more specific (2, 23).

In other experiments, we examined the effect of tyrosine kinase blockade on the transient $[\text{Ca}^{2+}]_i$ response to NE in the absence of extracellular Ca^{2+} . As previously established by us, this response, in afferent arterioles, consists of a clearly demonstrable immediate peak (32, 33). In the absence of Ca^{2+} entry, this transient is limited to release of Ca^{2+} from intracellular stores. We found that after 2-min pretreatment with 0 Ca, the transient peak to NE was reduced to $\sim 45\%$ of the peak response when Ca^{2+} entry is allowed. When afferent arterioles were pretreated with 0 Ca + Gen, this transient peak was totally abolished. Our findings are in agreement with earlier results for the rat aorta (1). We also noted an attenuation of the peak response, although not as pronounced, after 10-min pretreatment with Tyr-23 (50 μM) and 0 Ca. As previously mentioned, one study reported that the intracellular release

component of the Ca^{2+} response to ANG II was not affected by the tyrphostin-AG 1478, an agent considered to be specific for EGF receptor tyrosine kinase (5). This might indicate that the release component is not affected by plasma membrane EGF receptor kinase but by cytosolic tyrosine kinases as Tyr-23 does not distinguish between cytosolic and receptor tyrosine kinases. Another explanation is that release from intracellular stores plays a larger role in the NE response than that to ANG II, as has been indicated by other studies (17, 31, 32). Evidence implicates that tyrosine kinases act directly on IP_3 receptors of the sarcoplasmic reticulum (19) or upstream between the cell-surface G protein-coupled receptor and mobilization of intracellular Ca^{2+} (24, 27). The latter reports suggest that stimulation of rat VSMC with endothelin and ANG II activates tyrosine kinase-dependent production of IP_3 . It is noteworthy that Gen does not appear to affect $[\text{Ca}^{2+}]_i$ release activated directly by IP_3 or caffeine (24). One view is that activation of G protein-coupled receptors commonly tightly linked to phospholipase C (PLC)- β stimulates receptor or cytosolic tyrosine kinases that, in turn, activate PLC- γ , which leads to phosphoinositide hydrolysis and IP_3 production (27).

In contrast to Tyr-23, Gen does not affect baseline $[\text{Ca}^{2+}]_i$. The inactive analog Tyr-1 was also without effect in this regard. At present, we do not know whether the rise in baseline $[\text{Ca}^{2+}]_i$ with Tyr-23 reflects accelerated influx or retarded efflux. However, our results indicate that this increase in baseline $[\text{Ca}^{2+}]_i$ is at least partially due to impaired Ca^{2+} extrusion as the fall in baseline $[\text{Ca}^{2+}]_i$ after 10-min pretreatment with 0 Ca was reduced by concomitant Tyr-23 treatment. Furthermore, blockade of Ca^{2+} entry through nifedipine-sensitive channels does not blunt the increased baseline $[\text{Ca}^{2+}]_i$ due to Tyr-23.

In summary, our results indicate that two structurally different effective tyrosine kinase inhibitors, Gen and Tyr-23, but not inactive Tyr-1, buffer the $[\text{Ca}^{2+}]_i$ response to NE in rat afferent arteriolar VSMC. We noted similarities and differences that may reflect preferential actions of the inhibitors on different tyrosine kinases. Both Gen and Tyr-23 blunted the intracellular release of Ca^{2+} in response to NE. Both inhibitors impaired the Ca^{2+} entry response to NE; however, the mechanisms appear to differ. Gen affected a non-L-type Ca^{2+} entry pathway as well as voltage-gated L-type Ca^{2+} channels. Tyr-23 affected Ca^{2+} entry via L-type channels and the effect seems to be indirect, secondary to upstream events involving mobilization. Tyr-23 had no direct effect on high KCl-induced depolarization activation of Ca^{2+} entry, whereas Gen exerted partial inhibition on this entry pathway, suggesting a direct interaction with voltage-sensitive L-type Ca^{2+} entry channels. Taken together, our findings suggest that tyrosine phosphorylation is an important event in α -adrenoceptor-mediated control of renal vascular resistance. Tyrosine kinases may act at a proximal signal transduction site in α -adrenoceptor-induced Ca^{2+} recruitment via mobilization and entry pathways.

ACKNOWLEDGMENTS

The skillful technical assistance of A. Salomonsson is acknowledged.

GRANTS

These studies were supported by a National Institutes of Health Research Grant (HL-02334) from the National Heart, Lung, and Blood Institute, the Novo-Nordisk Foundation, the König-Petersen Foundation, the Berth von Kantzow's Foundation, and the Crafoord Foundation.

REFERENCES

- Abebe W and Agrawal DK. Role of tyrosine kinases in norepinephrine-induced contraction of vascular smooth muscle. *J Cardiovasc Pharmacol* 26: 153–159, 1995.
- Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, and Fukami Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 262: 5592–5595, 1987.
- Barajas L, Liu L, and Powers K. Anatomy of the renal innervation: intrarenal aspects and ganglia of origin. *Can J Physiol Pharmacol* 70: 735–749, 1992.
- Carmines PK, Fallet RW, Che Q, and Fujiwara K. Tyrosine kinase involvement in renal arteriolar constrictor responses to angiotensin II. *Hypertension* 37: 569–573, 2001.
- Che Q and Carmines PK. Angiotensin II triggers EGFR tyrosine kinase-dependent Ca^{2+} influx in afferent arterioles. *Hypertension* 40: 700–706, 2002.
- DiBona GF and Kopp UC. Neural control of renal function. *Physiol Rev* 77: 75–197, 1997.
- Di Salvo J, Gifford D, and Kokkinakis A. pp60c-src Kinase activity in bovine coronary extracts is stimulated by ATP. *Biochem Biophys Res Commun* 153: 388–394, 1988.
- Di Salvo J, Steusloff A, Semenchuk L, Satoh S, Kolquist K, and Pfitzer G. Tyrosine kinase inhibitors suppress agonist-induced contraction in smooth muscle. *Biochem Biophys Res Commun* 190: 968–974, 1993.
- Diliberto PA, Hubbert T, and Herman B. Early PDGF-induced alterations in cytosolic free calcium are required for mitogenesis. *Res Commun Chem Pathol Pharmacol* 72: 3–12, 1991.
- Duarte J, Ocete MA, Perez-Vizcaino F, Zarzuelo A, and Tamargo J. Effect of tyrosine kinase and tyrosine phosphatase inhibitors on aortic contraction and induction of nitric oxide synthase. *Eur J Pharmacol* 338: 25–33, 1997.
- Elberg G, Li J, Leibovitch A, and Shechter Y. Nonreceptor cytosolic protein tyrosine kinases from various rat tissues. *Biochim Biophys Acta* 1269: 299–306, 1995.
- Fang LH, Kwon SC, Zhang YH, and Ahn HY. Tyrosine kinase participates in vasoconstriction through a Ca^{2+} - and myosin light chain phosphorylation-independent pathway. *FEBS Lett* 512: 282–286, 2002.
- Fetscher C, Chen H, Schafers RF, Wambach G, Heusch G, and Michel MC. Modulation of noradrenaline-induced microvascular constriction by protein kinase inhibitors. *Naunyn Schmiedebergs Arch Pharmacol* 363: 57–65, 2001.
- Fleckenstein-Grun G. Calcium antagonism in vascular smooth muscle cells. *Pflügers Arch* 432: R53–R60, 1996.
- Gryniewicz G, Poenie M, and Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* 260: 3440–3450, 1985.
- Hughes AD and Wijetunge S. Role of tyrosine phosphorylation in excitation-contraction coupling in vascular smooth muscle. *Acta Physiol Scand* 164: 457–469, 1998.
- Incho EW, Imig JD, and Cook AK. Afferent and efferent arteriolar vasoconstriction to angiotensin II and norepinephrine involves release of Ca^{2+} from intracellular stores. *Hypertension* 29: 222–227, 1997.
- Iversen BM and Arendshorst WJ. ANG II and vasopressin stimulate calcium entry in dispersed smooth muscle cells of preglomerular arterioles. *Am J Physiol Renal Physiol* 274: F498–F508, 1998.
- Jayaraman T, Ondrias K, Ondriasova E, and Marks AR. Regulation of the inositol 1,4,5-trisphosphate receptor by tyrosine phosphorylation. *Science* 272: 1492–1494, 1996.
- Jin N, Siddiqui RA, English D, and Rhoades RA. Communication between tyrosine kinase pathway and myosin light chain kinase pathway in smooth muscle. *Am J Physiol Heart Circ Physiol* 271: H1348–H1355, 1996.
- Kornfeld M, Gutierrez AM, Gonzalez E, Salomonsson M, and Persson AE. Cell calcium concentration in glomerular afferent and efferent arterioles under the action of noradrenaline and angiotensin II. *Acta Physiol Scand* 151: 99–105, 1994.
- Lagaud GJL, Randriamboavonjy V, Roul G, Stoclet JC, and Andriantsitohaina R. Mechanism of Ca^{2+} release and entry during contraction elicited by norepinephrine in rat resistance arteries. *Am J Physiol Heart Circ Physiol* 276: H300–H308, 1999.
- Levitzki A and Gilon C. Tyrphostins as molecular tools and potential antiproliferative drugs. *Trends Pharmacol Sci* 12: 171–174, 1991.
- Liu CY and Sturek M. Attenuation of endothelin-1-induced calcium response by tyrosine kinase inhibitors in vascular smooth muscle cells. *Am J Physiol Cell Physiol* 270: C1825–C1833, 1996.
- Liu H, Li K, and Sperelakis N. Tyrosine kinase inhibitor, genistein, inhibits macroscopic L-type calcium current in rat portal vein smooth muscle cells. *Can J Physiol Pharmacol* 75: 1058–1062, 1997.
- Lyall RM, Zilberstein A, Gazit A, Gilon C, Levitzki A, and Schlessinger J. Tyrphostins inhibit epidermal growth factor (EGF)-receptor tyrosine kinase activity in living cells and EGF-stimulated cell proliferation. *J Biol Chem* 264: 14503–14509, 1989.
- Marrero MB, Paxton WG, Duff JL, Berk BC, and Bernstein KE. Angiotensin II stimulates tyrosine phosphorylation of phospholipase C- γ 1 in vascular smooth muscle cells. *J Biol Chem* 269: 10935–10939, 1994.
- Ollerstam A, Salomonsson M, and Persson AE. Reduced rat renal vascular response to angiotensin II after chronic inhibition of nNOS. *Acta Physiol Scand* 176: 245–252, 2002.
- Praddaude F, Marchetti J, Alhenc-Gelas F, and Ader J. Dissimilar mechanisms of Ca^{2+} response to bradykinin in different types of juxtamedullary glomerular arterioles. *Am J Physiol Renal Physiol* 277: F697–F705, 1999.
- Ramdas L, McMurray JS, and Budde RJ. The degree of inhibition of protein tyrosine kinase activity by tyrphostin 23 and 25 is related to their instability. *Cancer Res* 54: 867–869, 1994.
- Ruan X and Arendshorst WJ. Calcium entry and mobilization signaling pathways in ANG II-induced renal vasoconstriction in vivo. *Am J Physiol Renal Fluid Electrolyte Physiol* 270: F398–F405, 1996.
- Salomonsson M and Arendshorst WJ. Calcium recruitment in renal vasculature: NE effects on blood flow and cytosolic calcium concentration. *Am J Physiol Renal Physiol* 276: F700–F710, 1999.
- Salomonsson M and Arendshorst WJ. Norepinephrine-induced calcium signaling pathways in afferent arterioles of genetically hypertensive rats. *Am J Physiol Renal Physiol* 281: F264–F272, 2001.
- Salomonsson M, Brannstrom K, and Arendshorst WJ. α_1 -Adrenoceptor subtypes in rat renal resistance vessels: in vivo and in vitro studies. *Am J Physiol Renal Physiol* 278: F138–F147, 2000.
- Salomonsson M, Gonzalez E, Westerlund P, and Persson AE. Intracellular cytosolic free calcium concentration in the macula densa and in ascending limb cells at different luminal concentrations of sodium chloride and with added furosemide. *Acta Physiol Scand* 142: 283–290, 1991.
- Salomonsson M, Kornfeld M, Gutierrez AM, Magnusson M, and Persson AE. Effects of stimulation and inhibition of protein kinase C on the cytosolic calcium concentration in rabbit afferent arterioles. *Acta Physiol Scand* 161: 271–279, 1997.
- Salomonsson M, Oker M, Kim S, Zhang H, Faber JE, and Arendshorst WJ. α_1 -Adrenoceptor subtypes on rat afferent arterioles assessed by radioligand binding and RT-PCR. *Am J Physiol Renal Physiol* 281: F172–F178, 2001.
- Schlessinger J and Ullrich A. Growth factor signaling by receptor tyrosine kinases. *Neuron* 9: 383–391, 1992.
- Srivastava AK and St. Louis J. Smooth muscle contractility and protein tyrosine phosphorylation. *Mol Cell Biochem* 176: 47–51, 1997.
- Takasu N, Takasu M, Yamada T, and Shimizu Y. Epidermal growth factor (EGF) produces inositol phosphates and increases cytoplasmic free calcium in cultured porcine thyroid cells. *Biochem Biophys Res Commun* 151: 530–534, 1988.
- Thomas SM and Brugge JS. Cellular functions regulated by Src family kinases. *Annu Rev Cell Dev Biol* 13: 513–609, 1997.
- Tolloczko B, Tao FC, Zacour ME, and Martin JG. Tyrosine kinase-dependent calcium signaling in airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 278: L1138–L1145, 2000.
- Toma C, Jensen PE, Prieto D, Hughes A, Mulvany MJ, and Aalkjaer C. Effects of tyrosine kinase inhibitors on the contractility of rat mesenteric resistance arteries. *Br J Pharmacol* 114: 1266–1272, 1995.
- Touyz RM and Schiffrin EL. Tyrosine kinase signaling pathways modulate angiotensin II-induced calcium ($[Ca^{2+}]_i$) transients in vascular smooth muscle cells. *Hypertension* 27: 1097–1103, 1996.
- Tsuda T, Kawahara Y, Shii K, Koide M, Ishida Y, and Yokoyama M. Vasoconstrictor-induced protein-tyrosine phosphorylation in cultured vascular smooth muscle cells. *FEBS Lett* 285: 44–48, 1991.
- Walsh MP. Regulation of vascular smooth muscle tone. *Can J Physiol Pharmacol* 72: 919–936, 1994.
- Ward DT, Alder AC, Ohanian J, and Ohanian V. Noradrenaline-induced paxillin phosphorylation, ERK activation and MEK-regulated contraction in intact rat mesenteric arteries. *J Vasc Res* 39: 1–11, 2002.

48. **Ward DT, Ohanian J, Heagerty AM, and Ohanian V.** Phospholipase D-induced phosphatidate production in intact small arteries during nor-adrenaline stimulation: involvement of both G-protein and tyrosine-phosphorylation-linked pathways. *Biochem J* 307: 451–456, 1995.
49. **Wijetunge S, Aalkjaer C, Schachter M, and Hughes AD.** Tyrosine kinase inhibitors block calcium channel currents in vascular smooth muscle cells. *Biochem Biophys Res Commun* 189: 1620–1623, 1992.
50. **Wijetunge S and Hughes AD.** pp60c-src Increases voltage-operated calcium channel currents in vascular smooth muscle cells. *Biochem Biophys Res Commun* 217: 1039–1044, 1995.
51. **Yip KP and Marsh DJ.** $[Ca^{2+}]_i$ in rat afferent arteriole during constriction measured with confocal fluorescence microscopy. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F1004–F1011, 1996.
52. **Yokoshiki H, Sumii K, and Sperelakis N.** Inhibition of L-type calcium current in rat ventricular cells by the tyrosine kinase inhibitor, genistein and its inactive analog, daidzein. *J Mol Cell Cardiol* 28: 807–814, 1996.
53. **Zerrouk A, Auguet M, Dabire H, Brisac AM, Safar M, and Chabrier PE.** Differential effects of tyrosine kinase inhibitors on contraction and relaxation of the aortas of normotensive and hypertensive rats. *Eur J Pharmacol* 374: 49–58, 1999.

