p38 MAP kinase-mediated negative inotropic effect of HIV gp120 on cardiac myocytes

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Departments of 1Medicine and 2Physiology and Pharmacology, West Virginia University School of Medicine, Robert C. Byrd Health Sciences Center, Morgantown 26506; and 3Louis A. Johnson Department of Veterans Affairs Medical Center, Clarksburg, West Virginia 26301

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Kan, Hong, Zirong Xie, and Mitchell S. Finkel. p38 MAP kinase-mediated negative inotropic effect of HIV gp120 on cardiac myocytes. Am J Physiol Cell Physiol 286: C1–C7, 2004; 10.1152/ajpcell.00059.2003.—Myocardial dysfunction leading to dilated cardiomyopathy has been documented with surprisingly high frequency in human immunodeficiency virus (HIV)-infected individuals. p38 MAP kinase has been implicated as a mediator of myocardial dysfunction. We previously reported p38 MAP kinase activation by the HIV coat protein gp120 in neonatal rat cardiac myocytes. We now report the direct inotropic effects of HIV gp120 on adult rat ventricular myocytes (ARVM). ARVM were continuously superfused with gp120, and percent fractional shortening (FS) was determined by automated border detection and simultaneous intracellular ionized free Ca2+ concentration ([Ca2+]i) measured by fura 2-AM fluorescence: gp120 alone increased FS and increased [Ca2+]i, within 5 min and then depressed FS without a decrease in [Ca2+]i, by 20–60 min, which persisted for at least 2 h. Exposure of ARVM to gp120 also resulted in the phosphorylation of the upstream regulator of p38 MAP kinase MKK3/6, p38 MAP kinase itself, and its downstream effector, ATF-2, over a similar time course. ERK (p44/42) and JNK stress signaling pathways were not similarly activated. The effects of the p38 MAP kinase inhibitor were concentration dependent. SB-203580 (10 μM) blocked both p38 MAP kinase phosphorylation and the delayed negative inotropic effect of gp120. SB-203580 (5 μM) selectively blocked phosphorylation of ATF-2 without blocking the phosphorylation of MKK3/6 or p38 MAP kinase itself. SB-203580 (5 μM) administered before, with, or after gp120 blocked the negative inotropic effect of gp120 in ARVM. p38 MAP kinase activation may be a common stress-response mechanism contributing to myocardial dysfunction in HIV and other nonischemic as well as ischemic cardiomyopathies.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) type 1 is a retrovirus that carries two copies of its RNA genome that are transcribed into DNA inside infected cells and integrated into the host cell chromosome (17). HIV enters cells through a complex process that follows the direct binding of a viral envelope glycoprotein, gp120, that resides on the surface of the HIV virus (18). The HIV glycoprotein gp120 binds with high affinity to CD4 receptor-positive T lymphocytes, resulting in severe CD4+ T lymphocyte depletion and the development of the acquired immunodeficiency syndrome (AIDS) (18). Many AIDS-related deaths were initially due to opportunistic infections in severely immunocompromised patients. Recent therapeutic advances have now markedly improved the long-term survival of HIV-infected individuals. This improvement in prognosis has been accompanied by the recognition of other manifestations of HIV infection such as dementia and cardiomyopathy (2, 3, 5, 9).

Evidence is accumulating that HIV gp120 may also play an important pathogenic role in HIV dementia and cardiomyopathy. Direct effects of HIV gp120 on rat neurons have been reported (24, 25). These effects appear to involve changes in intracellular ionized free calcium and p38 MAP kinase activation (13, 19, 24, 34). Altered calcium homeostasis and p38 MAP kinase activation have also been implicated in myocardial dysfunction (8, 21, 27, 31, 35, 36, 40, 46). We previously reported (23) that HIV gp120 stimulates p38 MAP kinase activation in neonatal rat cardiac myocytes. The physiological consequences of these findings were not explored. We now report direct inotropic effects of recombinant HIV gp120 on adult rat ventricular myocytes (ARVM) mediated by a signaling pathway involving p38 MAP kinase activation. HIV gp120 may elicit a common stress response signaling pathway in ARVM that contributes to HIV and other cardiomyopathies.

MATERIALS AND METHODS

Materials. All reagents were purchased from Sigma (St. Louis, MO) or obtained from the National Institutes of Health (NIH) AIDS Repository (HIV-1 gp120; >99% pure) unless otherwise indicated. Adult male Sprague-Dawley rats (250–300 g) were purchased from Hilltop Lab Animals (Scottdale, PA) and housed in a room specifically dedicated for rats in the Animal Care Facility of the Robert C. Byrd Health Sciences Center of West Virginia University. Strict adherence to the protocol approved by the West Virginia University Animal Care and Use Committee was maintained throughout the study.

Statistical methods. Data represent the means ± SE of 12–15 different determinations derived from 12–15 individual myocytes from 7–9 separate myocyte preparations from 7–9 different rats. Analysis of variance (ANOVA) was used for multigroup comparisons. Values of P < 0.05 were considered statistically significant.

Adult rat ventricular myocytes. Cells were isolated from the hearts of adult male Sprague-Dawley rats (250–300 g) as previously reported by Kan et al. (22). Rats were anesthetized with pentobarbital sodium, and the hearts were removed rapidly and perfused with Krebs-Henseleit bicarbonate buffer (KHB) containing (in mM) 118.1 NaCl, 3.0 KCl, 1.8 CaCl2, 1.2 MgSO4, 1.0 KH2PO4, 27.3 NaHCO3, 10.0 glucose, and 2.5 pyruvic acid, pH 7.4, according to the method of Langendorff at a constant rate of 8 ml/min with a peristaltic pump. All buffer and enzyme solutions used during cell isolation were maintained at 37°C and preequilibrated with 95% O2-5% CO2. Hearts were perfused with KHB for 15 min, followed by changing to low-Ca2+ KHB containing (in mM) 105.1 NaCl, 3.0 KCl, 0.01 CaCl2, 1.2

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MgSO$_4$, 1.0 KH$_2$PO$_4$, 20.0 NaHCO$_3$, 10.0 glucose, 5.0 pyruvic acid, 10.0 taurine, and 5.0 mannitol, pH 7.4, for an additional 10 min. The hearts were then immersed in recirculating KHB with low Ca$^{2+}$, containing collagenase B (1.25 mg/ml; Boehringer Mannheim Biochemicals, Indianapolis, IN) for 40 min. The ventricles were minced and placed into a 50-ml centrifuge tube, adjusted to 25 ml with low-Ca$^{2+}$ KHB, and centrifuged at 500 g for 2 min. The supernatant was aspirated, and the concentration of Ca$^{2+}$ in KHB was increased in four increments (0.08, 0.6, 1.2, and 1.8 mM). Finally, the mixture was passed through 225-μm nylon mesh and centrifuged at 500 g for 2 min. The centrifuge procedure was repeated until the preparation was composed of at least 80% viable left ventricular myocytes. The myocytes exhibited typical striated and rod-shaped appearance when viewed by light microscope. Only those myocytes that were rod shaped, with striations and no blebs, and not spontaneously contracting were included for analyses (12). Physiological experiments were conducted with continuous superfusion of 95% O$_2$-5% CO$_2$ KHB buffer and electrically stimulated (95% O$_2$-5% CO$_2$) KHB alone maintained the same values for FS as an indicator of contractility and ratio of fura 2 Ca$^{2+}$ fluorescence as an indicator of [Ca$^{2+}$], were determined for each cell. Freshly isolated ARVM superfused with oxygenated (95% O$_2$-5% CO$_2$) KHB alone maintained the same values for FS and [Ca$^{2+}$], for several hours in preliminary experiments (Fig. 1). The data derived from control baseline experiments were consistent with the published literature (38, 44). The $K_d$ for radioligand binding of HIV gp120 to CD4$^+$ lymphocytes was reported to be 6 nM (39, 43). Accordingly, we used 1 μg/ml of HIV gp120 as we previously reported in neonatal myocytes (23), which corresponds to 8 nM. Effects on ARVM observed at this concentration would be consistent with potential consequences of gp120 binding to cardiac myocytes in competition with receptors on the surface of lymphocytes, macrophages, and dendritic cells. The continuous superfusion of ARVM with 1 μg/ml of recombinant gp120 resulted in an unprecedented biphasic inotropic effect: an immediate increase in FS within minutes and a subsequent decrease in FS beginning at 20–60 min and persisting for at least 2 h (Figs. 2 and 3). No effect was seen with another HIV recombinant protein, tat (data not shown). The use of continuous superfusion allowed each myocyte to serve as its own control. This approach

### RESULTS

Freshly isolated ARVM were continuously superfused with oxygenated (95% O$_2$-5% CO$_2$) KHB buffer and electrically stimulated at 0.5 Hz. Simultaneous measurements of percent FS as an indicator of contractility and ratio of fura 2 Ca$^{2+}$ fluorescence were consistent with the published literature [% fractional shortening (FS) = 12.6 ± 0.5; calcium ratio 1.48 ± 0.15; n = 12 myocytes from 7 rats].

**Fig. 1. Representative recordings of simultaneously determined changes in cell length and intracellular free ionized Ca$^{2+}$ concentration ([Ca$^{2+}$]) in electrically stimulated adult rat ventricular myocytes (ARVM) continuously superfused with oxygenated Krebs-Henseleit bicarbonate buffer (KHB) alone.**

- The ventricles were minced and placed into a 50-ml centrifuge tube, and centrifuged at 500 g for 2 min. The supernatant was aspirated, and the concentration of Ca$^{2+}$ in KHB was increased in four increments (0.08, 0.6, 1.2, and 1.8 mM). Finally, the mixture was passed through 225-μm nylon mesh and centrifuged at 500 g for 2 min. The centrifuge procedure was repeated until the preparation was composed of at least 80% viable left ventricular myocytes. The myocytes exhibited typical striated and rod-shaped appearance when viewed by light microscope. Only those myocytes that were rod shaped, with striations and no blebs, and not spontaneously contracting were included for analyses (12).
- Physiological experiments were conducted with continuous superfusion of 95% O$_2$-5% CO$_2$ KHB buffer and electrically stimulated (95% O$_2$-5% CO$_2$) KHB alone maintained the same values for FS and [Ca$^{2+}$], for several hours in preliminary experiments (Fig. 1). The data derived from control baseline experiments were consistent with the published literature (38, 44). The $K_d$ for radioligand binding of HIV gp120 to CD4$^+$ lymphocytes was reported to be 6 nM (39, 43). Accordingly, we used 1 μg/ml of HIV gp120 as we previously reported in neonatal myocytes (23), which corresponds to 8 nM. Effects on ARVM observed at this concentration would be consistent with potential consequences of gp120 binding to cardiac myocytes in competition with receptors on the surface of lymphocytes, macrophages, and dendritic cells. The continuous superfusion of ARVM with 1 μg/ml of recombinant gp120 resulted in an unprecedented biphasic inotropic effect: an immediate increase in FS within minutes and a subsequent decrease in FS beginning at 20–60 min and persisting for at least 2 h (Figs. 2 and 3). No effect was seen with another HIV recombinant protein, tat (data not shown). The use of continuous superfusion allowed each myocyte to serve as its own control. This approach

**Fig. 2. Representative recordings of simultaneously determined cell length and [Ca$^{2+}$]i in electrically stimulated ARVM continuously superfused with oxygenated KHB + HIV gp120 (1 μg/ml). The same experiment was repeated in 15 myocytes from 9 rats (%FS at baseline = 13.2 ± 0.8; calcium ratio = 1.42 ± 0.25).**
overcomes the limitations imposed by the cell-to-cell variability inherent in cardiac myocyte cell lengths. The initial positive inotropic effect of gp120 was associated with a corresponding increase in \( [\text{Ca}^{2+}]_i \) (Figs. 2 and 4). This is consistent with the most common mechanism of enhancing contractility by increasing \( [\text{Ca}^{2+}]_i \) (4). However, the delayed negative inotropic effect was not associated with a decrease in \( [\text{Ca}^{2+}]_i \) compared with controls (Figs. 2 and 4). A plausible mechanism by which binding of gp120 to the surface of a cardiac myocyte could lead to changes in contractile proteins remained to be determined.

HIV gp120 has been reported to activate p38 MAP kinase in rat neurons (24). We also previously reported (23) that HIV gp120 activates p38 MAP kinase in isolated neonatal rat cardiac myocytes. Therefore, we sought to determine whether the inotropic effect(s) of gp120 could be altered by the p38 MAP kinase inhibitor SB-203580. Pretreatment of myocytes with 10 \( \mu M \) SB-203580 completely blocked the negative inotropic effect of gp120 while having no effect on the initial positive inotropic effect (Fig. 5). SB-203580 alone had no inotropic effect. Interestingly, SB-203580 also had no effect on the initial gp120-mediated increase in \( [\text{Ca}^{2+}]_i \) (Fig. 6).

More direct evidence of a role for p38 MAP kinase in the negative inotropic effect of gp120 was provided by measuring phosphorylation of proteins involved in the p38 MAP kinase signaling pathway. Exposure of ARVM to gp120 (1 \( \mu g/ml \)) resulted in a time-dependent phosphorylation of p38 MAP kinase and its downstream effector, ATF-2 (Fig. 7). This pattern was selective for p38 MAP kinase and was not shared by p44/42 and JNK kinases (Fig. 7). HIV gp120 exposure had no apparent effect on p44/42 phosphorylation. JNK appears to be slightly and only transiently activated within a few minutes after exposure to gp120.

The contribution of the components of the p38 MAP kinase pathway to the negative inotropic effect of HIV gp120 on ARVM was further explored. Exposure of ARVM to gp120 (1 \( \mu g/ml \)) also resulted in phosphorylation of MKK3/6, an upstream activator of p38 (Fig. 8). A lower concentration of SB-203580 was used to ensure greater specificity of inhibition of p38 MAP kinase activity. Five micromolar SB-203580 selectively blocked the phosphorylation of ATF-2 by p38 MAP kinase without blocking the phosphorylation of either MKK3/6 or p38 MAP kinase (Fig. 7).

This lower concentration of SB-203580 (5 \( \mu M \)) also blocked the negative inotropic effect of gp120 as noted above for 10 \( \mu M \) SB-203580 (Figs. 5 and 8). In addition, the administration of 5 \( \mu M \) SB-203580 for 20 min before or 20 min after gp120 treatment blocked the gp120-induced negative inotropic effect (Fig. 9).
We previously reported (23) that the recombinant HIV coat protein gp120 activates p38 MAP kinase in neonatal rat cardiac myocytes. The physiological consequences of p38 MAP kinase activation were not explored in neonatal myocytes. We now report that gp120 directly activates a functional signaling pathway in ARVM. Continuous superfusion of ARVM with HIV gp120 transiently increases both \([\text{Ca}^{2+}]_i\) and percent FS over an initial 10-min period (Figs. 1–4). This is followed by a subsequent decrease in FS with no change in \([\text{Ca}^{2+}]_i\) that persists for at least 2 h (Figs. 2–4). The negative inotropic effect of HIV gp120 can be blocked by the p38 MAP kinase inhibitor SB-203580 at concentrations that correspond to the selective inhibition of p38 MAP kinase phosphorylation of ATF-2 (Figs. 5–8). The time course of p38 MAP kinase activation (but not p44/42 or JNK) corresponds to the negative inotropic effect of HIV gp120. Activation of p38 MAP kinase appears to be an important component of the pathways activated by ischemia in cardiac myocytes (31, 32, 35, 36). Thus the significance of this HIV gp120 signaling pathway in ARVM may extend beyond its potential relevance to HIV cardiomyopathy.

The development of a cardiomyopathy is a well-recognized complication of HIV infection that confers a worse prognosis for those afflicted with AIDS (3, 26, 29). An autopsy study found myocarditis in 52% and dilated cardiomyopathy in 10% of AIDS patients (1). Echocardiographic evidence of decreased FS (myocardial dysfunction) has been reported to predict an increase in mortality among HIV-infected children (2, 3, 8, 28).

A pathogenic role for gp120 in HIV cardiomyopathy is very similar to the role already proposed for gp120 in HIV dementia (5, 10). HIV gp120 has been reported to have CD4-independent effects on rat neurons that may be mediated through the \(N\)-methyl-D-aspartate (NMDA) receptor (10, 41). Drugs that either deplete (caffeine, thapsigargin) or block release from (dantrolene) intracellular Ca\(^{2+}\) stores were effective in blocking the rise in intracellular Ca\(^{2+}\) and cell death in rat neural tissue caused by gp120 (34). In addition, gp120 has been reported to regulate p38 MAP kinase activity in neurons (24, 25). Our results provide evidence for both Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent effects of gp120 in cardiac myocytes. Ca\(^{2+}\)-independent effects of gp120 have not been reported in neurons. This suggests differences between cardiac myocyte and neuronal receptors or signaling pathways and/or differences in the techniques used to study neurons and myocytes. These
observations in rat brain and heart support the view that clinically relevant insights into manifestations of HIV infection can be derived from studies in animal models that do not develop immunosuppression and death from HIV exposure. This may be true for HIV cardiomyopathy as well as dementia.

As noted above, this gp120 signaling pathway involves p38 MAP kinase activation, p38 MAP kinase is a member of a class of intracellular enzymes that phosphorylate proteins in response to inflammatory mediators (e.g., cytokines) and stress (e.g., ischemia) (40, 42). Evidence is rapidly accumulating in support of a pathogenic role for p38 MAP kinase in myocardial dysfunction in animal models and humans (8, 28). p38 MAP kinase activation has been implicated in ischemia, hypertrophy, apoptosis, and adrenergic signaling in cardiac myocytes (31, 32, 35, 45, 46). We now provide compelling evidence implicating p38 MAP kinase activation in the negative inotropic effect of gp120 in ARVM. A relatively brief and transient p38 MAP kinase response may confer early adaptive benefits by uncoupling Ca\(^{2+}\) release from myocyte contraction. This could conserve energy and diminish oxidative stress. However, the more prolonged activation of neurons by HIV gp120 has been shown to result in apoptosis (10, 24). Similarly, prolonged and repeated activation of p38 MAP kinase in cardiac myocytes by gp120 could result in HIV cardiomyopathy in vulnerable individuals.

Adrenergic signaling is characteristically blunted in humans as well as animal models with myocardial depression, cardiomyopathy, and chronic heart failure from a variety of causes (14, 16, 21). A physiological role for p38 MAP kinase in adrenergic signaling in cardiac myocytes was recently suggested by an elegant study in a transgenic mouse model lacking \(\beta_1\)-adrenergic receptors (46). Inhibition of p38 MAP kinase activation with SB-203580 was found to enhance the positive inotropic effect of the \(\beta_1\)-adrenergic agonist isoproterenol through the \(\beta_2\)-adrenergic receptor. A physiologically relevant role for p38 MAP kinase in adrenergic signaling in cardiac myocytes was further supported by studies in avian cardiac myocytes (33). Magne et al. (33) provided strong evidence for the regulation of \(\beta_2\)-adrenergic signaling through a MAP kinase/cPLA\(_2\) pathway. Human heart failure is associated with a relative decrease in \(\beta_1\) and an increase in \(\beta_2\)-adrenergic receptors (6, 20). Thus activation of p38 MAP kinase by HIV gp120 would lead to blunted autonomic responses typical of human cardiomyopathies and chronic heart failure.

A negative inotropic effect of p38 MAP kinase was reported recently by Liao et al. (28). Activation of p38 MAP kinase was achieved by adenoviral gene transfer of an activated mutant of its upstream kinase, MKK3bE. The authors reported a [Ca\(^{2+}\)]-independent negative inotropic effect of the activated MKK3bE mutant. This is remarkably similar to the effect we report as a result of HIV gp120. Liao et al. (28) concluded that troponin I phosphorylation was not responsible for the negative inotropic effect of p38 MAP kinase. Their conclusion was based on the results of their experiments that did not reveal direct phosphorylation of purified troponin I protein by p38 MAP kinase in vitro. These observations would suggest that p38 MAP kinase does not directly phosphorylate troponin I. However, p38 MAP kinase could still participate in an upstream process that results in troponin I phosphorylation. Future studies are warranted to further elucidate the details of the steps involved in the physiological consequences of p38 MAP kinase activation in cardiac myocytes.

The activation of p38 MAP kinase by phosphorylation through a MAPKK-dependent pathway is well established (40, 42). Ge et al. (15) recently discovered an alternative MAPKK-independent mechanism of activation of p38 MAP kinase by autophosphorylation. The results of our studies in ARVM could not definitively distinguish between the MAPKK-dependent and MAPKK-independent pathways. We identified phosphorylation of both MKK3/6 and p38 MAP kinase by gp120 (Fig. 7). The blockade of the inotropic effect of gp120 by SB-203580 (5 \(\mu\)M) was associated with minimal changes in MKK3/6 and p38 MAP kinase phosphorylation along with a decrease in ATF-2 phosphorylation (Fig. 7). These observations are consistent with either MKK3/6- or p38 MAP kinase-mediated phosphorylation and activation of p38 MAP kinase signaling by gp120 in cardiac myocytes.

Our data are also consistent with a recent report by Chen et al. (7) that HIV gp120 depresses rabbit ventricular myocyte contractility. However, the activation of intracellular signaling pathways by gp120 was not explored. Our data and those of Chen et al. (7) indicate the presence of a high-affinity (nM) HIV binding site on cardiac myocytes. The previously identified binding sites for HIV gp120 include the CD4, CXCR4, CCR5, and NMDA receptors (10, 30, 37). Our data suggest the presence of structures homologous to CD4, CXCR4, CCR5, or NMDA receptors on ARVM. Alternatively, ARVM possess a completely novel receptor for HIV gp120. A single report of immunolocalization of CXCR4 to human cardiac myocytes has been published (11). The immunohistochemical data from human heart failure patients raise the interesting possibility that CXCR4 is the receptor responsible for the physiological effects of HIV gp120 on ARVM. This recently identified binding site has no previously known function in the heart. Unfortunately, the currently available antibodies to human CXCR4 do not cross-react with the rat. This signaling pathway is presumably part of the repertoire that cardiac myocytes (and other cells) have evolved to respond to infectious and/or ischemic injury. Elucidation of this receptor signaling pathway in ARVM may provide fundamental insights into cardiac myocyte adaptation to stressful stimuli.

Controlling the activation and termination of this gp120 signaling pathway in myocytes has considerable potential clinical relevance. These studies are likely to provide new insights into the mechanisms contributing to myocardial depression seen in HIV infection as well as other cardiomyopathies. The basic mechanisms involved in p38 MAP kinase activation are relevant to chronic heart failure, ischemia, ischemic preconditioning, and adrenergic signaling in cardiac myocytes as well (35, 36, 46). Management of the HIV epidemic has brought many challenges and new insights into basic biology and medicine. This gp120 signaling pathway in cardiac myocytes may provide a novel therapeutic target for HIV as well as other cardiomyopathies.

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REFERENCES


